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Table of Contents

Abstract	4
Zusammenfassung	5
1. Introduction	6
1.1 Background and Rationale	6
1.2 Aim and Objectives	7
1.3 Methodology of Literature Search and Analysis.....	8
1.4 Structure of the Thesis.....	9
2. Cathepsins: Classification and Biological Functions	11
2.1 General Characteristics of Cathepsins.....	11
2.2 Classification of Cathepsins	15
2.3 Physiological Functions of Cathepsins.....	20
2.4 Regulation of Cathepsin Activity	27
3. Role of Cathepsins in Cancer Progression	33
3.1 Cathepsins and Tumor Cell Proliferation.....	33
3.2 Interaction between Cathepsins and the Tumor Microenvironment	37
3.3 Role of Cathepsins in Angiogenesis.....	42
3.4 Role of Cathepsins in Tumor Cell Survival	45
4. Cathepsin-Mediated Signaling Pathways	50
4.1 PI3K/Akt Signaling Pathway	50
4.2 MAPK/ERK Signaling Pathway	54
4.3 NF- κ B Signaling Pathway.....	59
4.4 TGF- β Signaling Pathway	63
4.5 Wnt/ β -catenin Signaling Pathway	66
5. Role of Cathepsins in Tumor Invasion and Metastasis	70
5.1 Extracellular Matrix Degradation.....	70
5.2 Epithelial–Mesenchymal Transition (EMT)	75
5.3 Tumor Cell Migration and Invasion.....	78
5.4 Formation of Metastatic Niches	81
6. Therapeutic Potential of Targeting Cathepsins	85
6.1 Cathepsin Inhibitors	85
6.2 Cathepsins as Biomarkers in Oncology.....	88
6.3 Current Approaches to Targeted Therapy	91
6.4 Limitations and Future Clinical Perspectives.....	94
7. Discussion	96
7.1 Summary of Current Scientific Evidence.....	96

7.2 Challenges and Contradictions in Current Research	97
7.3 Future Research Perspectives	100
8. Conclusions	103
References	105

Abstract

Cathepsins comprise a family of lysosomal proteases that are indispensable for normal cellular physiology, where they participate in intracellular protein turnover, maintenance of proteostasis, autophagy, and numerous other homeostatic processes. In recent years, extensive experimental and clinical evidence has demonstrated that disturbances in the expression, localization, or enzymatic activity of these proteases are closely associated with the initiation and progression of malignant neoplasms. This thesis provides a comprehensive analysis of contemporary scientific literature concerning the biological functions of cathepsins and their involvement in the molecular mechanisms underlying cancer development.

The review focuses primarily on the roles of cathepsins B, L, S, K, and D in fundamental processes that determine tumor progression, including uncontrolled cellular proliferation, extracellular matrix remodeling, epithelial–mesenchymal transition, angiogenesis, invasion, migration, and establishment of metastatic niches. Particular emphasis is placed on the interactions between cathepsins and the principal oncogenic signaling pathways, namely PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , and Wnt/ β -catenin, highlighting their contribution to the regulation of tumor cell survival, proliferation, metastatic potential, and adaptation to the tumor microenvironment.

The thesis further examines the clinical significance of cathepsins by summarizing current evidence supporting their application as diagnostic and prognostic biomarkers in oncology. In addition, recent advances in cathepsin-targeted therapeutic strategies are reviewed, including the development of selective enzyme inhibitors, cathepsin-responsive prodrugs, nanotechnology-based drug delivery platforms, and antibody–drug conjugates incorporating protease-cleavable linkers. Finally, the principal limitations of existing studies and the major challenges associated with translating experimental discoveries into routine clinical practice are critically discussed.

Overall, the analyzed evidence demonstrates that cathepsins function as key molecular regulators of tumor biology and represent promising candidates for the development of innovative diagnostic tools and personalized therapeutic strategies aimed at improving the management of malignant diseases.

Keywords: cathepsins, cancer, tumor progression, signaling pathways, PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , Wnt/ β -catenin, invasion, metastasis, biomarkers, targeted therapy.

Zusammenfassung

Cathepsine bilden eine Familie lysosomaler Proteasen, die für die normale Zellphysiologie unerlässlich sind. Sie sind am intrazellulären Proteinumsatz, der Aufrechterhaltung der Proteostase, der Autophagie und zahlreichen weiteren homöostatischen Prozessen beteiligt. In den letzten Jahren haben umfangreiche experimentelle und klinische Studien gezeigt, dass Störungen der Expression, Lokalisation oder enzymatischen Aktivität dieser Proteasen eng mit der Entstehung und dem Fortschreiten maligner Neoplasien verknüpft sind. Diese Arbeit bietet eine umfassende Analyse der aktuellen wissenschaftlichen Literatur zu den biologischen Funktionen der Cathepsine und ihrer Beteiligung an den molekularen Mechanismen der Krebsentwicklung. Der Fokus der Übersicht liegt auf der Rolle der Cathepsine B, L, S, K und D in grundlegenden Prozessen der Tumorprogression, darunter unkontrollierte Zellproliferation, Umbau der extrazellulären Matrix, epithelial-mesenchymale Transition, Angiogenese, Invasion, Migration und die Etablierung metastatischer Nischen. Besonderes Augenmerk liegt auf den Wechselwirkungen zwischen Cathepsinen und den wichtigsten onkogenen Signalwegen, namentlich PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β und Wnt/ β -Catenin. Dabei wird deren Beitrag zur Regulation des Überlebens, der Proliferation, des Metastasierungspotenzials und der Anpassung von Tumorzellen an die Tumormikroumgebung hervorgehoben.

Die Arbeit untersucht ferner die klinische Bedeutung von Cathepsinen, indem sie die aktuelle Evidenz für deren Anwendung als diagnostische und prognostische Biomarker in der Onkologie zusammenfasst. Darüber hinaus werden aktuelle Fortschritte bei Cathepsin-gerichteten Therapiestrategien dargestellt, darunter die Entwicklung selektiver Enzymhemmer, Cathepsin-responsiver Prodrugs, nanotechnologiebasierter Wirkstoffträgersysteme und Antikörper-Wirkstoff-Konjugate mit proteasespaltbaren Linkern. Abschließend werden die wichtigsten Limitationen bestehender Studien und die größten Herausforderungen bei der Übertragung experimenteller Erkenntnisse in die klinische Routinepraxis kritisch diskutiert.

Die analysierten Daten zeigen insgesamt, dass Cathepsine als wichtige molekulare Regulatoren der Tumorbiologie fungieren und vielversprechende Kandidaten für die Entwicklung innovativer Diagnoseverfahren und personalisierter Therapieansätze zur Verbesserung der Behandlung maligner Erkrankungen darstellen.

Schlüsselwörter: Cathepsine, Krebs, Tumorprogression, Signalwege, PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , Wnt/ β -Catenin, Invasion, Metastasierung, Biomarker, zielgerichtete Therapie.

1. Introduction

1.1 Background and Rationale

Cancer remains one of the most significant health problems worldwide and continues to rank among the leading causes of morbidity and mortality despite continuous improvements in diagnostic methods, molecular research, and individualized treatment strategies. Over recent decades, advances in cancer biology have greatly improved understanding of the molecular events underlying malignant transformation, demonstrating that tumor initiation and progression result from complex interactions involving genetic alterations, dysregulated signaling pathways, and changes within the tumor microenvironment. Rather than representing a single pathological event, carcinogenesis is now recognized as a multistep process characterized by sustained cellular proliferation, resistance to regulatory mechanisms, extracellular matrix remodeling, and dynamic communication between malignant cells and surrounding stromal and immune components.

Among the numerous molecular factors involved in these processes, cathepsins have emerged as an important group of proteolytic enzymes with functions extending well beyond their traditional lysosomal role. Under normal physiological conditions, cathepsins contribute to intracellular protein turnover, autophagy, antigen processing, apoptosis, tissue remodeling, immune responses, and maintenance of cellular homeostasis. However, growing experimental and clinical evidence indicates that disturbances in their expression, localization, or enzymatic activity accompany the development of many malignancies and actively contribute to tumor progression. These observations suggest that cathepsins should be regarded not only as lysosomal proteases but also as active regulators of cancer-associated biological processes.

Particular attention has been focused on cathepsins B, L, S, K, and D because numerous studies have demonstrated their aberrant expression in a wide variety of human cancers, including breast, colorectal, lung, pancreatic, liver, and brain tumors, as well as melanoma. Increased expression or activity of these enzymes has repeatedly been correlated with greater tumor aggressiveness, enhanced invasive behavior, higher metastatic potential, and poorer clinical prognosis. Current research has further revealed that the biological significance of cathepsins extends beyond degradation of extracellular matrix components. These enzymes are capable of processing growth factors, cytokines, cell-surface receptors, and additional regulatory molecules, thereby influencing multiple signaling events that control tumor development and adaptation.

The expanding knowledge of cathepsin biology has highlighted their close relationship with intracellular signaling pathways that regulate malignant behavior. Experimental studies demonstrate functional interactions between cathepsins and several major oncogenic signaling networks, including PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , and Wnt/ β -catenin. Through direct or indirect modulation of these pathways, cathepsins participate in the regulation of cell proliferation, survival, apoptosis, migration, invasion, angiogenesis, epithelial–mesenchymal transition, and metastatic spread. Accordingly, these enzymes are increasingly recognized as multifunctional signaling modulators that influence multiple stages of cancer progression rather than acting solely as proteases responsible for protein degradation.

The growing appreciation of these diverse biological functions has also strengthened interest in the clinical significance of cathepsins. Numerous investigations indicate that altered expression of individual cathepsins may serve as a useful indicator of tumor progression, metastatic capacity, and patient prognosis, supporting their potential use as diagnostic or prognostic biomarkers. At the same time, advances in the development of selective cathepsin inhibitors, enzyme-activated prodrugs, and cathepsin-responsive drug-delivery systems have identified these proteases as promising therapeutic targets within precision oncology. Nevertheless, important questions remain unresolved. The molecular mechanisms through which cathepsins regulate individual signaling pathways, the variability of their functions among different tumor types, and the influence of the tumor microenvironment continue to be actively investigated, while inconsistencies among experimental and clinical findings have limited the translation of current knowledge into routine clinical practice.

Considering the increasing body of evidence linking cathepsins to the molecular mechanisms of tumor progression, a comprehensive synthesis of current scientific data is both timely and necessary. Integrating recent experimental and clinical findings may provide a more complete understanding of how cathepsins regulate signaling pathways involved in cancer growth, invasion, and metastasis, clarify their value as biomarkers and therapeutic targets, and identify promising directions for future research in molecular oncology and the development of targeted anticancer therapies.

1.2 Aim and Objectives

The primary aim of this thesis was to provide a comprehensive review and critical evaluation of the current scientific literature concerning the involvement of cathepsins in signaling pathways that regulate cancer growth, invasion, and metastatic progression, while

also assessing their emerging significance as diagnostic biomarkers and potential molecular targets for anticancer therapy.

To accomplish this aim, the following objectives were defined:

- to review the current understanding of the classification, structural characteristics, and physiological roles of cathepsins;
- to examine the molecular mechanisms responsible for regulating cathepsin expression and enzymatic activity under both normal physiological conditions and pathological states;
- to evaluate the contribution of cathepsins to key aspects of tumor progression, including malignant cell proliferation, angiogenesis, and resistance to cell death;
- to investigate the interactions between cathepsins and the principal intracellular signaling pathways involved in carcinogenesis;
- to assess the role of cathepsins in extracellular matrix remodeling, tumor cell migration, local invasion, and metastatic dissemination;
- to critically summarize available evidence regarding the clinical application of cathepsins as biomarkers for cancer diagnosis and prognosis, as well as their potential use as therapeutic targets;
- to identify existing knowledge gaps, methodological limitations, and promising directions for future research aimed at improving the clinical translation of cathepsin-based strategies in oncology.

1.3 Methodology of Literature Search and Analysis

This master's thesis was prepared as a narrative literature review based on a structured search and critical evaluation of contemporary scientific publications. The principal source of bibliographic data was the PubMed database, which was used to identify peer-reviewed articles relevant to the biological functions of cathepsins and their involvement in cancer-associated signaling pathways. The search strategy employed combinations of keywords related to cathepsins, tumor progression, carcinogenesis, intracellular signaling pathways, angiogenesis, metastasis, epithelial–mesenchymal transition, biomarkers, and targeted anticancer therapy.

The selection process primarily included publications written in English and published in international peer-reviewed journals. Preference was given to recently published review papers, systematic reviews, meta-analyses, and original experimental studies that provide up-to-date evidence in the field. At the same time, several highly cited landmark studies were

incorporated to ensure adequate coverage of the fundamental concepts that have shaped current understanding of cathepsin biology and its relevance to cancer research.

The collected literature was examined through comparative analysis, evidence synthesis, and critical interpretation of findings reported by different research groups. Particular emphasis was placed on identifying consensus and discrepancies among published studies, evaluating the molecular mechanisms through which cathepsins regulate signaling pathways involved in tumor development, and assessing their contribution to cancer growth, invasion, metastasis, and interactions with the tumor microenvironment. In addition, the available evidence regarding the diagnostic, prognostic, and therapeutic significance of cathepsins was systematically evaluated to provide an integrated overview of their potential clinical applications in modern oncology.

1.4 Structure of the Thesis

The master's thesis is organized into an introduction, six main chapters, a discussion, conclusions, and a reference list. This structure provides a logical progression from the fundamental biological characteristics of cathepsins to their involvement in cancer-related molecular mechanisms and their potential clinical significance.

The introduction outlines the scientific background of the study, explains its relevance, defines the research aim and objectives, and describes the methodology used for the literature search and analysis. Chapter 2 presents the general characteristics of cathepsins, including their classification, physiological functions, and the principal mechanisms controlling their expression and enzymatic activity. Chapter 3 examines the contribution of cathepsins to cancer progression, with particular emphasis on their roles in tumor cell proliferation, survival, angiogenesis, and interactions with the tumor microenvironment. Chapter 4 analyzes the major intracellular signaling pathways influenced by cathepsins and discusses their involvement in the molecular regulation of carcinogenesis. Chapter 5 focuses on the participation of cathepsins in extracellular matrix degradation, epithelial–mesenchymal transition, tumor invasion, migration, and metastatic dissemination. Chapter 6 reviews current therapeutic strategies targeting cathepsins, evaluates their potential use as diagnostic and prognostic biomarkers, and discusses recent advances together with future perspectives for their clinical application.

The discussion integrates and critically interprets the findings presented throughout the thesis, emphasizing areas of agreement, unresolved questions, and existing limitations within the current body of scientific evidence. Finally, the conclusions summarize the principal outcomes of the literature review, highlight the significance of cathepsins in cancer-associated

signaling pathways, and outline their potential implications for future research and the development of novel anticancer therapies.

2. Cathepsins: Classification and Biological Functions

2.1 General Characteristics of Cathepsins

Cathepsins comprise a heterogeneous group of intracellular proteolytic enzymes that are found predominantly within the endolysosomal system of eukaryotic cells. Traditionally, these enzymes have been recognized for their central role in intracellular protein degradation, a process that is essential for preserving proteostasis and maintaining normal cellular function (1, 2). For many years, cathepsins were considered to be nonspecific lysosomal hydrolases whose principal purpose was the recycling of damaged or unnecessary proteins. However, advances in molecular biology, cell biology, and proteomic research have substantially expanded this concept. Current evidence demonstrates that cathepsins are involved in a broad spectrum of physiological and pathological processes, including autophagy, antigen presentation, immune regulation, extracellular matrix remodeling, inflammatory responses, apoptosis, and the development and progression of cancer (1–3).

According to the catalytic amino acid responsible for peptide bond cleavage, human cathepsins are divided into three principal enzymatic classes. The largest group consists of cysteine proteases belonging to the papain family and includes cathepsins B, C, F, H, K, L, O, S, V, W, and X/Z (1). A second group comprises the aspartic proteases, represented by cathepsins D and E, whereas cathepsins A and G belong to the serine protease family (1, 4). This classification is based on fundamental differences in catalytic mechanisms, structural organization, substrate specificity, intracellular trafficking, and susceptibility to enzymatic inhibition. Consequently, it provides the basis for understanding both the normal physiological functions and the pathological significance of individual cathepsins (1, 4).

Despite their structural and functional diversity, members of the cathepsin family share a largely conserved biosynthetic pathway. They are initially synthesized as inactive precursor proteins containing three major structural regions: an N-terminal signal peptide, an inhibitory propeptide, and a catalytic domain that subsequently forms the mature enzyme (1, 5). During intracellular synthesis, the propeptide blocks access to the catalytic site, thereby preventing premature proteolytic activity before the enzyme reaches its appropriate intracellular destination (1, 5). Following translation, newly synthesized cathepsins are transported primarily to lysosomes through the mannose-6-phosphate receptor-dependent pathway, although certain enzymes may also utilize alternative trafficking routes (1, 6). Proteolytic removal of the inhibitory propeptide then converts the inactive precursor into an enzymatically active mature

protease. This activation may occur through autocatalytic cleavage or through processing by other lysosomal proteases (1, 5).

The enzymatic activity of cathepsins is strongly influenced by the physicochemical characteristics of their surrounding environment. Most lysosomal cathepsins display maximal catalytic efficiency within the acidic conditions of endosomes and lysosomes, where low pH favors optimal substrate hydrolysis (1, 4). Nevertheless, individual members of the family differ considerably in their biochemical properties. For example, cathepsin S retains substantial proteolytic activity at near-neutral pH, enabling efficient function within extracellular tissues (2). Similarly, cathepsin B may remain enzymatically active after its release from lysosomes, although its extracellular activity depends on several factors, including local pH, substrate availability, and the biochemical composition of the surrounding microenvironment (2, 7).

Increasing evidence indicates that the biological significance of cathepsins extends well beyond their established role in intracellular protein degradation. Following lysosomal redistribution or secretion into the extracellular space, these enzymes contribute to extracellular matrix remodeling, activation and maturation of biologically active proteins, regulation of inflammatory responses, and modulation of signaling pathways involved in intercellular communication (2, 3). Rather than functioning solely as degradative enzymes, extracellular cathepsins selectively process numerous regulatory molecules, including cytokines, chemokines, growth factors, cell-surface receptors, and adhesion proteins. Through these proteolytic modifications, they influence multiple physiological and pathological processes, making them increasingly attractive candidates for use as diagnostic biomarkers and therapeutic targets in oncology, inflammatory disorders, and other human diseases (2, 3).

Although lysosomes remain the principal intracellular site of cathepsin accumulation, current evidence demonstrates that their intracellular distribution is considerably more versatile than previously recognized. Under physiological conditions, mature enzymes are concentrated mainly within lysosomes; however, in response to cellular activation or pathological stimuli they may also be detected in endosomes, secretory vesicles, the cytoplasm, the nucleus, the plasma membrane, and the extracellular environment (1–3). This dynamic intracellular trafficking reflects the multifunctional nature of cathepsins and emphasizes that their biological activities are not restricted to lysosomal proteolysis. Their presence in multiple cellular compartments enables them to participate in tissue remodeling, regulation of cell-to-cell communication, and modulation of intracellular signaling pathways, thereby substantially expanding their functional importance within both normal physiology and disease (2, 3).

Under physiological conditions, lysosomal membrane integrity ensures that cathepsins remain confined within the lysosomal lumen, thereby preventing inappropriate proteolytic activity in other cellular compartments. This strict compartmentalization is crucial for maintaining cellular homeostasis, since the escape of lysosomal proteases into the cytoplasm may profoundly alter cellular function and viability. Disturbances in lysosomal membrane integrity, commonly referred to as lysosomal membrane permeabilization (LMP), together with impaired lysosomal acidification or abnormalities in intracellular trafficking, facilitate the partial or complete redistribution of cathepsins beyond the lysosomal compartment (1, 8, 9). The biological consequences of this process are largely determined by the extent of membrane damage. Limited permeabilization may activate adaptive stress-response pathways that enhance cell survival, whereas severe lysosomal disruption causes extensive release of cathepsins into the cytosol. Under these conditions, cathepsins contribute to mitochondrial dysfunction, proteolytic modification of Bcl-2 family proteins, activation of caspase-dependent signaling, and induction of apoptosis, necrosis, or other forms of regulated cell death (1, 6). Therefore, preservation of lysosomal membrane stability is considered essential for normal cellular integrity and survival.

In addition to their intracellular activities, several cathepsins can be actively secreted into the extracellular environment through regulated cellular pathways. This phenomenon has been described most extensively for cathepsins B, L, S, and K, whose extracellular concentrations increase substantially during physiological tissue remodeling as well as in pathological conditions such as inflammation, wound repair, and malignant transformation (2, 9). In cancer cells, elevated extracellular secretion has been associated with defects in lysosomal trafficking, impaired acidification of intracellular organelles, and alterations in secretory vesicle function (2, 9). Experimental studies conducted by M. Aufy and colleagues demonstrated that treatment with enniatin B and beauvericin disrupts normal lysosomal physiology by increasing intralysosomal pH, impairing the trafficking of cathepsin B toward lysosomes, and promoting its redistribution into both the cytoplasm and the extracellular compartment. Interestingly, similar alterations were not observed for cathepsins D and L, indicating that intracellular sorting mechanisms differ considerably among individual members of the cathepsin family and may contribute to their distinct biological functions (9).

Another characteristic feature distinguishing individual cathepsins is their marked substrate specificity. Although all cathepsins catalyze proteolytic cleavage of peptide bonds, they differ substantially in substrate preference, catalytic efficiency, and the mechanisms by which proteins are processed. Some family members primarily function as endopeptidases that

cleave peptide bonds within protein molecules, whereas others possess exopeptidase activity and sequentially remove amino acid residues from the amino- or carboxyl-terminal ends of polypeptides (1, 8). These enzymatic differences largely determine the physiological roles of individual cathepsins and form the basis for their functional classification according to substrate specificity and catalytic behavior.

Equally essential for normal cellular physiology are the regulatory mechanisms that tightly control cathepsin activity and prevent excessive proteolysis. Maintenance of proteostasis depends not only on regulated enzyme synthesis, intracellular trafficking, and proteolytic activation, but also on endogenous inhibitory proteins. Among these, members of the cystatin family represent the principal physiological inhibitors of cysteine cathepsins. By binding with high affinity to the catalytic sites of these enzymes, cystatins reversibly suppress proteolytic activity and prevent uncontrolled degradation of cellular proteins (1, 3). In mammals, this regulatory network includes intracellular stefins, represented by cystatins A and B, secreted type II cystatins such as cystatins C, D, E/M, F, S, SA, and SN, together with high-molecular-weight kininogens that regulate cathepsin activity in both intracellular and extracellular compartments (1, 3). Through coordinated interactions among these molecules, cells maintain a balanced relationship between cathepsin synthesis, activation, inhibition, and biological function.

Maintenance of the equilibrium between cathepsins and their endogenous inhibitors is indispensable for normal tissue homeostasis. Excessive expression of proteases or reduced production of inhibitory proteins disturbs this finely controlled balance, resulting in abnormal proteolytic activity. Such dysregulation promotes degradation of extracellular matrix components, enhances inflammatory responses, disrupts tissue architecture, and alters the activity of numerous signaling molecules involved in cellular regulation (2, 3). In malignant tumors, imbalance within the cathepsin–cystatin system has consistently been associated with increased tumor invasiveness, stimulation of angiogenesis, induction of epithelial–mesenchymal transition, and enhanced metastatic dissemination (2, 3). Consequently, contemporary studies increasingly emphasize that evaluation of cathepsin biology should consider not only the expression levels of individual enzymes but also the overall balance between proteases and their endogenous inhibitors.

The biological functions of cathepsins should also be viewed within the context of the broader cellular proteolytic network rather than as isolated enzymatic activities. These proteases interact closely with matrix metalloproteinases, serine proteases, caspases, calpains, and several additional protease systems that collectively regulate intracellular protein turnover,

extracellular matrix remodeling, immune function, inflammatory signaling, and numerous intracellular signaling pathways (1–3). The coordinated activity of these proteolytic systems forms an integrated regulatory network that enables cells to preserve tissue homeostasis while adapting efficiently to physiological demands and pathological stress.

Overall, current scientific evidence demonstrates that cathepsins comprise a structurally and functionally diverse family of proteolytic enzymes whose biological significance extends far beyond their traditional lysosomal functions. Besides maintaining intracellular protein homeostasis, they participate in autophagy, immune regulation, tissue remodeling, extracellular communication, and multiple signaling processes that influence both normal physiology and disease. Conversely, disturbances in cathepsin expression, intracellular trafficking, or enzymatic activity contribute directly to the pathogenesis of numerous disorders, particularly malignant neoplasms (1–3). Because of their remarkable structural diversity, distinct catalytic properties, and specialized biological functions, systematic classification of cathepsins is essential for understanding the specific contribution of each family member to health and disease. These classification principles are considered in greater detail in the following section.

2.2 Classification of Cathepsins

Human cathepsins comprise a structurally diverse family of proteolytic enzymes whose classification is based primarily on the nature of the catalytic residue involved in peptide bond cleavage, the structural organization of the active site, evolutionary relationships, and the biochemical mechanism underlying proteolysis (1, 4). According to the currently accepted biochemical classification, human cathepsins are grouped into three principal catalytic classes: cysteine proteases, aspartic proteases, and serine proteases (1, 4). This categorization reflects more than differences in enzyme nomenclature, as each class possesses characteristic catalytic mechanisms, substrate selectivity, intracellular localization, optimal conditions for enzymatic activity, and distinct susceptibility to pharmacological inhibitors. Collectively, these properties determine both the physiological roles and pathological significance of individual cathepsins (1, 4, 10).

Among these groups, cysteine cathepsins constitute the largest and functionally most diverse subgroup. They belong to the papain superfamily and utilize a highly conserved cysteine residue within the catalytic center as the principal nucleophile responsible for peptide bond hydrolysis (1, 10). This subgroup includes cathepsins B, C, F, H, K, L, O, S, V, W, and X/Z (1, 3). Although all members share the same fundamental catalytic mechanism, they differ considerably in substrate specificity, tissue distribution, intracellular trafficking, and biological

activity. Such diversity enables individual cysteine cathepsins to perform specialized functions in intracellular protein degradation, extracellular matrix remodeling, immune regulation, tumor invasion, and numerous signaling pathways associated with cellular adaptation and disease progression (1, 3, 11).

Cysteine cathepsins may also be subdivided according to their mode of proteolytic activity. Depending on the position at which peptide bonds are cleaved, they function as endopeptidases, exopeptidases, or enzymes possessing characteristics of both catalytic types (1, 10). Endopeptidases hydrolyze peptide bonds within the interior of protein molecules, whereas exopeptidases progressively remove amino acids or short peptide fragments from either the amino- or carboxyl-terminal ends of polypeptides (1, 10). Cathepsins L, S, K, V, F, and O are predominantly classified as endopeptidases. In contrast, cathepsin C primarily functions as a dipeptidyl peptidase, cathepsin H exhibits mainly aminopeptidase activity, and cathepsin X/Z acts principally as a carboxypeptidase (1, 12, 13). Cathepsin B possesses unique catalytic versatility because the presence of its characteristic occluding loop enables it to function both as an endopeptidase and as a carboxydipeptidase, thereby substantially broadening the range of substrates that it can process (1, 10).

Compared with the extensive cysteine subgroup, the aspartic cathepsin family includes only two members, cathepsins D and E. Their catalytic mechanism differs fundamentally from that of cysteine proteases because peptide bond cleavage is mediated by two conserved aspartic acid residues located within the active site rather than by a catalytic cysteine residue (4, 14). Cathepsin D is one of the major lysosomal proteases involved in intracellular protein degradation, maintenance of tissue homeostasis, and regulation of programmed cell death (14). In addition to these physiological functions, this enzyme has attracted considerable attention in cancer research because elevated expression and secretion have been reported in several malignancies, particularly breast cancer, where increased cathepsin D levels are associated with greater tumor aggressiveness and less favorable clinical outcomes (15, 16). In contrast, cathepsin E is localized predominantly within endosomal compartments and contributes mainly to antigen processing and regulation of immune responses rather than to conventional lysosomal protein degradation (3, 4).

The serine cathepsin subgroup is represented exclusively by cathepsins A and G, both of which possess biological properties that distinguish them from other members of the cathepsin family (4). Cathepsin A, also known as protective protein/carboxypeptidase, forms part of the lysosomal multienzyme complex together with β -galactosidase and neuraminidase. Within this complex, it stabilizes the associated enzymes while simultaneously participating in

the metabolism of biologically active peptides (3, 4). Cathepsin G exhibits an even more specialized pattern of expression. Rather than functioning primarily as a lysosomal enzyme, it is stored within the azurophilic granules of neutrophils, where it contributes to antimicrobial defense, regulation of inflammatory responses, and innate immune function (16, 17). Consequently, cathepsin G is generally regarded as an immune-associated serine protease instead of a classical lysosomal hydrolase.

The functional specialization observed among individual cathepsins further illustrates the remarkable diversity of this enzyme family. Cathepsin K is expressed predominantly in osteoclasts, where it serves as the principal collagenolytic enzyme responsible for degradation of type I collagen during physiological bone resorption (18). Cathepsin S plays a crucial role in professional antigen-presenting cells by cleaving the invariant chain of major histocompatibility complex class II molecules, thereby facilitating antigen presentation to T lymphocytes (18, 19, 20). Cathepsin C, also referred to as dipeptidyl peptidase I, is indispensable for activation of several neutrophil serine proteases, including neutrophil elastase, proteinase 3, and cathepsin G (12, 21). By comparison, cathepsin X/Z is expressed mainly in immune cells, where it regulates cell adhesion, migration, and intracellular signaling through interactions with integrin receptors (13, 22).

For easier comparison of individual family members, the principal biochemical and biological characteristics of the major human cathepsins are presented in Table 1. The table summarizes their catalytic classification, enzymatic activity, predominant localization, tissue distribution, and major physiological functions, thereby illustrating the functional diversity that exists within the cathepsin family.

Table 1. Classification of the Major Human Cathepsins and Their Biological Characteristics

Cathepsin	Catalytic Class	Type of Enzymatic Activity	Primary Localization / Expression	Major Biological Functions
Cathepsin B	Cysteine protease	Endopeptidase / carboxydipeptidase	Lysosomes, endosomes, extracellular space	Intracellular proteolysis, extracellular matrix (ECM) degradation and remodeling, facilitation of tumor invasion
Cathepsin C (DPPI)	Cysteine protease	Dipeptidyl peptidase	Lysosomes, immune cells	Proteolytic activation of serine proteases in neutrophils and

				cytotoxic lymphocytes
Cathepsin F	Cysteine protease	Endopeptidase	Predominantly lysosomal	Intracellular protein turnover and protein processing
Cathepsin H	Cysteine protease	Aminopeptidase / endopeptidase	Lysosomes	Processing of protein substrates and maintenance of cellular proteostasis
Cathepsin K	Cysteine protease	Endopeptidase with collagenolytic activity	Osteoclasts	Degradation of type I collagen during physiological bone resorption
Cathepsin L	Cysteine protease	Endopeptidase	Lysosomes and other intracellular compartments	Lysosomal proteolysis, autophagy, and protein maturation
Cathepsin O	Cysteine protease	Endopeptidase	Lysosomes	Poorly characterized cathepsin presumed to participate in intracellular protein degradation
Cathepsin S	Cysteine protease	Endopeptidase	Antigen-presenting cells, macrophages, dendritic cells	Cleavage of the MHC class II invariant chain, antigen presentation, and regulation of immune responses
Cathepsin V	Cysteine protease	Endopeptidase	Thymus, epithelial tissues	Participation in immune regulation and intracellular protein processing
Cathepsin W	Cysteine protease	Endopeptidase	NK cells, cytotoxic T lymphocytes	Support of the cytotoxic activity of immune cells
Cathepsin X/Z	Cysteine protease	Carboxypeptidase	Mainly expressed in immune cells	Regulation of cell adhesion, migration, and integrin-dependent signaling
Cathepsin D	Aspartic protease	Endopeptidase	Lysosomes, secretory vesicles	Lysosomal protein degradation, maintenance of tissue homeostasis, regulation of programmed cell death, and

				involvement in tumor progression
Cathepsin E	Aspartic protease	Endopeptidase	Endosomes, immune cells	Processing of antigens and modulation of immune responses
Cathepsin A	Serine protease	Carboxypeptidase	Lysosomal multienzyme complex	Stabilization of β -galactosidase and neuraminidase together with metabolism of biologically active peptides
Cathepsin G	Serine protease	Endopeptidase	Azurophilic granules of neutrophils	Antimicrobial defense, regulation of inflammatory reactions, and modulation of immune cell function

Source: Compiled by the author based on data from PubMed/PMC publications (1, 3, 4, 10, 12–14,16–22).

The classification presented above demonstrates that cathepsins should be regarded as a highly heterogeneous family of proteolytic enzymes rather than as a uniform group of lysosomal hydrolases. Although all members catalyze proteolytic reactions, they differ markedly in catalytic mechanisms, substrate specificity, expression profiles, intracellular localization, and biological function (1, 3, 4, 10). These differences enable individual cathepsins to participate not only in general lysosomal protein degradation but also in numerous specialized physiological processes that vary among tissues, organs, and cell types.

The specialized biological functions of individual cathepsins further emphasize the complexity of this enzyme family. Cathepsin K is the major protease responsible for collagen degradation during physiological bone remodeling, whereas cathepsin S performs an essential function in antigen presentation through proteolytic processing of major histocompatibility complex class II molecules. Cathepsin C serves as a key regulator of immune cell function by activating several neutrophil serine proteases, while cathepsin D contributes both to intracellular protein turnover and to multiple mechanisms associated with malignant transformation and tumor progression (12, 14, 19–21). Consequently, comprehensive characterization of any individual cathepsin requires consideration of several interrelated features, including catalytic class, structural organization, substrate preference, tissue-specific

expression, intracellular localization, and its contribution to physiological regulation as well as pathological processes.

2.3 Physiological Functions of Cathepsins

The physiological functions of cathepsins are considerably broader than the intracellular degradation of proteins traditionally associated with lysosomes. Although these enzymes were initially described as lysosomal hydrolases responsible for proteolysis, current knowledge indicates that they participate in numerous biological processes required for normal cellular activity and tissue homeostasis. Besides maintaining protein quality control, cathepsins contribute to autophagy, regulation of immune responses, antigen presentation, turnover of extracellular matrix components, tissue regeneration, and modulation of intracellular signaling pathways (1, 3, 10). According to V. Turk and colleagues (1), these diverse functions are coordinated through an integrated regulatory system that controls every stage of the cathepsin life cycle, including biosynthesis, intracellular transport, maturation of inactive precursors, compartment-specific activation, and inhibition by endogenous protease inhibitors. Such precise regulation ensures efficient proteolysis while preventing uncontrolled degradation of cellular constituents.

Among the numerous physiological roles of cathepsins, maintenance of intracellular proteostasis is one of the most fundamental. Proteostasis represents a highly coordinated network responsible for controlling protein synthesis, folding, post-translational modification, degradation, and recycling, thereby preserving the integrity of the cellular proteome. Failure of this system leads to the accumulation of damaged, misfolded, or aggregated proteins that interfere with normal cellular function and promote the development of various pathological conditions (3, 4). As noted by T. Yadati et al. (3), intracellular protein degradation is achieved mainly through two complementary proteolytic systems: the ubiquitin–proteasome pathway and the lysosomal compartment containing cathepsins. Whereas the proteasome primarily eliminates soluble cytoplasmic proteins, lysosomal cathepsins possess the capacity to degrade substrates that cannot be efficiently processed by proteasomes, including large protein aggregates, membrane-associated proteins, extracellular macromolecules internalized by endocytosis, and damaged intracellular organelles.

Efficient lysosomal degradation depends on several mechanisms responsible for delivering substrates into the endolysosomal compartment. Proteins and other cellular material reach lysosomes through endocytosis, phagocytosis, and different forms of autophagy, where they are subsequently exposed to a coordinated system of lysosomal proteases. Rather than

acting independently, multiple cathepsins function simultaneously, each recognizing a distinct spectrum of substrates. As described by K. Brix (4), the complementary activities of these enzymes ensure almost complete degradation of complex protein molecules into amino acids and short peptides. After transport back into the cytoplasm, these degradation products become available for new protein synthesis, production of biologically active metabolites, and metabolic pathways involved in energy generation. Consequently, lysosomal proteolysis not only removes dysfunctional cellular components but also provides essential molecular substrates that support continuous renewal of the intracellular proteome and maintenance of metabolic homeostasis (1, 3, 4).

Autophagy represents another physiological process in which cathepsins perform an indispensable function. This highly conserved intracellular quality-control mechanism enables cells to eliminate damaged organelles, aggregated proteins, and intracellular pathogens through lysosome-dependent degradation. During autophagy, targeted cytoplasmic material is first enclosed within double-membrane autophagosomes, which subsequently fuse with lysosomes to form autolysosomes. Y. Uchiyama (23) demonstrated that degradation of the enclosed material occurs only after activation of lysosomal cathepsins within these autolysosomal compartments. Thus, the completion of autophagic flux depends directly on the proteolytic activity of lysosomal enzymes. When cathepsin function is impaired, degradation of autophagic cargo becomes incomplete, resulting in disruption of intracellular quality-control mechanisms and progressive accumulation of undegraded cellular material.

The essential contribution of cathepsins to autophagy has been confirmed by numerous experimental studies. M. Jung et al. (24) reported that pharmacological inhibition of cysteine cathepsins B and L markedly suppresses autophagic flux, leading to intracellular accumulation of autophagic vesicles together with elevated expression of LC3-II and p62, both widely recognized molecular indicators of defective autophagy. Similar observations were obtained by M. Tatti et al. (25), who demonstrated that fibroblasts lacking cathepsins B and D exhibit a profound reduction in lysosomal degradative capacity. Restoration of normal cathepsin expression largely reversed these abnormalities and re-established efficient autophagic activity. Collectively, these findings confirm that functional lysosomal cathepsins are indispensable for proper operation of the autophagy–lysosome pathway and for preservation of intracellular homeostasis.

The physiological significance of cathepsin-mediated autophagy extends beyond simple degradation of damaged cellular structures. Lysosomal proteolysis also plays an important role in metabolic adaptation during unfavorable environmental conditions. Under nutrient

deprivation, oxidative stress, or other forms of cellular stress, activation of autophagy generates amino acids and additional metabolic intermediates through cathepsin-dependent degradation of intracellular substrates. These products can subsequently be redirected toward biosynthetic pathways or utilized as energy sources to sustain ATP production. In this way, cathepsins contribute not only to intracellular recycling but also to the metabolic flexibility that enables cells to survive periods of environmental stress. Consistent with this concept, abnormalities in cathepsin expression or enzymatic activity have been implicated in the pathogenesis of numerous disorders, including neurodegenerative diseases, metabolic dysfunction, chronic inflammatory conditions, and malignant tumors, emphasizing the central importance of these enzymes in maintaining normal cellular physiology (1, 3).

In addition to maintaining intracellular protein homeostasis, cathepsins play indispensable roles in both innate and adaptive immune responses. Within cells of the immune system, their activity extends far beyond nonspecific degradation of lysosomal contents and involves highly selective proteolytic processing of proteins required for antigen presentation, immune cell maturation, and regulation of immune signaling pathways (26–29). Through these specialized functions, cathepsins contribute to immune surveillance, facilitate appropriate activation of immune responses, and help preserve the balance between effective host defense and immunological tolerance.

Among the different members of the cathepsin family, cathepsin S has been studied most extensively because of its pivotal function in the major histocompatibility complex (MHC) class II antigen presentation pathway. During the synthesis of MHC class II molecules, the peptide-binding groove is occupied by the invariant chain (Ii), which prevents premature loading of endogenous peptides and directs the newly assembled complex toward the endolysosomal compartment. H. Driessen et al. (26) demonstrated that, once the complex reaches late endosomes, cathepsin S sequentially degrades the invariant chain, ultimately leaving only the class II-associated invariant chain peptide (CLIP). This intermediate is subsequently exchanged for antigen-derived peptides through the activity of HLA-DM, allowing mature MHC class II molecules to display processed antigens on the cell surface for recognition by CD4⁺ T lymphocytes. Consequently, the proteolytic activity of cathepsin S represents a critical step linking intracellular antigen processing with activation of adaptive immune responses.

Although cathepsin S is regarded as the principal enzyme responsible for invariant chain degradation in professional antigen-presenting cells, this process involves coordinated participation of several additional cathepsins. As summarized by C. Honey and H. Rudensky

(29), cathepsins L, F, and V also contribute to invariant chain processing, although their relative importance varies according to cell lineage, tissue distribution, and developmental stage of antigen-presenting cells. For instance, cathepsin L plays an essential role within cortical thymic epithelial cells, where it participates in positive selection of developing thymocytes and contributes to formation of a functional T-cell repertoire (28). By contrast, dendritic cells, macrophages, and other professional antigen-presenting cells depend primarily on cathepsin S for efficient MHC class II maturation. Such division of functions allows different immune cell populations to employ distinct proteolytic mechanisms while maintaining the precision required for effective immune recognition and self-tolerance.

Cathepsins are equally important for the proper functioning of innate immunity. Within this branch of the immune system, cathepsin C, also known as dipeptidyl peptidase I (DPPI), occupies a central regulatory position because it activates several serine proteases that are essential for neutrophil antimicrobial activity, including neutrophil elastase, proteinase 3, and cathepsin G (17, 21). These enzymes are synthesized as inactive precursors and require proteolytic removal of N-terminal dipeptides before they acquire full catalytic activity. This maturation step is mediated specifically by cathepsin C during neutrophil development. Experimental work conducted by A. M. Adkison et al. (21) demonstrated that deficiency of cathepsin C prevents activation of these serine proteases, resulting in marked impairment of neutrophil antimicrobial function and diminished inflammatory responses. Similar conclusions were reported by C. T. N. Pham (17), who identified cathepsin C as the principal regulator responsible for generating the proteolytic machinery required for efficient innate immune defense.

Beyond antigen presentation and activation of immune proteases, cathepsins influence numerous aspects of immune cell communication by regulating the activity of signaling molecules involved in inflammatory responses. Experimental studies have shown that macrophages, dendritic cells, B lymphocytes, and other immune cells utilize cathepsin-mediated proteolysis to process cytokines, chemokines, membrane receptors, and additional regulatory proteins that coordinate intercellular signaling. According to T. Yadati et al. (3), selective cleavage of these substrates enables cathepsins to modulate both the intensity and duration of immune activation, thereby shaping the overall inflammatory response. Rather than serving solely as degradative enzymes, cathepsins therefore function as important modulators of immune signaling networks that integrate intracellular proteolysis with mechanisms governing immune homeostasis.

The contribution of cathepsins to immune regulation illustrates the remarkable functional versatility of this enzyme family. Their activity is required not only for lysosomal degradation of proteins but also for precise proteolytic events that determine antigen recognition, maturation of immune cells, activation of antimicrobial defense mechanisms, and regulation of cytokine-mediated communication. Through coordinated participation in these processes, cathepsins help ensure that immune responses remain sufficiently effective to eliminate pathogens while avoiding excessive activation that could compromise normal tissue integrity (3, 17, 21, 26–29).

In addition to their intracellular functions, cathepsins contribute substantially to the physiological remodeling of the extracellular matrix (ECM), a process that is indispensable for normal tissue maintenance, embryonic development, wound repair, and adaptation to mechanical stress. The extracellular matrix is not a static structural scaffold but a highly dynamic network whose composition is continuously modified through a balance between synthesis and proteolytic degradation. Preservation of this equilibrium is essential for maintaining tissue architecture and supporting controlled cell migration, differentiation, and regeneration. A growing body of evidence indicates that several cathepsins remain enzymatically active after being secreted from lysosomes, allowing them to participate directly in these extracellular remodeling events (30).

The biological activities of extracellular cathepsins differ considerably from their classical lysosomal functions. Rather than serving exclusively as enzymes responsible for protein degradation, secreted cathepsins regulate the composition of the extracellular environment by selectively processing structural proteins and signaling molecules. M. Vizovišek et al. (30) demonstrated that extracellular cysteine cathepsins efficiently hydrolyze major components of the extracellular matrix while simultaneously modifying proteins involved in cell adhesion, migration, and intercellular communication. Through controlled proteolytic processing of these substrates, cathepsins alter both the biochemical composition and the biological properties of the surrounding microenvironment. Consequently, their extracellular activity should be regarded as a regulatory mechanism that supports tissue plasticity rather than merely a degradative process.

The importance of this function becomes particularly evident during tissue regeneration. Successful wound healing requires coordinated migration of epithelial cells together with localized degradation and reconstruction of extracellular matrix components. These events enable cells to migrate into the damaged area while simultaneously restoring normal tissue architecture. H. Büth et al. (31) demonstrated that cathepsin B released by migrating

keratinocytes plays a central role in this process by generating localized zones of extracellular proteolysis at the leading edge of regenerating tissue. Inhibition of cathepsin B activity significantly delayed wound closure in an in vitro scratch model, indicating that efficient tissue repair depends on appropriate extracellular proteolytic activity. The authors further suggested that the contribution of cathepsin B extends beyond direct degradation of matrix proteins because its activity initiates secondary proteolytic cascades involving additional extracellular matrix-remodeling enzymes. These findings illustrate that tightly regulated extracellular proteolysis is essential for coordinated cell migration and successful restoration of tissue integrity.

Cathepsins are also indispensable for physiological bone remodeling, another process that requires continuous and precisely regulated turnover of extracellular matrix components. Normal skeletal homeostasis is maintained through a balance between bone formation by osteoblasts and bone resorption by osteoclasts. Within this remodeling cycle, cathepsin K performs a highly specialized function owing to its exceptional capacity to degrade type I collagen, the predominant structural protein of the organic bone matrix (32). This enzyme is expressed almost exclusively by activated osteoclasts, where it represents the principal protease responsible for enzymatic degradation of collagen during bone resorption. As emphasized by A. G. Costa et al. (32), physiological remodeling of skeletal tissue depends largely on the tightly regulated expression and activity of cathepsin K. Disruption of these regulatory mechanisms impairs normal bone turnover and contributes to the development of pathological alterations in bone structure and mineral metabolism.

Taken together, these observations demonstrate that extracellular cathepsin activity is essential for preserving normal tissue homeostasis. Through selective remodeling of extracellular matrix components and controlled processing of regulatory proteins, cathepsins participate in wound healing, tissue regeneration, maintenance of skeletal integrity, and adaptation of tissues to physiological demands. Their ability to modify both structural and signaling molecules illustrates that extracellular cathepsins function as regulators of tissue remodeling rather than simply as degradative proteases (30–32).

For comparative purposes, the principal physiological functions of the major members of the cathepsin family are summarized in Table 2. The table illustrates the characteristic tissue distribution of individual cathepsins together with their predominant biological roles in proteostasis, autophagy, immune regulation, extracellular matrix remodeling, tissue repair, bone homeostasis, and intracellular signaling.

Table 2. Major Physiological Functions of Individual Cathepsins

Cathepsin	Major Cell Types / Tissues	Major Physiological Functions
Cathepsin B	Widely expressed in most cell types; keratinocytes; immune cells	Intralysosomal protein degradation, autophagy, extracellular matrix (ECM) remodeling, contribution to cell migration during wound repair
Cathepsin C (DPPI)	Neutrophils, cytotoxic immune cells	Proteolytic activation of neutrophil elastase, proteinase 3, and cathepsin G; preservation of innate immune effector activity
Cathepsin D	Broadly distributed in tissues; lysosomes	Lysosomal protein degradation, maintenance of autolysosomal function, regulation of programmed cell death
Cathepsin K	Osteoclasts	Degradation of type I collagen, mediation of bone resorption, maintenance of physiological bone remodeling
Cathepsin L	Lysosomes; epithelial tissues; thymus	Maintenance of proteostasis, autophagy, intracellular protein processing, regulation of positive T-cell selection
Cathepsin S	Dendritic cells, macrophages, professional antigen-presenting cells	Proteolytic processing of the MHC class II invariant chain, antigen presentation, regulation of adaptive immune responses
Cathepsin G	Neutrophils	Antimicrobial defense, modulation of inflammatory responses, participation in innate immune proteolytic cascades
Cysteine cathepsins (overall)	Endolysosomal compartments; extracellular environment	Extracellular matrix (ECM) remodeling, processing of inflammatory mediators, regulation of cellular signaling pathways

Source: Compiled by the author based on data from PubMed publications (1, 3, 4, 8, 10, 17, 21, 23–35).

Overall, current evidence clearly indicates that cathepsins are essential regulators of physiological processes operating at both the cellular and tissue levels. Their biological activities encompass intracellular protein degradation, maintenance of proteostasis, completion of autophagy, antigen processing, regulation of innate and adaptive immunity, extracellular matrix remodeling, tissue repair, bone remodeling, and modulation of intracellular signaling pathways. Because these functions are closely interconnected, disturbances affecting cathepsin expression, intracellular trafficking, compartmentalization, activation, or catalytic activity may disrupt cellular homeostasis and promote the development of numerous diseases. Consequently, understanding the mechanisms that regulate cathepsin biosynthesis, maturation, intracellular transport, activation, inhibition, and spatial distribution is fundamental for distinguishing their

normal physiological roles from the pathological activities observed in human disorders. These regulatory mechanisms are discussed in the following section.

2.4 Regulation of Cathepsin Activity

The biological activity of cathepsins is determined not only by their synthesis and proteolytic activation but also by a complex regulatory system that precisely controls the intracellular localization, catalytic competence, and temporal activity of these enzymes. Because cathepsins possess considerable proteolytic potential, their function must be tightly restricted to appropriate intracellular compartments in order to preserve cellular integrity. This regulation involves coordinated control of intracellular trafficking, lysosomal acidification, post-translational maturation, and endogenous inhibitory mechanisms that collectively ensure efficient proteolysis while preventing damage to normal cellular structures (1, 3, 36).

One of the key regulatory processes governing cathepsin function is their accurate intracellular targeting after biosynthesis and maturation. Under physiological conditions, newly synthesized enzymes are transported from the Golgi apparatus to lysosomes, where they exert their principal biological functions. The predominant mechanism responsible for this transport is the mannose-6-phosphate receptor (M6PR)-dependent pathway, which recognizes mannose-6-phosphate residues attached to newly synthesized lysosomal enzymes and directs them to the endolysosomal system. T. Brulke and J. S. Bonifacino (37) identified recognition of mannose-6-phosphate by M6P receptors as the central molecular event controlling lysosomal sorting of hydrolases. Efficient operation of this pathway ensures that mature cathepsins accumulate within lysosomes, whereas disruption of M6PR-mediated transport results in defective intracellular targeting, reduced lysosomal enzyme content, and increased extracellular secretion of cathepsins (37).

Although the mannose-6-phosphate pathway represents the major route for lysosomal delivery, accumulating evidence indicates that it is not the only mechanism responsible for intracellular distribution of cathepsins. Several members of this enzyme family can also reach their intracellular destinations through alternative trafficking pathways involving adaptor protein complexes, specialized sorting receptors, and secretory vesicles that function independently of classical M6PR-mediated transport (4, 36). The presence of multiple transport mechanisms enables cells to regulate the localization of individual cathepsins according to tissue-specific functions, developmental stage, and changing physiological requirements. As emphasized by K. Brix et al. (4), alterations in intracellular trafficking frequently represent one of the earliest molecular abnormalities preceding dysregulation of cathepsin activity,

highlighting the importance of precise spatial organization for maintaining normal proteolytic function.

Another fundamental prerequisite for normal cathepsin activity is preservation of the acidic environment within lysosomes. Most members of the cathepsin family exhibit maximal catalytic efficiency at a pH between approximately 4.5 and 5.5, corresponding to the physiological conditions present in late endosomes and lysosomes (1, 4). Maintenance of this acidic microenvironment depends on the continuous activity of the vacuolar H⁺-ATPase (V-ATPase), which actively transports hydrogen ions into the lysosomal lumen through ATP-dependent proton pumping. Any disturbance of lysosomal acidification compromises both maturation and catalytic activity of cathepsins, leading to reduced degradative capacity and progressive accumulation of undegraded intracellular material (3, 4). Consequently, maintenance of lysosomal acidity is essential not only for optimal activity of individual enzymes but also for preservation of normal lysosomal function as a whole.

Despite belonging to the same protease family, individual cathepsins differ markedly in their sensitivity to environmental pH. Cathepsin S represents one of the best-characterized examples of this functional diversity because, unlike most cysteine cathepsins, it preserves substantial enzymatic activity under near-neutral conditions (3, 8). This unique biochemical property enables cathepsin S to function efficiently outside lysosomes, where it participates in antigen presentation, remodeling of the extracellular matrix, and regulation of intercellular signaling. Cathepsin B likewise retains catalytic activity following its release from lysosomes; however, its proteolytic efficiency depends much more strongly on local physicochemical conditions, substrate accessibility, and the biochemical composition of the surrounding tissue microenvironment (1, 3). These differences illustrate that pH sensitivity contributes significantly to the functional specialization and physiological diversity observed among individual cathepsins.

Regulation of cathepsin activity is further reinforced by endogenous inhibitory proteins that limit excessive proteolysis under physiological conditions. The most important of these regulators are members of the cystatin family, which act as the principal endogenous inhibitors of cysteine cathepsins. By binding reversibly to the catalytic cleft of the enzyme, cystatins prevent substrate access to the active site and thereby suppress proteolytic activity. According to V. Turk et al. (1), maintenance of normal cellular homeostasis depends on a dynamic equilibrium between active cathepsins and their endogenous inhibitors. Disturbance of this balance may produce two fundamentally different pathological outcomes. Excessive protease activity results in uncontrolled degradation of intracellular and extracellular proteins, whereas

excessive inhibition impairs lysosomal proteolysis and promotes accumulation of undegraded substrates within the cell.

Among the currently identified endogenous inhibitors, cystatin C, together with stefins A and B and high-molecular-weight kininogens, has been investigated most extensively. Although these regulatory proteins differ with respect to tissue distribution, intracellular localization, and inhibitory specificity, they function collectively to maintain physiological control over cathepsin activity. Cystatin C, in particular, is considered one of the most potent endogenous inhibitors because of its broad tissue expression and exceptionally high affinity for several cysteine cathepsins. By restricting excessive proteolytic activity in both intracellular and extracellular environments, cystatin C plays a crucial role in preserving tissue integrity, maintaining proteolytic homeostasis, and protecting cells from inappropriate protein degradation.

Maintenance of lysosomal membrane integrity represents another fundamental mechanism controlling the physiological activity of cathepsins. Under normal conditions, the lysosomal membrane forms an effective barrier that confines proteolytic enzymes to the lysosomal lumen and prevents contact with cytoplasmic substrates. Such compartmentalization is essential because unrestricted release of lysosomal proteases may profoundly alter intracellular signaling and compromise cell viability. However, oxidative stress, toxic compounds, inflammatory mediators, and other damaging stimuli can impair membrane stability, resulting in lysosomal membrane permeabilization (LMP) and partial or complete leakage of cathepsins into the cytoplasm (2, 3).

The biological consequences of LMP are closely related to the severity of membrane damage. When lysosomal permeabilization is limited, moderate release of cathepsins may activate adaptive signaling pathways that promote cellular survival and increase resistance to environmental stress. In contrast, extensive disruption of lysosomal membranes leads to massive redistribution of cathepsins into the cytosol, where these enzymes initiate proteolytic cascades associated with mitochondrial dysfunction, activation of apoptosis, and other forms of regulated cell death (2, 3). Consequently, preservation of lysosomal membrane stability is indispensable for maintaining spatial control of cathepsin activity and preventing inappropriate proteolysis outside the lysosomal compartment.

The importance of intracellular compartmentalization has been demonstrated experimentally by M. Aufy and colleagues (9). Their investigation showed that exposure of cultured cells to the mycotoxins enniatin B and beauvericin disturbed normal lysosomal function by increasing intralysosomal pH and impairing intracellular trafficking of cathepsin

B. As a result, the enzyme was redistributed from lysosomes to both the cytoplasm and the extracellular environment. Interestingly, similar alterations were not observed for cathepsins D and L, indicating that individual members of the cathepsin family differ considerably in the mechanisms governing intracellular sorting and compartment-specific localization. These findings emphasize that even relatively subtle disturbances in lysosomal trafficking can substantially modify enzyme localization, catalytic activity, and biological function, underscoring the importance of spatial regulation in maintaining physiological protease homeostasis (9).

In addition to intracellular trafficking and compartmentalization, cathepsin activity is influenced by several post-translational and environmental regulatory mechanisms. Proper N-linked glycosylation is required for correct folding of newly synthesized enzymes, stabilization of their three-dimensional structure, and successful intracellular transport. Equally important is the attachment of mannose-6-phosphate residues, which enables recognition by lysosomal sorting receptors and ensures efficient delivery of cathepsins to their physiological destination (37). Defects affecting either glycosylation or mannose-6-phosphate modification impair intracellular trafficking and reduce the efficiency of lysosomal proteolysis.

The functional properties of cathepsins are also modulated by changes in the intracellular and extracellular biochemical environment. Variations in cellular redox status, interactions with regulatory proteins, substrate availability, and alterations in the physicochemical characteristics of the surrounding tissue microenvironment may all influence catalytic efficiency and substrate specificity. Rather than functioning as constitutively active enzymes, cathepsins respond dynamically to fluctuations in cellular conditions, allowing lysosomal proteolysis to adapt rapidly to changing physiological requirements (1, 3, 36). This flexibility is particularly important in tissues exposed to metabolic stress, inflammation, or rapid remodeling, where proteolytic activity must be continuously adjusted without compromising cellular integrity.

Current evidence therefore indicates that regulation of cathepsin activity involves a highly integrated network of complementary mechanisms operating at multiple biological levels. Control begins with transcriptional regulation of gene expression and continues through biosynthesis of inactive precursor proteins, post-translational modification, intracellular trafficking, proteolytic maturation, maintenance of lysosomal acidity, inhibition by endogenous regulatory proteins, and precise compartmentalization of mature enzymes (1, 3, 36). The coordinated interaction of these mechanisms ensures that cathepsins remain catalytically active

only under appropriate physiological conditions while protecting intracellular structures from uncontrolled proteolytic damage.

Conversely, disruption of any component within this regulatory network may profoundly alter the biological behavior of cathepsins. Abnormal intracellular trafficking, defective lysosomal acidification, impaired compartmentalization, excessive extracellular secretion, or imbalance between cathepsins and their endogenous inhibitors can all lead to inappropriate proteolytic activity. Increasing evidence indicates that these alterations represent early molecular events contributing to the development of numerous pathological conditions, particularly malignant transformation, tumor progression, and metastatic dissemination (3, 10, 36).

Table 3 summarizes the principal mechanisms responsible for regulating cathepsin activity, including gene expression, zymogen synthesis, post-translational modifications, lysosomal targeting, proteolytic activation, maintenance of acidic pH, endogenous inhibitory systems, preservation of lysosomal membrane integrity, and spatial compartmentalization. Together, these regulatory processes ensure that cathepsins perform their physiological functions with high efficiency while minimizing the risk of uncontrolled proteolysis.

Table 3. Major Mechanisms Regulating Cathepsin Activity

Regulatory Mechanism	Main Characteristics	Biological Significance
Regulation of gene expression	Modulation of cathepsin synthesis by cytokines, growth factors, hormones, and intracellular signaling pathways	Enables enzyme expression to adapt to the physiological requirements of the cell
Zymogen synthesis	Formation of inactive precursor proteins (preprocathepsins and procathepsins)	Prevents premature proteolytic activity within the cell
Post-translational modifications	N-linked glycosylation and addition of mannose-6-phosphate (M6P) residues	Ensures correct maturation and intracellular trafficking of cathepsins
Lysosomal trafficking	Mannose-6-phosphate receptor (M6PR)-dependent transport together with alternative intracellular sorting pathways	Directs cathepsins to their appropriate intracellular compartments
Proteolytic activation	Removal of the inhibitory propeptide in the acidic lysosomal environment	Converts inactive precursors into catalytically active enzymes
Acidic microenvironment	Maintenance of lysosomal pH (4.5–5.5) by V-ATPase	Provides optimal conditions for proteolytic activity

Endogenous inhibitors	Cystatins, stefins, and kininogens	Prevent excessive cathepsin-dependent proteolysis
Lysosomal membrane integrity	Preservation of lysosomal membrane stability and prevention of lysosomal membrane permeabilization (LMP)	Restricts lysosomal enzymes to their physiological intracellular compartment
Spatial localization	Regulation of intracellular distribution and controlled extracellular secretion	Ensures compartment-specific biological functions of cathepsins

Source: Compiled by the author based on data from references (1, 3, 4, 6, 10, 36–38).

Overall, the available scientific evidence demonstrates that physiological regulation of cathepsins relies on the coordinated interaction of numerous complementary mechanisms rather than on a single regulatory process. Tight control of gene expression, synthesis of inactive zymogens, post-translational processing, intracellular trafficking, lysosomal acidification, enzymatic activation, endogenous inhibition, and compartment-specific localization collectively preserves intracellular proteostasis and normal tissue homeostasis. Failure of any of these regulatory mechanisms disturbs the balance of proteolytic activity, creating conditions that favor pathological processes, including malignant transformation, tumor invasion, and metastatic progression. Understanding these regulatory pathways therefore provides the molecular basis for explaining how dysregulation of cathepsins contributes to cancer development, which is examined in the following chapter.

3. Role of Cathepsins in Cancer Progression

3.1 Cathepsins and Tumor Cell Proliferation

One of the defining characteristics of malignant tumors is their ability to sustain continuous proliferation despite the presence of physiological mechanisms that normally restrict cell division. In healthy tissues, progression through the cell cycle is tightly controlled by regulatory networks that coordinate proliferation with tissue requirements and preserve homeostasis. During malignant transformation, however, these regulatory systems become progressively disrupted, allowing cancer cells to proliferate independently of normal growth constraints while simultaneously acquiring resistance to antiproliferative signals. Although cathepsins were originally investigated primarily as lysosomal proteases involved in intracellular protein turnover and degradation of extracellular matrix components, extensive experimental evidence now indicates that their biological functions extend to the regulation of molecular pathways governing tumor cell proliferation. As summarized by T. Yadati et al. (3), abnormal expression and activity of several cathepsins are common features of many human cancers and are closely associated with signaling mechanisms that stimulate cell-cycle progression, suppress apoptosis, and maintain the proliferative phenotype characteristic of malignant cells.

Rather than influencing tumor growth through a single isolated mechanism, cathepsins participate in an extensive regulatory network that integrates intracellular signaling with reciprocal interactions between tumor cells and their surrounding microenvironment. Their proteolytic activity modifies numerous biologically important substrates, including growth factors, membrane receptors, extracellular matrix proteins, and signaling mediators. By processing these molecules, cathepsins regulate multiple pathways that collectively determine whether malignant cells continue to proliferate. In addition, these enzymes affect intercellular communication and alter transcriptional programs responsible for controlling cell-cycle progression. M. M. Mohamed and B. F. Sloane (39) emphasized that the ability of cathepsins to coordinate extracellular and intracellular signaling events creates a molecular environment that continuously favors tumor expansion and supports progressive cancer development.

Among the various members of the cathepsin family, cathepsin B has been investigated most extensively because of its strong association with enhanced proliferative activity in malignant tumors. Elevated expression of this protease has been reported in numerous human cancers, including breast, colorectal, hepatocellular, and non-small cell lung carcinomas, as

well as melanoma and glioblastoma (39). Nevertheless, increased expression alone does not fully explain its biological significance. Malignant transformation is frequently accompanied by profound alterations in the intracellular trafficking of cathepsin B, resulting in its redistribution from lysosomes toward the plasma membrane and extracellular compartment. According to M. M. Mohamed and B. F. Sloane (39), this abnormal localization greatly broadens the functional repertoire of cathepsin B by enabling direct interactions with extracellular components of the tumor microenvironment. Consequently, the enzyme contributes not only to accelerated tumor cell proliferation but also to the acquisition of additional malignant properties, including enhanced migratory and invasive potential.

The transition of cathepsin B from an intracellular lysosomal enzyme to an extracellular protease represents another important event during tumor progression. Once secreted into the tumor microenvironment, cathepsin B no longer functions solely in intracellular proteolysis but actively modifies the biochemical composition of the surrounding tissue. C. S. Gondi and J. S. Rao (40) demonstrated that extracellular accumulation of cathepsin B generates localized regions of intense proteolytic activity capable of releasing growth factors that remain bound to extracellular matrix components. Liberation and activation of these signaling molecules provide malignant cells with continuous autocrine and paracrine stimulation, thereby maintaining persistent proliferative signaling and supporting uncontrolled tumor expansion.

Clinical observations further reinforce the importance of cathepsin B in malignant growth. In a comprehensive review of experimental and clinical evidence, N. Aggarwal and B. F. Sloane (41) concluded that elevated cathepsin B expression is consistently associated with higher proliferative activity, poor tumor differentiation, increased biological aggressiveness, and less favorable clinical outcomes across multiple cancer types. These findings indicate that cathepsin B has considerable clinical relevance, serving not only as an indicator of disease progression but also as a promising target for the development of novel therapeutic strategies aimed at suppressing tumor growth.

The principal mechanisms through which cathepsins stimulate tumor cell proliferation are illustrated schematically in Figure 1.

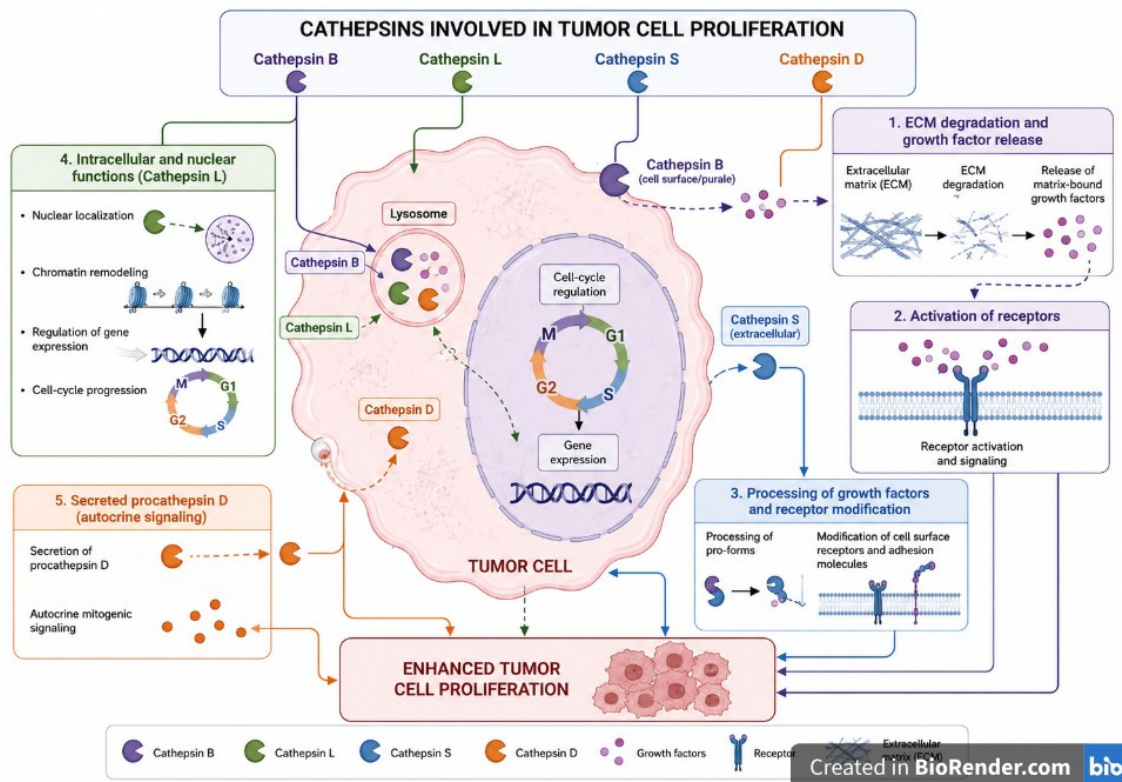


Figure 1. Major mechanisms of cathepsin involvement in tumor cell proliferation

Created by the author using BioRender.com.

The figure summarizes the principal mechanisms through which cathepsins B, L, S, and D contribute to tumor cell proliferation. It illustrates their intracellular and extracellular activities, including extracellular matrix degradation, growth factor release, receptor activation, regulation of gene expression and cell-cycle progression, as well as autocrine signaling, which collectively promote sustained malignant cell growth.

Although cathepsin B has been investigated most extensively, it is not the only member of the cathepsin family involved in regulating tumor growth. A growing body of evidence demonstrates that several additional cathepsins contribute to maintenance of the proliferative phenotype through distinct yet complementary molecular mechanisms. Among these, cathepsin L has attracted particular attention because its biological effects are mediated predominantly within intracellular compartments rather than through extracellular proteolysis. Besides its classical localization in lysosomes, cathepsin L has been detected in the cytoplasm and nucleus, where it participates in chromatin remodeling, processing of regulatory proteins, and modulation of cell-cycle progression. This broader intracellular distribution distinguishes cathepsin L from many other members of the family and enables it to influence multiple mechanisms controlling malignant cell proliferation.

Experimental findings consistently support the role of cathepsin L as a positive regulator of tumor growth. Increased expression of this enzyme enhances the proliferative capacity of cancer cells, whereas its genetic silencing or pharmacological inhibition suppresses cell growth and reduces tumor expansion. H. Goulet et al. (42) reported pronounced overexpression of cathepsin L in several highly aggressive malignancies, including breast, colorectal, and pancreatic cancers as well as melanoma. Moreover, elevated enzyme levels were associated with unfavorable prognosis, reduced patient survival, and increased biological aggressiveness of tumors. These observations indicate that cathepsin L functions not only as an important intracellular regulator of proliferative signaling but also as a clinically valuable prognostic marker and a potential therapeutic target for molecularly directed anticancer treatment.

Another cathepsin closely associated with tumor proliferation is cathepsin S, although its biological role differs substantially from those of cathepsins B and L. Because cathepsin S retains significant enzymatic activity under near-neutral pH conditions, it remains functionally active after secretion into the extracellular environment. In addition to malignant cells, this enzyme is released by macrophages, fibroblasts, and other stromal cells that constitute the tumor microenvironment. T. Wilkinson et al. (43) demonstrated that extracellular cathepsin S regulates tumor growth by proteolytically activating growth-associated molecules, modifying cell-surface receptors, and remodeling the surrounding extracellular environment. These activities strengthen reciprocal communication between cancer cells and stromal components, creating a positive feedback loop that continuously reinforces proliferative signaling and promotes sustained tumor growth.

The aspartic protease cathepsin D also plays an important role in the regulation of malignant proliferation. Although traditionally regarded as a lysosomal enzyme responsible for intracellular protein degradation, subsequent studies have demonstrated that cathepsin D possesses additional extracellular signaling functions that contribute directly to tumor development. H. Rochefort (44) showed that breast cancer cells actively secrete procathepsin D, which functions as an autocrine mitogenic factor capable of stimulating proliferation of neighboring malignant cells. These observations were later confirmed by M. Fusek et al. (45), who demonstrated that procathepsin D isolated from the secretome of ZR-75-1 breast cancer cells exhibits pronounced mitogenic activity. Collectively, these findings indicate that cathepsin D should not be viewed solely as a lysosomal hydrolase but also as an extracellular signaling molecule that enhances communication within the tumor microenvironment and promotes cancer cell proliferation.

Taken together, available evidence indicates that sustained tumor proliferation results from the coordinated activity of several cathepsins rather than from the action of a single protease. Each member of the family contributes through a distinct molecular mechanism while simultaneously interacting with complementary regulatory pathways. Cathepsin B primarily establishes a proteolytically active microenvironment that facilitates continuous growth stimulation, whereas cathepsin L regulates intracellular mechanisms controlling cell-cycle progression and gene expression. Cathepsin S strengthens proliferative signaling by promoting communication between malignant cells and stromal elements of the tumor microenvironment, while cathepsin D additionally functions as a secreted mitogenic factor that enhances autocrine stimulation of tumor growth. Acting together, these enzymes form an integrated proteolytic network that supports persistent cell proliferation and creates favorable molecular conditions for subsequent stages of cancer progression, including invasion and metastasis.

3.2 Interaction between Cathepsins and the Tumor Microenvironment

The biological behavior of malignant tumors is determined not only by the intrinsic properties of cancer cells but also by continuous interactions with the surrounding tumor microenvironment (TME). This complex ecosystem comprises stromal fibroblasts, immune cells, endothelial cells, pericytes, extracellular matrix (ECM) components, and numerous soluble mediators that establish an extensive network of reciprocal communication with malignant cells. Through these interactions, the tumor microenvironment regulates virtually every stage of cancer progression, including uncontrolled proliferation, local tissue invasion, angiogenesis, metastatic dissemination, and the response to anticancer therapy. Within this dynamic environment, cathepsins have emerged as key proteolytic regulators that influence extracellular matrix remodeling, pericellular proteolysis, intercellular communication, and activation of signaling molecules that collectively determine tumor behavior (39).

One of the major distinctions between normal tissues and malignant lesions is the profound alteration in the localization and distribution of cathepsins. Besides exhibiting elevated expression levels, many members of the cathepsin family undergo changes in intracellular trafficking that result in their accumulation on the plasma membrane, secretion into the extracellular space, or expression by non-malignant cells residing within the tumor stroma, including immune and stromal cell populations (39, 46). According to L. Sevenich and J. A. Joyce (47), extracellular proteolytic activity within tumors arises from the coordinated contribution of multiple cell types rather than from cancer cells alone. This concept has fundamentally changed the current understanding of tumor biology by demonstrating that the

tumor microenvironment is an active participant in malignant progression rather than a passive structural scaffold surrounding the tumor.

Among the numerous proteases operating within the tumor microenvironment, cysteine cathepsins occupy a particularly important position because of their broad substrate specificity and ability to regulate multiple biological processes simultaneously. M. M. Mohamed and B. F. Sloane (39) reported that cathepsins B, L, S, and K participate in degradation of extracellular matrix proteins, activation of additional protease systems, regulation of cell adhesion, and modulation of signaling pathways responsible for communication between malignant and stromal cells. Through these coordinated activities, cathepsins generate a microenvironment that facilitates continuous tumor expansion, promotes local invasion, and prepares surrounding tissues for subsequent metastatic dissemination. Nevertheless, the contribution of each individual cathepsin varies according to tumor type, cellular composition of the stroma, and the predominant cellular source of enzyme production (39).

The interaction between cathepsins and immune cells represents another essential aspect of tumor microenvironment regulation. Experimental evidence indicates that these enzymes are produced not only by malignant cells but also by numerous immune cell populations infiltrating tumors. T. Jakoš et al. (46) demonstrated that macrophages, dendritic cells, and other myeloid-derived cells express cysteine cathepsins that participate in antigen presentation, regulation of innate and adaptive immunity, tissue remodeling, and establishment of an immunosuppressive microenvironment. Consequently, cathepsins should be viewed as multifunctional mediators that coordinate communication among tumor cells, immune cells, and extracellular matrix components rather than as proteases originating exclusively from malignant tissue.

The biological importance of enzyme origin has been clearly illustrated in experimental models of cancer progression. O. Vasiljeva et al. (48) demonstrated that both tumor-derived and macrophage-derived cathepsin B contribute significantly to mammary tumor growth and pulmonary metastasis in mice. These findings indicate that the biological consequences of cathepsin activity depend not only on overall enzyme abundance but also on the specific cell populations responsible for its production. This observation provides a possible explanation for the variable therapeutic responses observed following pharmacological inhibition of cathepsins in different tumor types, where the cellular composition of the tumor microenvironment differs considerably.

The principal cellular sources of cathepsins and their major functions within the tumor microenvironment are summarized schematically in Figure 2.

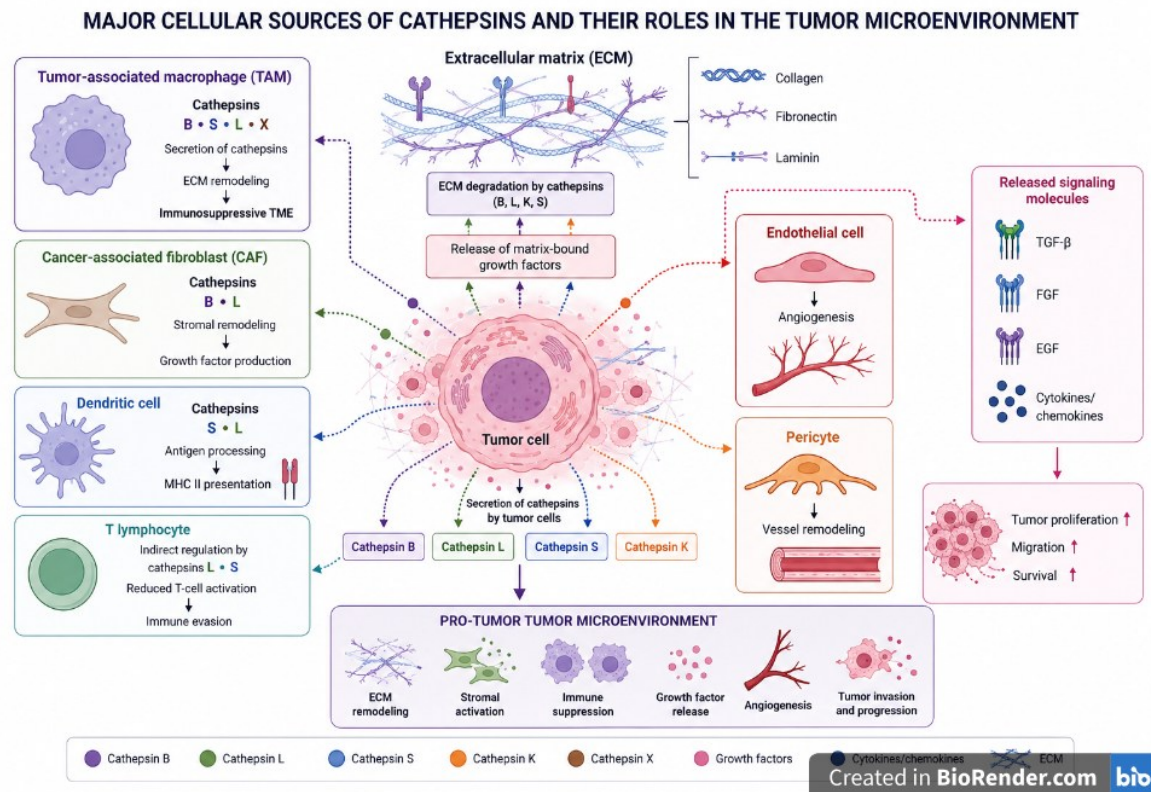


Figure 2. Major cellular sources of cathepsins and their roles in the tumor microenvironment

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This figure presents the principal cellular sources of cathepsins within the tumor microenvironment and the main biological processes regulated by these enzymes during tumor progression.

Among the non-malignant constituents of the tumor microenvironment, tumor-associated macrophages (TAMs) represent one of the most abundant and biologically influential sources of extracellular cathepsins in many solid malignancies. These cells actively secrete several members of the cathepsin family, particularly cathepsins B, S, L, and X, thereby contributing substantially to the proteolytic remodeling of the surrounding tissue. L. Sevenich and J. A. Joyce (47) demonstrated that macrophage-derived cathepsins promote degradation of extracellular matrix components, modify interactions between neighboring cells, and establish a stromal environment that favors continuous tumor growth. The level of protease expression in TAMs is strongly regulated by local conditions, including hypoxia, inflammatory mediators, and growth factors, allowing macrophages to adapt their proteolytic activity to the changing biological demands of the developing tumor.

One of the best-characterized functions of cathepsins within the tumor microenvironment is their participation in extracellular matrix remodeling. Under physiological conditions, the extracellular matrix provides structural support, preserves tissue architecture, and regulates communication between adjacent cells. During malignant transformation, however, this highly organized structure undergoes continuous degradation and reconstruction, resulting in profound alterations of the tumor stroma. M. Vizovišek et al. (30) demonstrated that several cathepsins efficiently degrade major extracellular matrix constituents, including collagen, laminin, fibronectin, and elastin, while simultaneously processing numerous matrix-associated regulatory proteins. These proteolytic modifications alter not only the biochemical composition of the extracellular matrix but also its physical properties, reducing mechanical barriers that normally restrict tumor cell movement and thereby facilitating local invasion.

The biological consequences of extracellular matrix degradation extend well beyond structural remodeling. Proteolytic cleavage of matrix components also regulates the availability of numerous signaling molecules that remain immobilized within the extracellular matrix under normal conditions. M. M. Mohamed and B. F. Sloane (39) demonstrated that cathepsin-mediated proteolysis promotes the release and activation of growth factors belonging to the transforming growth factor- β (TGF- β), fibroblast growth factor (FGF), and epidermal growth factor (EGF) families, together with additional signaling molecules that stimulate malignant cell proliferation, survival, and migration. Consequently, the extracellular matrix is transformed from a passive structural scaffold into an active reservoir of biological signals that continuously reinforce tumor progression.

Another stromal population profoundly influenced by cathepsin activity consists of cancer-associated fibroblasts (CAFs). As the principal producers of extracellular matrix components within tumors, CAFs play a fundamental role in shaping stromal architecture and regulating tumor–stroma interactions. According to L. Sevenich and J. A. Joyce (47), cathepsins indirectly modulate fibroblast activity by remodeling the extracellular matrix and altering the signaling molecules that mediate communication between malignant and stromal cells. Activated fibroblasts subsequently increase the synthesis of extracellular matrix proteins, cytokines, and growth factors, thereby amplifying stromal remodeling and establishing conditions that further support tumor progression. These reciprocal interactions create a self-reinforcing cycle in which cathepsin activity and fibroblast activation continuously stimulate one another.

Recent studies have further demonstrated that cathepsins contribute to the development of an immunosuppressive tumor microenvironment, one of the major mechanisms by which

malignant cells evade immune surveillance. T. Jakoš et al. (46) reported that cathepsins expressed by myeloid-derived cells regulate several immunological processes, including antigen presentation, dendritic cell maturation, T-lymphocyte activation, and production of inflammatory mediators. Through coordinated modulation of these pathways, cathepsins participate in reprogramming local immune responses, ultimately reducing the effectiveness of antitumor immunity and facilitating immune escape. These observations emphasize that cathepsins influence tumor progression not only through direct effects on malignant cells but also by reshaping immune regulation within the surrounding microenvironment.

For easier comparison of the principal mechanisms through which cathepsins influence individual components of the tumor microenvironment, these interactions are summarized in Table 4.

Table 4. Major mechanisms of cathepsin interactions with components of the tumor microenvironment

Tumor microenvironment component	Major cathepsins	Biological effect
Extracellular matrix (ECM)	Cathepsins B, L, K, S	Degradation of collagen, laminin, fibronectin, and other extracellular matrix proteins; remodeling of the ECM
Tumor-associated macrophages (TAMs)	Cathepsins B, S, L, X	Secretion of proteolytic enzymes, promotion of tumor growth, and remodeling of the tumor microenvironment
Cancer-associated fibroblasts (CAFs)	Cathepsins B, L	Regulation of stromal remodeling and modulation of tumor–stroma interactions
Dendritic cells	Cathepsins S, L	Antigen processing and regulation of MHC class II-mediated antigen presentation
T lymphocytes	Cathepsins L, S	Indirect modulation of T-cell activation and adaptive immune responses
Growth factors sequestered within the ECM	Cathepsins B, L	Release and activation of biologically active growth factors
Cytokines and chemokines	Cathepsins B, S	Proteolytic regulation of local intercellular signaling

Source: Compiled by the author based on data from references (39, 46, 47, 30).

Collectively, current evidence indicates that cathepsins represent central regulators of tumor microenvironment dynamics rather than simple lysosomal proteases involved in intracellular protein degradation. Through coordinated effects on extracellular matrix remodeling, stromal activation, immune cell function, and intercellular communication, these enzymes orchestrate complex interactions between malignant cells and their surrounding tissues. The resulting microenvironment supports sustained tumor growth, facilitates local invasion, promotes immune evasion, and creates favorable conditions for disease progression.

An additional consequence of cathepsin-mediated remodeling is the regulation of the bioavailability of signaling molecules within the tumor microenvironment. Degradation of extracellular matrix components is accompanied by liberation of growth factors, cytokines, and chemokines that subsequently activate intracellular signaling pathways in both malignant and stromal cells. This continuous release of biologically active mediators reinforces reciprocal communication among the various cellular components of the tumor, establishing a positive feedback mechanism that perpetuates proliferation, stromal remodeling, and local invasion. Thus, cathepsins influence tumor biology not only through direct proteolytic activity but also by controlling the molecular signals that coordinate communication throughout the tumor ecosystem.

Particular attention has also been directed toward the involvement of cathepsins in tumor angiogenesis. By remodeling extracellular matrix components, regulating the availability of proangiogenic growth factors, and modifying the biochemical characteristics of the tumor stroma, these proteases create conditions that favor endothelial cell activation and formation of new blood vessels. Because adequate vascularization is indispensable for continued tumor expansion and progression, cathepsins have become recognized as important molecular regulators of angiogenesis. The specific mechanisms through which these enzymes influence neovascularization are discussed in detail in the following section.

3.3 Role of Cathepsins in Angiogenesis

Formation of a functional vascular network is an essential prerequisite for continued tumor progression because rapidly proliferating malignant cells require a constant supply of oxygen and nutrients while simultaneously eliminating metabolic waste products. Once a tumor exceeds the diffusion limits of surrounding tissues, passive nutrient exchange becomes insufficient to sustain further growth. To overcome this limitation, malignant tissues activate angiogenic programs that promote the development of new blood vessels through coordinated interactions between cancer cells, stromal elements, endothelial cells, and extracellular matrix

components. This process is regulated by numerous molecular mediators, including vascular endothelial growth factor (VEGF), extracellular matrix remodeling enzymes, and signaling pathways controlling endothelial cell migration and proliferation. Increasing evidence indicates that cathepsins play an important regulatory role throughout this process by modifying the extracellular matrix, degrading basement membranes, regulating the availability of angiogenic mediators, and directly influencing endothelial cell function (49–51).

Experimental studies have demonstrated that angiogenesis is not driven by nonspecific proteolytic activity but instead depends on the specialized biological functions of individual cathepsins. A landmark investigation conducted by V. Gocheva et al. (49) using a multistage model of tumor development showed that different cysteine cathepsins contribute to distinct phases of tumor progression. Selective deletion of individual enzymes produced markedly different effects on activation of the angiogenic switch, subsequent tumor expansion, and invasive behavior. These findings demonstrated that the role of cathepsins in neovascularization cannot be explained simply by increased protease activity. Rather, each member of the family performs a specific biological function that collectively determines the efficiency of tumor vascular development.

Among the cathepsins implicated in angiogenesis, cathepsin S has received particular attention because of its prominent role in regulating vascular formation within malignant tissues. B. Wang et al. (50) demonstrated that genetic deficiency of cathepsin S significantly suppresses tumor angiogenesis and delays tumor progression in murine models. This effect was accompanied by reduced formation of angiogenic islets and impaired development of a functional vascular network within tumors. The authors attributed these changes to altered endothelial cell activity together with decreased local availability of proangiogenic mediators. These observations identify cathepsin S as one of the principal molecular regulators linking extracellular proteolysis with vascular development in the tumor microenvironment.

The major mechanisms through which cathepsins contribute to tumor angiogenesis are illustrated schematically in Figure 3.

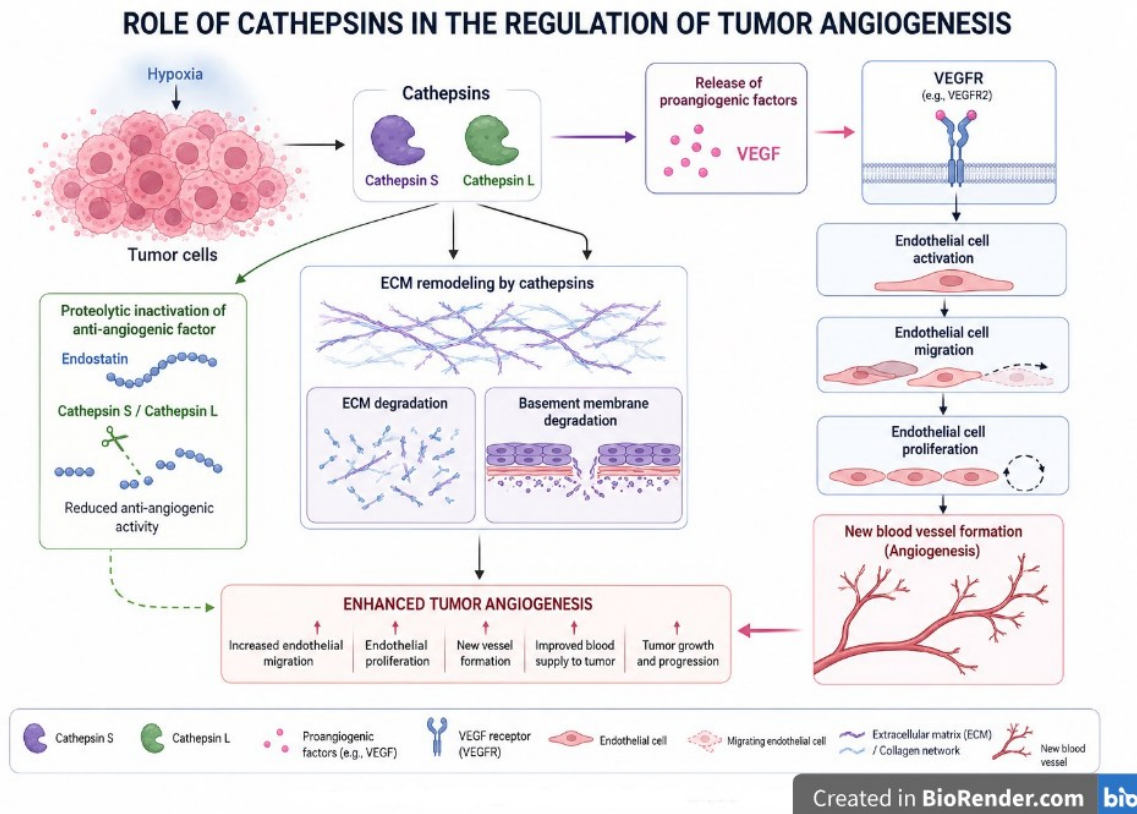


Figure 3. Role of cathepsins in the regulation of tumor angiogenesis

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Cathepsins S and L promote tumor angiogenesis by remodeling the extracellular matrix, facilitating the release of proangiogenic factors, enhancing VEGF-mediated signaling, and reducing the activity of endogenous antiangiogenic molecules. These coordinated mechanisms stimulate endothelial cell activation, migration, proliferation, and the formation of new blood vessel

Importantly, the angiogenic activity of cathepsin S is not limited to enzymes produced by malignant cells. Evidence obtained using a syngeneic colorectal carcinoma model demonstrated that both tumor-derived and host-derived cathepsin S actively participate in vascular development. D. M. Small et al. (51) reported that cathepsin S synthesized by stromal cells within the tumor microenvironment contributes substantially to neovascularization together with the enzyme produced by cancer cells. These findings further support the concept that tumor angiogenesis is a cooperative process involving multiple cellular populations rather than a phenomenon driven exclusively by malignant cells. Stromal and immune cells therefore represent important contributors to cathepsin-dependent regulation of vascular growth.

Another member of the cathepsin family that has been strongly implicated in angiogenesis is cathepsin L. Experimental work performed by D. R. Sudhan et al. (52) demonstrated that inhibition of cathepsin L markedly reduces angiogenic activity in breast cancer models, suggesting that this enzyme represents a promising molecular target for antiangiogenic therapy. Additional mechanistic insight was provided by T. Pan et al. (53), who showed that cathepsin L promotes angiogenesis in gastric carcinoma through activation of the CDP/Cux/VEGF-D signaling pathway. By regulating expression of genes associated with vascular development, cathepsin L establishes a direct molecular connection between lysosomal proteases and transcriptional control of angiogenesis.

Cathepsins promote vascular development not only by enhancing proangiogenic signaling but also by weakening endogenous mechanisms that normally suppress angiogenesis. F. Veillard et al. (54) demonstrated that cathepsins S and L proteolytically cleave endostatin, thereby reducing its biological activity as an endogenous inhibitor of blood vessel formation. As a consequence, these enzymes simultaneously stimulate angiogenesis through two complementary mechanisms: increasing the activity of proangiogenic pathways while diminishing the effectiveness of intrinsic antiangiogenic regulators. This dual mode of action substantially amplifies vascular development within malignant tissues.

Collectively, current evidence indicates that the contribution of cathepsins to tumor angiogenesis is mediated through multiple interrelated biological mechanisms rather than through a single signaling pathway. By remodeling extracellular matrix components, degrading basement membranes, facilitating endothelial cell migration, regulating VEGF-dependent signaling, and modulating endogenous inhibitors of angiogenesis, these proteases create conditions that support formation of an extensive tumor vascular network. Although the strongest experimental evidence currently concerns cathepsins S and L, available data suggest that additional members of the cathepsin family also participate in angiogenic regulation, with their relative contribution depending on tumor type, disease stage, and the cellular composition of the tumor microenvironment.

3.4 Role of Cathepsins in Tumor Cell Survival

Long-term persistence of malignant cells depends not only on their ability to proliferate but also on their capacity to survive under the unfavorable conditions that characterize the tumor microenvironment. During tumor progression, cancer cells are continuously exposed to hypoxia, nutrient limitation, oxidative stress, immune surveillance, and cytotoxic therapies. To remain viable, they must activate adaptive mechanisms that preserve cellular homeostasis while

simultaneously suppressing pathways leading to programmed cell death. Increasing evidence indicates that cathepsins are actively involved in these adaptive processes. Depending on the cellular context, they may either enhance tumor cell survival by supporting autophagy, metabolic adaptation, and therapeutic resistance or promote cell death following disruption of lysosomal integrity (55–57).

A central mechanism linking cathepsins to the regulation of cell survival is lysosomal membrane permeabilization (LMP). Under physiological conditions, lysosomal membranes maintain strict compartmentalization of cathepsins, thereby preventing inappropriate proteolytic activity within the cytoplasm. However, various stress stimuli can compromise lysosomal stability and allow lysosomal enzymes to escape into the cytosol. C. Oberle et al. (56) demonstrated that numerous apoptotic stimuli induce destabilization of lysosomal membranes, leading to cytoplasmic release of cathepsins and activation of signaling pathways associated with programmed cell death. Importantly, the cellular outcome depends largely on the magnitude of lysosomal damage. Limited membrane permeabilization primarily modifies intracellular signaling and may still permit cellular adaptation, whereas extensive disruption results in massive leakage of lysosomal proteases and promotes apoptosis or necrotic cell death (56).

Because of this relationship, maintenance of lysosomal integrity is of particular importance for malignant cells exposed to chronic environmental stress. Functional lysosomes preserve intracellular proteostasis by ensuring efficient degradation of damaged proteins, supporting completion of autophagic flux, and preventing uncontrolled redistribution of lysosomal proteases. Consequently, tumor cell survival depends on maintaining a delicate balance between controlled lysosomal proteolysis and preservation of membrane integrity. Moderate alterations in lysosomal permeability may facilitate adaptive responses that improve cellular resistance, whereas severe membrane destabilization converts cathepsins from mediators of survival into initiators of regulated cell death.

Among the individual members of the cathepsin family, cathepsin B has been studied most extensively with respect to mechanisms governing tumor cell survival. A comprehensive review by O. Mijanović et al. (55) summarized evidence demonstrating that cathepsin B participates in multiple processes associated with malignant progression, including regulation of autophagy, stimulation of tumor growth, angiogenesis, invasion, metastatic dissemination, and resistance to anticancer therapy. Nevertheless, the biological activity of cathepsin B is highly context dependent. As emphasized by both O. Mijanović et al. (55) and C. Oberle et al. (56), its effects are determined by several factors, including intracellular localization, the

biological characteristics of the tumor cell, and the intensity of environmental stress. Depending on these conditions, cathepsin B may either enhance adaptive mechanisms that promote survival or participate directly in lysosome-mediated activation of cell death pathways.

The close relationship between cathepsin B, autophagy, and apoptosis has been demonstrated in several experimental studies. P. Bhoopathi et al. (58) investigated primitive neuroectodermal tumor (PNET) cells and showed that expression of SPARC initially triggers an autophagic response, which subsequently progresses to apoptosis through mechanisms requiring cathepsin B activity. These findings suggest that cathepsin B functions as an important molecular link connecting two sequential cellular responses to stress. Under conditions in which protective autophagy is no longer sufficient to restore homeostasis, cathepsin B contributes to the transition toward irreversible programmed cell death.

Another important regulator of tumor cell survival is cathepsin L, particularly in the context of anticancer therapy. Increased activity of this enzyme has been associated with enhanced tolerance to therapeutic stress and development of treatment resistance in several malignancies. X. Zheng et al. (59) demonstrated that pharmacological inhibition of cathepsin L significantly reduces drug resistance in both cultured cells and experimental animal models, indicating direct involvement of this protease in molecular pathways responsible for chemoresistance. Similar observations were reported by N. Yang et al. (60), who found that inhibition of cathepsin L markedly increased the sensitivity of glioma cells to ionizing radiation and temozolomide. Together, these findings identify cathepsin L as a critical regulator of cellular adaptation during anticancer treatment and support its potential value as a therapeutic target for overcoming treatment resistance.

The biological functions of cathepsin D, the major lysosomal aspartic protease, are likewise characterized by considerable functional diversity. According to V. Vetvicka et al. (61), tumor-secreted procathepsin D possesses mitogenic activity that promotes malignant cell growth and supports long-term survival. At the same time, evidence indicates that cathepsin D may also participate in mechanisms leading to cell death under specific stress conditions. C. Marques et al. (62) demonstrated that acetate-induced apoptosis in colorectal carcinoma cells is accompanied by lysosomal membrane permeabilization and subsequent release of cathepsin D into the cytoplasm. These observations illustrate the dual biological role of cathepsin D, which may either facilitate tumor progression or contribute to activation of apoptotic pathways depending on the characteristics of the tumor microenvironment and the nature of the cellular stress response.

The principal mechanisms through which cathepsins regulate lysosomal integrity, autophagy, apoptosis, and resistance to anticancer therapy are summarized schematically in Figure 4.

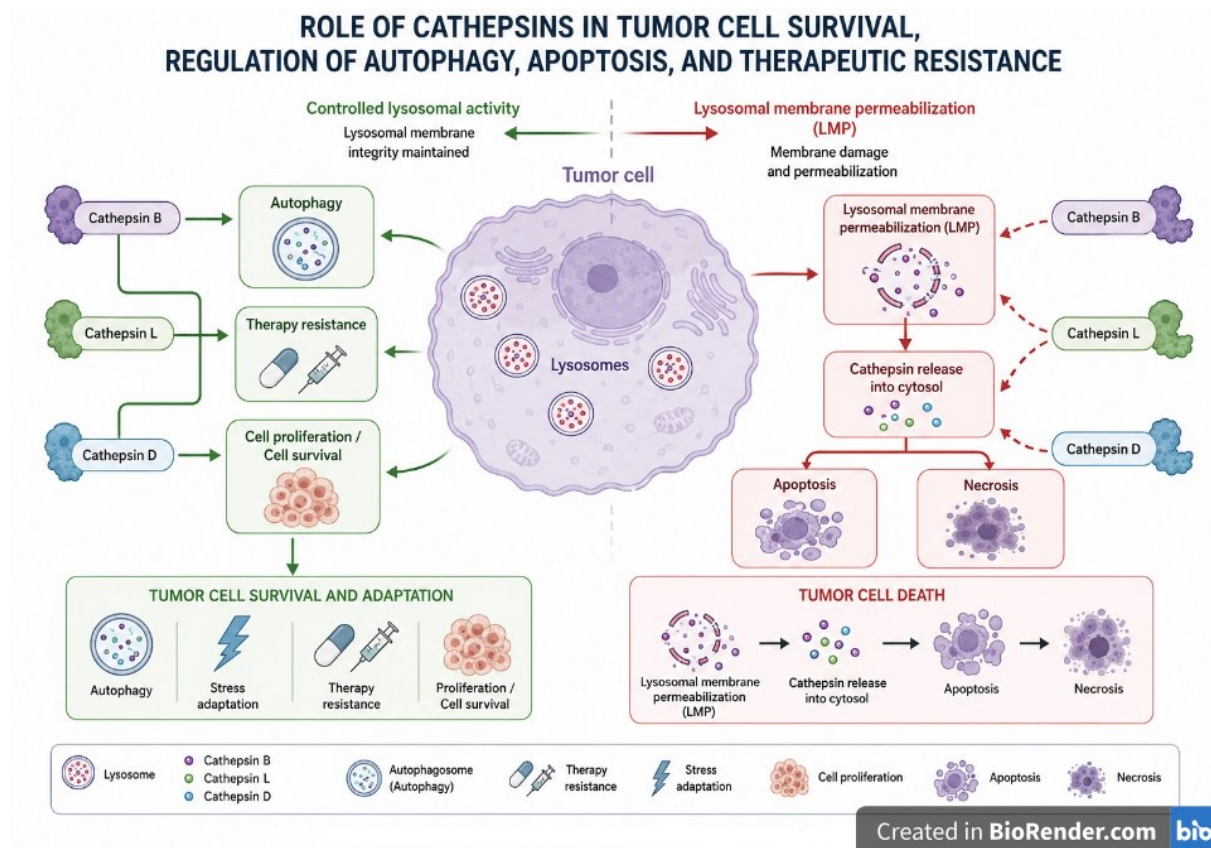


Figure 4. Role of cathepsins in tumor cell survival, regulation of autophagy, apoptosis, and therapeutic resistance

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The figure illustrates the dual role of cathepsins in determining tumor cell fate. Under conditions of preserved lysosomal integrity, cathepsins support autophagy, cellular adaptation, and therapeutic resistance, whereas lysosomal membrane permeabilization leads to the release of cathepsins into the cytosol and activation of apoptosis or necrosis.

Overall, current evidence demonstrates that the influence of cathepsins on tumor cell survival is highly dependent on the biological context in which these enzymes operate. Under conditions of moderate cellular stress, cathepsins support lysosomal function, maintain autophagic degradation, facilitate metabolic adaptation, and increase resistance to anticancer therapy. In contrast, severe disruption of lysosomal membrane integrity leads to redistribution of cathepsins into the cytoplasm, where they activate signaling pathways responsible for apoptosis and other forms of regulated cell death. This functional duality places cathepsins at

the intersection of molecular mechanisms governing cellular adaptation and elimination, making them attractive candidates for the development of novel therapeutic strategies aimed at selectively disrupting tumor cell survival while preserving normal tissue homeostasis.

4. Cathepsin-Mediated Signaling Pathways

4.1 PI3K/Akt Signaling Pathway

Among the intracellular signaling systems implicated in carcinogenesis, the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway is considered one of the most important regulators of malignant cell behavior because it integrates signaling events that govern proliferation, cellular growth, metabolism, survival, and migration. Under physiological conditions, activation of this cascade is initiated by the interaction of extracellular growth factors with receptor tyrosine kinases located on the plasma membrane. Ligand binding stimulates phosphoinositide 3-kinase (PI3K), which catalyzes the formation of phosphatidylinositol-3,4,5-trisphosphate (PIP3) from membrane phospholipids. The accumulation of PIP3 at the inner surface of the plasma membrane recruits protein kinase B (Akt), allowing its phosphorylation and subsequent activation. Activated Akt then transmits signals to numerous downstream effectors that regulate progression through the cell cycle, protein biosynthesis, apoptosis, autophagy, and maintenance of cellular metabolic balance. Persistent activation of this signaling network has therefore become recognized as one of the characteristic molecular abnormalities observed in a wide variety of human cancers (63, 64).

Under normal physiological circumstances, signaling through the PI3K/Akt cascade is maintained within strict limits by several inhibitory mechanisms, among which the phosphatase and tensin homolog (PTEN) plays a central regulatory role. PTEN antagonizes PI3K by dephosphorylating PIP3, thereby restricting recruitment and activation of Akt and preventing excessive propagation of downstream signals. Loss of PTEN function or constitutive Akt activation disrupts this regulatory equilibrium, leading to continuous stimulation of proliferative pathways, suppression of apoptotic signaling, activation of mammalian target of rapamycin (mTOR)-dependent protein synthesis, and acquisition of malignant cellular characteristics. Consequently, alterations affecting the PI3K/Akt/PTEN regulatory axis are now regarded as key molecular events underlying both tumor initiation and subsequent cancer progression (64).

Recent investigations have demonstrated that cathepsins influence PI3K/Akt signaling through mechanisms that extend well beyond their traditional lysosomal degradative functions. Rather than acting solely as intracellular proteases, several members of the cathepsin family regulate both extracellular and intracellular signaling by modifying membrane receptor activity, remodeling the extracellular matrix, and controlling the local availability of biologically active

growth factors. Through these complementary mechanisms, cathepsins establish conditions that favor prolonged activation of the PI3K/Akt pathway. T. Yadati et al. (3) emphasized that cathepsins should be regarded as multifunctional signaling regulators that connect extracellular proteolytic activity with intracellular molecular pathways responsible for adaptation of malignant cells to the constantly evolving tumor microenvironment.

Among all members of the family, cathepsin B has been investigated most extensively as a regulator of PI3K/Akt signaling. In a comprehensive review, Z. Yan et al. (65) summarized evidence from numerous experimental studies demonstrating a close association between elevated cathepsin B expression and enhanced activation of the PI3K/Akt pathway across multiple human malignancies. Sustained signaling through this cascade was consistently linked with accelerated cellular proliferation, increased resistance to apoptosis, enhanced invasive behavior, and stimulation of angiogenesis. Importantly, both pharmacological inhibition and genetic silencing of cathepsin B significantly reduced Akt phosphorylation in several experimental tumor models. These observations strongly support the concept that cathepsin B functions upstream of Akt and contributes directly to maintenance of persistent PI3K/Akt signaling during malignant progression (65).

The ability of cathepsin B to sustain activation of the PI3K/Akt pathway is not explained exclusively by its intracellular enzymatic activity. Following secretion into the tumor microenvironment, cathepsin B actively degrades extracellular matrix components, leading to proteolytic release of signaling molecules that are normally sequestered within the matrix. These molecules include numerous cytokines and growth factors capable of activating receptor tyrosine kinases, which serve as the principal upstream initiators of PI3K/Akt signaling. Consequently, extracellular cathepsin B enhances intracellular signaling indirectly by altering the biochemical composition of the surrounding microenvironment and strengthening communication between neighboring cells. Through this mechanism, extracellular matrix remodeling becomes functionally integrated with activation of intracellular pathways that promote malignant proliferation (39, 65).

Although cathepsin B remains the best-characterized regulator of this signaling cascade, accumulating evidence suggests that additional members of the cathepsin family also contribute to sustained PI3K/Akt activation. T. Yadati et al. (3) reported that increased expression of cathepsins L, S, and K frequently accompanies activation of signaling networks associated with cellular proliferation, survival, and metabolic adaptation. While the precise molecular mechanisms underlying these interactions remain incompletely understood, current findings indicate that these proteases primarily influence PI3K/Akt signaling through regulation of

receptor-mediated pathways, remodeling of extracellular matrix architecture, and modulation of local concentrations of biologically active growth factors. These observations suggest that multiple cathepsins cooperate to establish a signaling environment favorable for continued tumor progression.

One of the major biological consequences of sustained PI3K/Akt activation is the suppression of programmed cell death. Following activation, Akt phosphorylates several proteins involved in apoptotic signaling, including Bad, FoxO transcription factors, and glycogen synthase kinase-3 β (GSK-3 β), thereby inhibiting their proapoptotic activity and prolonging cellular survival (63, 64). Accordingly, persistent expression of cathepsins that maintain PI3K/Akt signaling indirectly contributes to acquisition of an antiapoptotic phenotype, which is considered one of the defining molecular characteristics of malignant transformation.

In addition to suppressing apoptosis, the PI3K/Akt signaling cascade plays a pivotal role in regulating cellular metabolism and biosynthetic activity through activation of the mechanistic target of rapamycin (mTOR). Acting downstream of Akt, mTOR coordinates multiple anabolic processes that are essential for rapid tumor growth, including protein synthesis, ribosome biogenesis, and cellular metabolism. D. A. Fruman et al. (63) demonstrated that activation of the PI3K/Akt/mTOR axis markedly increases production of proteins required for cell-cycle progression while simultaneously enhancing the capacity of malignant cells to adapt to hypoxia, nutrient deprivation, and therapeutic stress. Through these coordinated effects, persistent signaling within this pathway provides tumor cells with the metabolic flexibility necessary to maintain continuous proliferation despite adverse conditions in the tumor microenvironment. Consequently, sustained activation of the PI3K/Akt/mTOR network is now regarded as one of the principal molecular mechanisms driving malignant progression (63).

An important aspect of the relationship between cathepsins and the PI3K/Akt pathway is that their interaction is bidirectional rather than unidirectional. While cathepsins promote activation of PI3K/Akt signaling, the signaling cascade itself also regulates the expression and activity of several cathepsin family members. According to T. Yadati et al. (3) and Z. Yan et al. (65), activation of PI3K/Akt influences cathepsin expression through modulation of transcription factors, metabolic pathways, and gene regulatory programs associated with tumor adaptation. As a consequence, increased activity of one component reinforces activation of the other, generating a positive regulatory feedback loop that continuously strengthens proliferative signaling, enhances resistance to environmental stress, and improves the long-term survival of malignant cells within the tumor microenvironment. This reciprocal regulation may explain

why simultaneous overactivation of both cathepsins and the PI3K/Akt pathway is frequently observed in highly aggressive cancers.

The principal molecular mechanisms through which cathepsins promote activation of the PI3K/Akt signaling pathway are illustrated schematically in Figure 5.

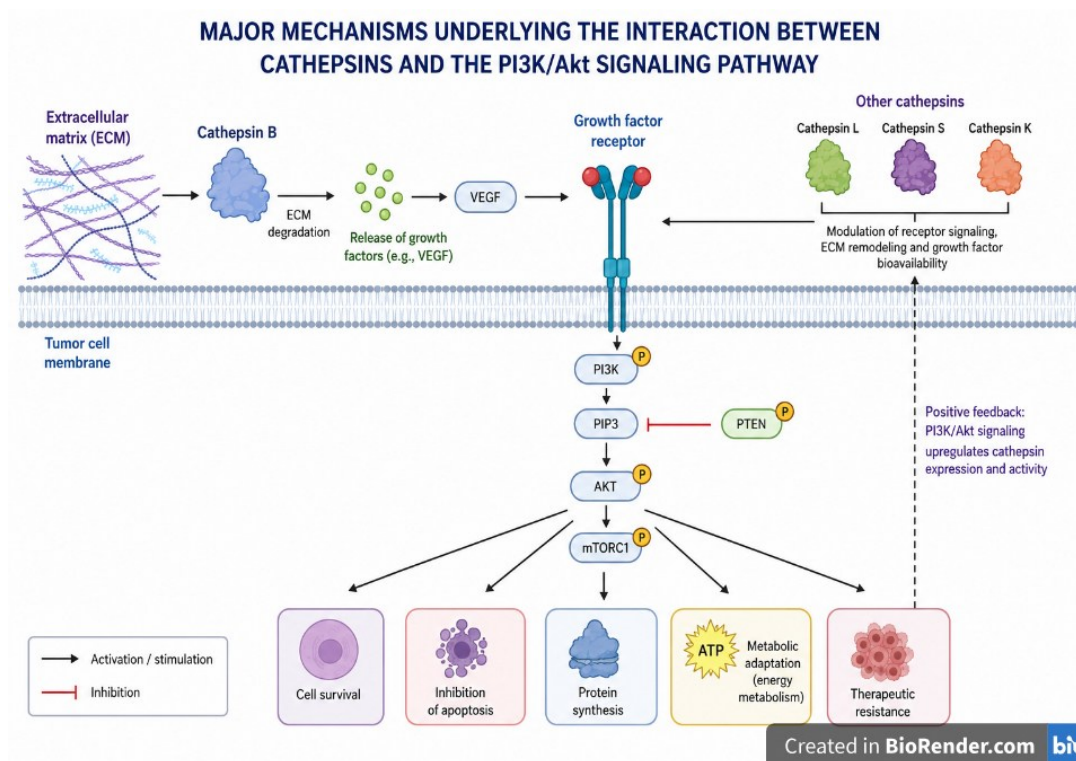


Figure 5. Major mechanisms underlying the interaction between cathepsins and the PI3K/Akt signaling pathway

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Cathepsins promote activation of the PI3K/Akt signaling pathway by remodeling the extracellular matrix, increasing the availability of growth factors, and modulating receptor-mediated signaling. Persistent activation of this pathway enhances cell survival, protein synthesis, metabolic adaptation, and therapeutic resistance while suppressing apoptosis.

Taken together, current experimental and clinical evidence demonstrates that the PI3K/Akt pathway represents one of the most important molecular interfaces linking cathepsin activity with malignant progression. Rather than affecting this signaling cascade through a single mechanism, cathepsins regulate PI3K/Akt activation at multiple levels. Their biological effects include remodeling of the extracellular matrix, modulation of growth factor bioavailability, regulation of receptor tyrosine kinase signaling, and facilitation of intercellular communication within the tumor microenvironment. Through the combined action of these

processes, cathepsins establish molecular conditions that favor persistent activation of proliferative signaling.

Among the individual family members, cathepsin B currently has the strongest experimental support as an upstream regulator of PI3K/Akt signaling. Nevertheless, increasing evidence indicates that cathepsins L, S, and K also contribute to maintenance of this pathway through complementary molecular mechanisms. Instead of acting independently, these proteases appear to function cooperatively, creating a proteolytic network that sustains intracellular signaling required for malignant growth and adaptation.

Overall, the available data indicate that the functional interaction between cathepsins and the PI3K/Akt pathway plays a central role in multiple aspects of tumor biology. By simultaneously enhancing proliferation, suppressing apoptosis, promoting metabolic adaptation, and increasing cellular resistance to environmental stress, the cathepsin–PI3K/Akt axis provides cancer cells with significant biological advantages during tumor progression. These observations identify this regulatory network as a promising target for future therapeutic strategies aimed at disrupting several cancer-promoting mechanisms through a single molecular intervention.

4.2 MAPK/ERK Signaling Pathway

The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway is one of the central molecular systems through which cells translate extracellular stimuli into coordinated biological responses. This signaling cascade regulates numerous physiological processes, including cell proliferation, differentiation, migration, survival, and acquisition of invasive properties. Under normal conditions, activation of the pathway begins when extracellular growth factors interact with receptor tyrosine kinases located on the cell membrane. Receptor stimulation initiates sequential activation of the RAS–RAF–MEK–ERK signaling cascade, culminating in phosphorylation of ERK. Activated ERK subsequently translocates into the nucleus, where it regulates the activity of multiple transcription factors, including Elk-1, c-Fos, c-Myc, AP-1, and other proteins responsible for controlling cell-cycle progression, protein synthesis, and proliferative activity (66, 67).

Persistent dysregulation of the MAPK/ERK pathway is among the most frequently observed molecular alterations in human cancer. According to S. Dhillon et al. (66), continuous activation of this signaling network promotes uncontrolled proliferation, suppresses apoptotic responses, enhances invasive behavior, facilitates epithelial-to-mesenchymal transition (EMT), and contributes to the development of resistance to anticancer therapy. Owing to its broad

influence on malignant cell biology, the MAPK/ERK pathway is now regarded, together with the PI3K/Akt cascade, as one of the principal signaling systems responsible for maintaining the malignant phenotype of cancer cells (66, 67).

Although cathepsins are not integral components of the kinase cascade itself, numerous studies indicate that they exert a significant regulatory influence on MAPK/ERK signaling through indirect mechanisms. Rather than participating directly in intracellular phosphorylation reactions, these proteases modify the extracellular environment in ways that facilitate receptor-mediated signal transduction. Their biological activities include degradation of extracellular matrix proteins, regulation of growth factor bioavailability, modulation of membrane receptor activity, and continuous remodeling of the tumor microenvironment. T. Yadati et al. (3) proposed that these proteolytic functions establish a functional bridge linking extracellular matrix remodeling with intracellular signaling pathways responsible for tumor progression.

Among the various members of the cathepsin family, cathepsin B currently exhibits the strongest experimental association with activation of the MAPK/ERK pathway. In a comprehensive review, Z. Yan et al. (65) summarized findings from numerous experimental studies showing that increased expression of cathepsin B is consistently accompanied by enhanced activity of several oncogenic signaling cascades, including MAPK/ERK. Importantly, both pharmacological inhibition and genetic suppression of cathepsin B reduce ERK phosphorylation, decrease proliferative activity, and attenuate the invasive phenotype of malignant cells. These observations strongly support the hypothesis that cathepsin B functions as an upstream regulator capable of maintaining sustained MAPK/ERK signaling during tumor progression.

One of the principal mechanisms through which cathepsins stimulate MAPK/ERK activation involves proteolytic remodeling of the extracellular matrix. Degradation of structural matrix proteins, including collagen, laminin, fibronectin, and related extracellular components, results in the release of numerous signaling molecules that are normally sequestered within the matrix. Once liberated, these growth factors interact with receptor tyrosine kinases and trigger intracellular activation of the RAS–RAF–MEK–ERK cascade. Consequently, cathepsin-mediated remodeling of the tumor microenvironment indirectly enhances MAPK/ERK signaling by increasing the local concentration of extracellular stimulatory molecules capable of activating receptor-dependent pathways (30, 39).

Activation of ERK subsequently induces extensive changes in the transcriptional profile of malignant cells. S. Dhillon et al. (66, 67) demonstrated that phosphorylated ERK promotes expression of cyclin D1, c-Myc, multiple matrix metalloproteinases (MMPs), and additional

proteins involved in epithelial-to-mesenchymal transition (EMT), cell migration, and tumor invasion. These transcriptional changes reinforce malignant progression while simultaneously enhancing remodeling of the surrounding extracellular environment. Consequently, a positive regulatory circuit is established in which cathepsin-mediated extracellular proteolysis promotes MAPK/ERK activation, whereas MAPK/ERK signaling increases production of molecules that further modify the tumor microenvironment and facilitate continued disease progression.

The biological consequences of this interaction are not restricted to regulation of cellular proliferation. M. M. Mohamed and B. F. Sloane (39) demonstrated that degradation of extracellular matrix proteins by cathepsins not only releases matrix-associated growth factors but also alters the structural organization and mechanical properties of tumor tissue. These changes facilitate migration and invasion of malignant cells while creating favorable conditions for vascular development. Simultaneously, activation of the MAPK/ERK pathway stimulates endothelial cell proliferation, enhances production of proangiogenic mediators, and promotes formation of functional tumor vasculature, thereby supporting continuous tumor expansion (39).

Current evidence further indicates that MAPK/ERK signaling operates as part of an integrated signaling network rather than as an isolated molecular pathway. Extensive functional crosstalk exists between MAPK/ERK and other oncogenic cascades, particularly PI3K/Akt and NF- κ B, allowing malignant cells to compensate for inhibition of one pathway by activating alternative signaling mechanisms. T. Yadati et al. (3) together with Z. Yan et al. (65) suggested that continuous remodeling of the extracellular environment by cathepsins may simultaneously promote activation of several intracellular signaling pathways. Through this coordinated regulation, cathepsins enhance the biological plasticity of malignant cells and improve their ability to survive under unfavorable conditions within the tumor microenvironment.

The principal mechanisms through which cathepsins regulate activation of the MAPK/ERK signaling pathway are summarized schematically in Figure 6.

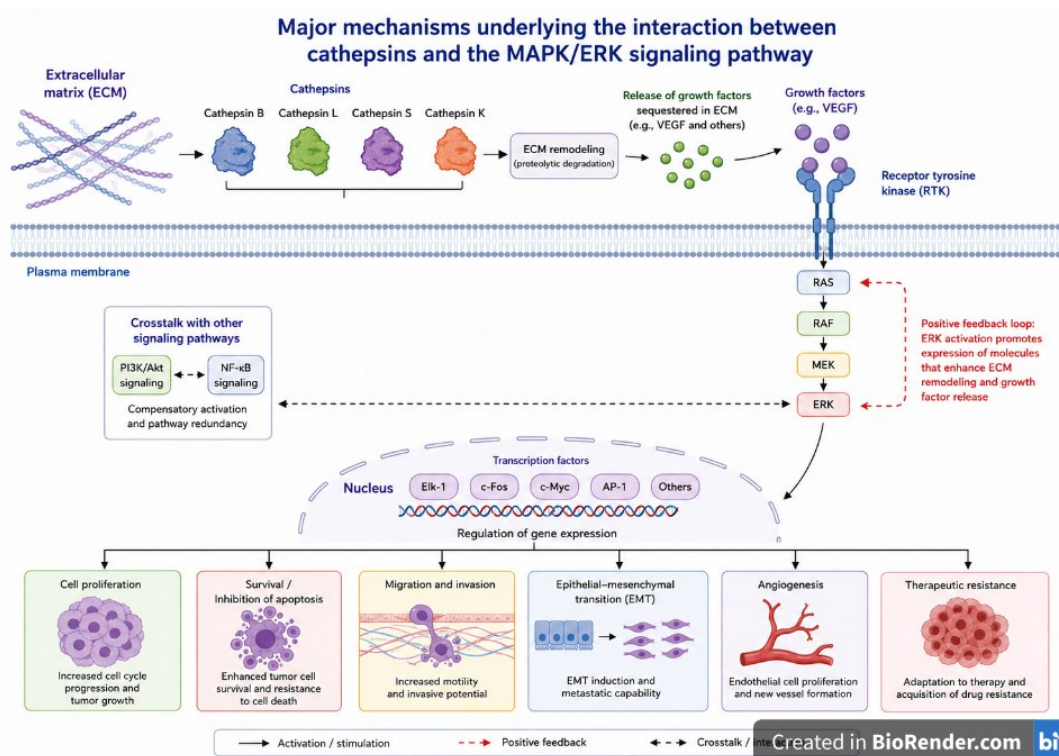


Figure 6. Major mechanisms underlying the interaction between cathepsins and the MAPK/ERK signaling pathway

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Cathepsins enhance MAPK/ERK signaling by promoting extracellular matrix remodeling, increasing the availability of growth factors, and facilitating receptor tyrosine kinase activation. Sustained ERK signaling subsequently regulates gene expression associated with proliferation, migration, epithelial–mesenchymal transition, angiogenesis, and therapeutic resistance.

Collectively, current experimental and clinical findings indicate that the influence of cathepsins on MAPK/ERK signaling is mediated predominantly through indirect regulatory processes rather than through direct participation in the kinase cascade itself. Instead of functioning as structural components of the RAS–RAF–MEK–ERK pathway, cathepsins modify the extracellular environment in ways that facilitate receptor-dependent signal transduction. Their ability to degrade extracellular matrix components, regulate the release of matrix-bound growth factors, and remodel the biochemical landscape of the tumor microenvironment creates favorable conditions for sustained activation of upstream signaling events that ultimately converge on ERK.

Among the different members of the cathepsin family, cathepsin B currently possesses the strongest experimental evidence supporting its role in regulation of MAPK/ERK signaling.

Numerous studies have demonstrated that increased expression or activity of this protease is consistently associated with enhanced ERK phosphorylation, whereas inhibition of cathepsin B suppresses activation of the pathway and attenuates malignant cellular behavior. These observations identify cathepsin B as one of the principal extracellular regulators capable of maintaining prolonged MAPK/ERK signaling during tumor progression (65).

Persistent activation of the MAPK/ERK cascade subsequently initiates a broad spectrum of molecular events that collectively reinforce the malignant phenotype. Continuous ERK signaling stimulates tumor cell proliferation, promotes epithelial-to-mesenchymal transition (EMT), enhances migratory and invasive capacities, supports angiogenesis, and increases the ability of cancer cells to adapt to environmental stress and therapeutic intervention (39, 66, 67). As these biological processes develop simultaneously, they progressively strengthen the aggressive characteristics of malignant tumors and facilitate disease progression.

An important feature of this regulatory system is the reciprocal relationship between extracellular proteolysis and intracellular signaling. Cathepsin-mediated remodeling of the tumor microenvironment enhances activation of MAPK/ERK, while the downstream transcriptional consequences of ERK activation further stimulate production of molecules that promote extracellular matrix remodeling, cell migration, and angiogenesis. This bidirectional interaction establishes a self-reinforcing signaling network that continuously amplifies malignant behavior and supports long-term tumor development.

Taken together, available evidence demonstrates that the cathepsin–MAPK/ERK axis represents an important molecular interface linking extracellular remodeling with intracellular oncogenic signaling. Rather than acting through isolated mechanisms, cathepsins coordinate multiple biological processes that collectively sustain activation of MAPK/ERK-dependent pathways. Through regulation of receptor-mediated signaling, modification of extracellular matrix architecture, control of growth factor availability, and facilitation of intercellular communication, these proteases contribute significantly to the maintenance of malignant cell proliferation, invasion, angiogenesis, and resistance to adverse microenvironmental conditions.

From a clinical perspective, these observations further emphasize the therapeutic relevance of this signaling network. Simultaneous targeting of cathepsin activity and MAPK/ERK signaling may represent a promising strategy for disrupting several complementary mechanisms responsible for tumor progression. By interfering with both extracellular proteolytic remodeling and intracellular oncogenic signaling, such approaches could potentially reduce tumor growth, limit metastatic dissemination, and improve the efficacy of existing anticancer therapies.

4.3 NF- κ B Signaling Pathway

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway is one of the key molecular systems responsible for coordinating inflammatory responses, immune regulation, cellular survival, and adaptation to environmental stress. In unstimulated cells, NF- κ B transcription factors are retained in the cytoplasm through association with inhibitory proteins of the I κ B family, which prevent their nuclear translocation. Upon exposure to proinflammatory cytokines, growth factors, microbial components, or oxidative stress, the I κ B kinase (IKK) complex becomes activated and phosphorylates I κ B proteins, targeting them for ubiquitin-dependent proteasomal degradation. Degradation of I κ B releases NF- κ B dimers—most commonly the p65/p50 complex—which subsequently translocate into the nucleus and activate transcription of numerous genes involved in cell proliferation, apoptosis resistance, cytokine synthesis, angiogenesis, migration, and other adaptive cellular responses (68, 69).

Constitutive activation of the NF- κ B pathway is recognized as one of the characteristic molecular abnormalities observed in a wide range of human malignancies. M. S. Hayden and S. Ghosh (68) demonstrated that persistent NF- κ B signaling enables tumor cells to evade programmed cell death, sustain chronic inflammatory responses, tolerate adverse conditions within the tumor microenvironment, and develop resistance to anticancer therapies. Because of its ability to simultaneously regulate inflammation and cellular survival, NF- κ B is widely regarded as a crucial molecular bridge linking chronic inflammatory processes with tumor initiation, progression, and therapeutic failure.

Although cathepsins do not form structural elements of the canonical NF- κ B signaling cascade, increasing evidence indicates that they substantially influence its activity through indirect regulatory mechanisms. Rather than participating directly in intracellular phosphorylation events, these proteases modify signaling upstream of NF- κ B by processing signaling molecules, remodeling the extracellular matrix, regulating cytokine production, and altering communication between malignant cells and surrounding stromal or immune cell populations. T. Yadati et al. (3) proposed that the close interaction between extracellular proteolysis and inflammatory signaling represents one of the principal mechanisms through which cathepsins promote malignant progression.

Among the members of the cathepsin family, cathepsin B has demonstrated the strongest experimental association with activation of NF- κ B signaling. A comprehensive review by Z. Yan et al. (65) summarized numerous experimental studies showing that elevated cathepsin B expression is frequently accompanied by increased activity of several oncogenic signaling

pathways, including NF- κ B. Importantly, inhibition of cathepsin B activity—either pharmacologically or through genetic suppression—consistently decreases NF- κ B activation, reduces production of proinflammatory cytokines, and suppresses proliferation of malignant cells. These findings strongly suggest that cathepsin B functions as an important upstream regulator responsible for maintaining sustained NF- κ B-dependent transcription during tumor development.

One of the principal mechanisms connecting cathepsins with NF- κ B activation involves regulation of the inflammatory cytokine network within the tumor microenvironment. Through selective proteolytic processing, cathepsins modify the biological activity of cytokines and chemokines while simultaneously promoting the release of signaling molecules that are normally sequestered within extracellular matrix components. As a consequence, the local concentrations of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and other inflammatory mediators increase markedly. Because these cytokines are potent activators of the NF- κ B pathway, cathepsin-mediated remodeling indirectly sustains prolonged signaling not only in malignant cells but also in stromal and immune cell populations that comprise the tumor microenvironment (3, 68).

The functional relationship between cathepsins and NF- κ B signaling is particularly evident in tumor-associated macrophages (TAMs), which represent one of the dominant immune cell populations infiltrating many solid tumors. T. Jakoš et al. (46) demonstrated that cathepsins expressed by myeloid-derived cells regulate inflammatory signaling, antigen processing, and production of mediators responsible for intercellular communication. Through these biological activities, cathepsins promote sustained activation of NF- κ B within the tumor microenvironment, thereby maintaining chronic inflammation while simultaneously contributing to formation of an immunosuppressive niche that supports malignant progression.

An important characteristic of this molecular relationship is its bidirectional nature. In addition to being regulated by cathepsins, the NF- κ B pathway itself controls the expression of several cathepsin family members. M. S. Hayden and S. Ghosh (68) demonstrated that NF- κ B regulates transcription of numerous genes associated with inflammatory responses, tissue remodeling, and cellular adaptation. Activation of this transcriptional program in malignant cells creates conditions favorable for increased expression of selected proteases. As emphasized by Z. Yan et al. (65), elevated cathepsin activity subsequently enhances cytokine production and further stimulates NF- κ B signaling, thereby establishing a self-amplifying positive feedback loop that continuously reinforces inflammatory signaling throughout tumor progression.

The principal molecular mechanisms underlying the interaction between cathepsins and the NF- κ B signaling pathway are summarized schematically in Figure 7.

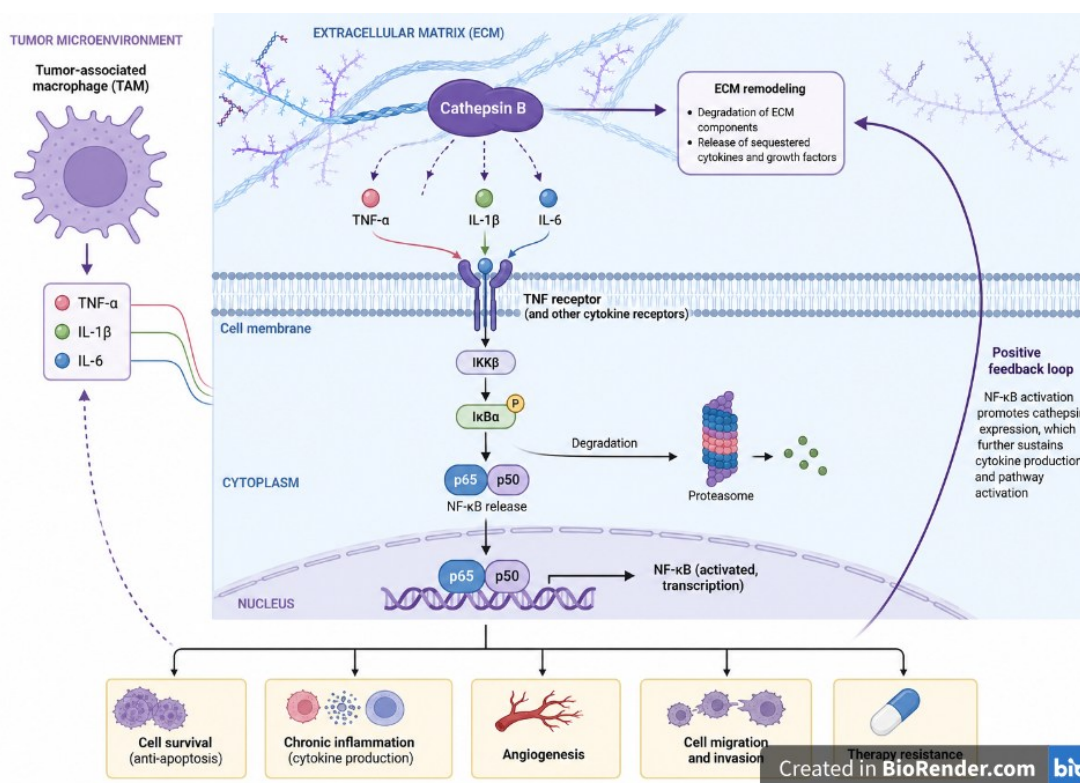


Figure 7. Major mechanisms underlying the interaction between cathepsins and the NF- κ B signaling pathway

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Cathepsin B promotes NF- κ B activation by enhancing extracellular matrix remodeling and regulating the production of proinflammatory cytokines, including TNF- α , IL-1 β , and IL-6. Sustained NF- κ B signaling supports chronic inflammation, cell survival, angiogenesis, migration, invasion, and the development of therapeutic resistance.

Collectively, available experimental and clinical evidence indicates that the NF- κ B pathway constitutes one of the major signaling systems through which cathepsins influence the biological behavior of malignant tumors. Rather than acting as integral elements of the canonical signaling cascade, these proteases regulate NF- κ B activity by modifying the inflammatory landscape of the tumor microenvironment. Their ability to remodel extracellular tissues, regulate the availability of cytokines and chemokines, and influence communication between malignant, stromal, and immune cells establishes conditions that favor persistent activation of NF- κ B-dependent transcription.

Among the individual members of the cathepsin family, cathepsin B currently possesses the strongest experimental support for involvement in this regulatory process. Increased expression of cathepsin B has repeatedly been associated with sustained NF- κ B activation, whereas inhibition of the enzyme attenuates inflammatory signaling and reduces transcription of genes that promote malignant progression. These findings identify cathepsin B as one of the principal proteolytic regulators linking extracellular remodeling with intracellular inflammatory signaling (65).

Continuous activation of the NF- κ B pathway has profound biological consequences for tumor development. Persistent signaling promotes chronic inflammation, suppresses apoptotic responses, enhances tumor cell survival, facilitates adaptation to metabolic and therapeutic stress, and contributes to the acquisition of resistance against anticancer treatment (68, 69). Through simultaneous regulation of these processes, NF- κ B supports maintenance of the malignant phenotype and enables tumor cells to survive within the constantly changing conditions of the tumor microenvironment.

An additional characteristic of this regulatory network is the existence of a self-reinforcing molecular circuit between cathepsins and NF- κ B signaling. Cathepsin-mediated remodeling of the tumor microenvironment increases production and activity of inflammatory mediators that stimulate NF- κ B, whereas activation of NF- κ B induces expression of genes encoding proteases and other molecules involved in tissue remodeling and inflammatory regulation. Consequently, activation of one system continuously strengthens the activity of the other, generating a positive feedback mechanism that amplifies inflammation, promotes extracellular matrix remodeling, and accelerates malignant progression (65, 68).

Current evidence therefore supports the concept that cathepsins function not only as proteolytic enzymes but also as important modulators of inflammatory signaling within tumors. By regulating cytokine availability, extracellular matrix turnover, immune cell activity, and intercellular communication, these proteases indirectly sustain NF- κ B activation and thereby influence numerous biological processes essential for cancer progression.

Taken together, the available data demonstrate that the cathepsin–NF- κ B axis represents an important molecular interface connecting extracellular proteolysis with intracellular inflammatory signaling. Through coordinated regulation of chronic inflammation, cellular survival, resistance to apoptosis, and adaptation to therapeutic stress, this signaling network contributes substantially to tumor development and progression. These observations further strengthen the rationale for therapeutic strategies aimed at simultaneously targeting cathepsin

activity and NF- κ B signaling in order to disrupt complementary mechanisms supporting malignant growth and improve the effectiveness of anticancer treatment.

4.4 TGF- β Signaling Pathway

The transforming growth factor-beta (TGF- β) signaling pathway is one of the principal molecular systems responsible for maintaining tissue homeostasis through regulation of cell proliferation, differentiation, apoptosis, and extracellular matrix turnover. Its biological role, however, undergoes a profound transformation during cancer development. In normal tissues and early stages of tumorigenesis, TGF- β primarily functions as a tumor suppressor by inhibiting epithelial cell proliferation and promoting cellular differentiation. As malignant transformation progresses, this protective activity gradually diminishes, and TGF- β acquires predominantly protumorigenic properties. In advanced cancers, activation of the pathway promotes epithelial-to-mesenchymal transition (EMT), stimulates extracellular matrix remodeling, suppresses antitumor immune responses, enhances angiogenesis, and facilitates metastatic dissemination (70, 71).

Signal transduction through the TGF- β pathway is initiated when the ligand binds to the type II transforming growth factor-beta receptor (TGFB β R2). Ligand binding induces recruitment and phosphorylation of the type I receptor (TGFB β R1), which subsequently activates the intracellular mediators Smad2 and Smad3. These proteins associate with Smad4, and the resulting complex translocates into the nucleus, where it regulates transcription of numerous genes involved in tumor progression. In addition to this canonical Smad-dependent mechanism, TGF- β also activates several noncanonical signaling pathways, including PI3K/Akt, MAPK/ERK, Rho GTPases, and NF- κ B. Simultaneous engagement of multiple signaling networks largely explains the broad spectrum of biological responses elicited by TGF- β in malignant tissues (70, 72).

Although cathepsins are not structural components of the TGF- β signaling cascade, accumulating evidence demonstrates that they exert a substantial indirect influence on its activity by reshaping the tumor microenvironment. Their regulatory effects are mediated primarily through extracellular matrix degradation, modulation of growth factor bioavailability, and regulation of communication between malignant cells and stromal populations. T. Yadati et al. (3) emphasized that cathepsin-dependent proteolysis alters the molecular composition of the tumor microenvironment, thereby establishing conditions that favor activation of signaling pathways supporting malignant progression.

Among the members of the cathepsin family, cathepsin B has shown the strongest experimental association with TGF- β signaling. M. Yin et al. (73) demonstrated that cathepsin B regulates both the expression and biological activity of TGF- β within the melanoma microenvironment. Pharmacological inhibition of cathepsin B significantly attenuated TGF- β signaling, reduced activation of cancer-associated fibroblasts (CAFs), and markedly suppressed invasive growth of melanoma cells. These findings indicate that cathepsin B functions as an important molecular intermediary facilitating TGF- β -dependent communication between malignant cells and stromal components.

Further support for this interaction was provided by A. Reisenauer et al. (74), who investigated the influence of TGF- β 1 on cathepsin B expression in myeloid tumor cells. Their experiments showed that stimulation with TGF- β 1 markedly increased both synthesis and enzymatic activity of cathepsin B. Enhanced cathepsin B expression was accompanied by increased migratory capacity, greater invasive potential, and reduced susceptibility of tumor cells to apoptosis. Collectively, these observations demonstrate that the interaction between cathepsin B and TGF- β signaling is bidirectional. While activation of TGF- β promotes cathepsin B expression, increased cathepsin B activity simultaneously reinforces the tumor-promoting effects mediated by the TGF- β pathway, creating a positive regulatory feedback loop.

Another important point of convergence between cathepsins and TGF- β signaling is extracellular matrix remodeling. Proteolytic degradation of matrix components by cathepsins B, L, and K continuously alters both the structural organization and biomechanical properties of the tumor stroma. These modifications facilitate interactions between signaling molecules and neighboring cells while increasing accessibility of growth factors that are normally sequestered within extracellular matrix components. According to M. M. Mohamed and B. F. Sloane (39), extracellular matrix remodeling not only promotes local invasion but also establishes a microenvironment that strongly favors activation of signaling pathways, among which TGF- β occupies a particularly important position.

The biological interaction between cathepsins and TGF- β becomes especially evident during the activation and maintenance of cancer-associated fibroblasts (CAFs). TGF- β represents one of the principal molecular stimuli responsible for fibroblast activation, whereas cathepsin-mediated remodeling of the surrounding extracellular matrix generates the structural environment necessary to sustain CAF function. Once activated, CAFs increase production of extracellular matrix proteins together with numerous cytokines and growth factors, thereby strengthening reciprocal communication between stromal and malignant cells. The resulting

positive feedback mechanism continuously reinforces stromal remodeling and creates conditions that favor persistent tumor progression (71, 73).

The principal mechanisms through which cathepsins regulate TGF- β signaling are summarized schematically in Figure 8.

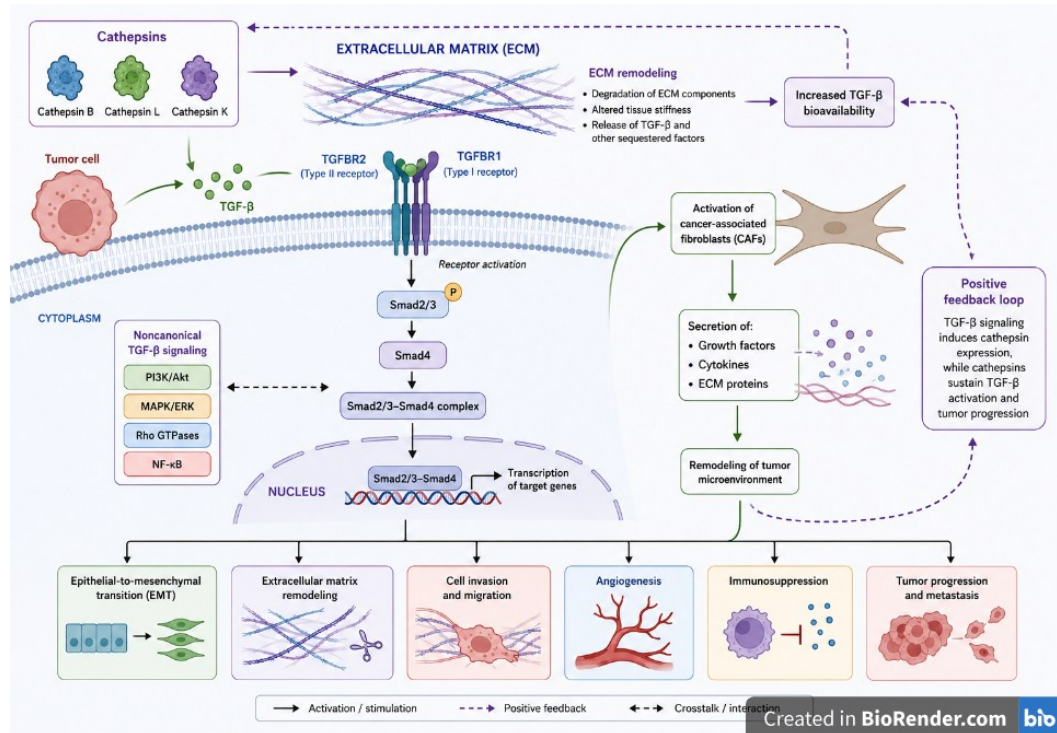


Figure 8. Major mechanisms underlying the interaction between cathepsins and the TGF- β signaling pathway

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Cathepsins regulate TGF- β signaling by remodeling the extracellular matrix, increasing TGF- β bioavailability, and promoting the activation of cancer-associated fibroblasts. These interactions enhance epithelial–mesenchymal transition, angiogenesis, immunosuppression, and tumor progression through both canonical and noncanonical TGF- β signaling.

Collectively, current experimental and clinical evidence demonstrates that cathepsins function as important indirect regulators of TGF- β signaling throughout malignant progression. Rather than participating directly in the intracellular Smad signaling cascade, these proteases modulate pathway activity by controlling extracellular matrix remodeling, regulating local TGF- β bioavailability, and facilitating reciprocal communication between tumor cells and stromal populations.

Among the individual members of the cathepsin family, cathepsin B currently exhibits the strongest experimental association with the tumor-promoting activities of TGF- β . Through

stimulation of cancer-associated fibroblast activation, enhancement of extracellular matrix remodeling, and maintenance of a microenvironment that supports sustained TGF- β signaling, cathepsin B significantly contributes to invasive tumor growth. These findings identify the cathepsin B–TGF- β axis as an important molecular mechanism governing stromal remodeling, malignant progression, and establishment of a protumorigenic tumor microenvironment, highlighting its potential value as a target for future therapeutic intervention.

4.5 Wnt/ β -catenin Signaling Pathway

The Wnt/ β -catenin signaling pathway is one of the major regulatory systems controlling embryonic development, tissue homeostasis, cellular differentiation, proliferation, and maintenance of stem cell populations. In the absence of Wnt ligands, intracellular levels of β -catenin remain low because the protein is continuously phosphorylated and targeted for degradation by a multiprotein destruction complex consisting of adenomatous polyposis coli (APC), Axin, casein kinase 1 (CK1), and glycogen synthase kinase-3 β (GSK-3 β). Activation of the pathway occurs when Wnt proteins bind to Frizzled receptors together with the LRP5/6 co-receptors, resulting in inactivation of the destruction complex. Consequently, β -catenin accumulates within the cytoplasm and subsequently translocates into the nucleus, where it interacts with transcription factors of the TCF/LEF family to initiate expression of genes involved in cell-cycle progression, proliferation, survival, and preservation of stem cell characteristics (75, 76).

Constitutive activation of Wnt/ β -catenin signaling is recognized as one of the most frequent molecular abnormalities associated with human cancer. Aberrant stimulation of this pathway has been documented in numerous malignancies, including colorectal carcinoma, hepatocellular carcinoma, melanoma, and breast cancer. According to H. Clevers and R. Nusse (75), sustained activation of Wnt signaling promotes uncontrolled cellular proliferation, suppresses differentiation, preserves populations of cancer stem cells, and enhances resistance to anticancer therapy. Because of its extensive influence on multiple aspects of tumor biology, the Wnt/ β -catenin pathway is now regarded as one of the core signaling networks driving malignant transformation and disease progression.

Current evidence indicates that cathepsins regulate Wnt/ β -catenin signaling predominantly through indirect mechanisms rather than by direct interaction with components of the signaling cascade. These proteases are not involved in assembly or function of the β -catenin destruction complex itself. Instead, they reshape the extracellular environment by remodeling extracellular matrix architecture, modifying cell–cell adhesion, altering

intercellular junctions, and regulating the local availability of signaling molecules. Through these biological activities, cathepsins establish conditions that facilitate sustained activation of Wnt-dependent signaling.

Among the individual members of the cathepsin family, cathepsin B has shown the strongest experimental association with the Wnt/ β -catenin pathway. Z. Yan et al. (65) summarized evidence indicating that increased expression of cathepsin B in malignant tumors is closely associated with activation of signaling networks that promote tumor cell proliferation, invasion, and epithelial-to-mesenchymal transition (EMT). According to the authors, these biological effects arise primarily from cathepsin B-mediated remodeling of the extracellular matrix together with disruption of cell–cell adhesion, both of which facilitate efficient activation of Wnt/ β -catenin signaling.

The relationship between cathepsins and epithelial-to-mesenchymal transition (EMT) provides an important explanation for the biological consequences of Wnt pathway activation. Stimulation of Wnt/ β -catenin signaling induces extensive phenotypic reprogramming characterized by downregulation of E-cadherin and increased expression of N-cadherin, vimentin, and other mesenchymal markers. H. Clevers and R. Nusse (75) demonstrated that these molecular alterations markedly enhance migratory and invasive capacities of malignant cells while facilitating metastatic dissemination. Cathepsins further reinforce these processes by degrading extracellular matrix proteins and weakening intercellular adhesion, thereby generating a tissue environment that supports the phenotypic changes initiated by Wnt/ β -catenin signaling (30, 39).

Another important biological consequence of Wnt pathway activation is the maintenance of cancer stem cell (CSC) populations. R. Nusse and H. Clevers (76) reported that sustained Wnt/ β -catenin signaling preserves the self-renewal capacity of CSCs, maintains their long-term proliferative potential, and contributes significantly to resistance against chemotherapeutic agents. Because cathepsins actively remodel the tumor microenvironment and regulate communication between malignant cells and their surrounding stem cell niches, increased proteolytic activity may indirectly support maintenance of CSC populations by establishing conditions favorable for persistent Wnt signaling (3, 39).

The Wnt/ β -catenin pathway should also be considered within the broader context of interconnected oncogenic signaling networks. Rather than functioning independently, it exhibits extensive crosstalk with several additional signaling pathways, including PI3K/Akt, MAPK/ERK, and TGF- β . Together, these integrated molecular systems coordinate fundamental processes associated with malignant progression, including tumor growth, local

invasion, and metastatic dissemination (72, 75). Within this complex signaling landscape, cathepsins contribute primarily by modifying the extracellular environment, thereby facilitating activation and long-term maintenance of multiple complementary signaling cascades.

The principal mechanisms through which cathepsins regulate Wnt/ β -catenin signaling are summarized schematically in Figure 9.

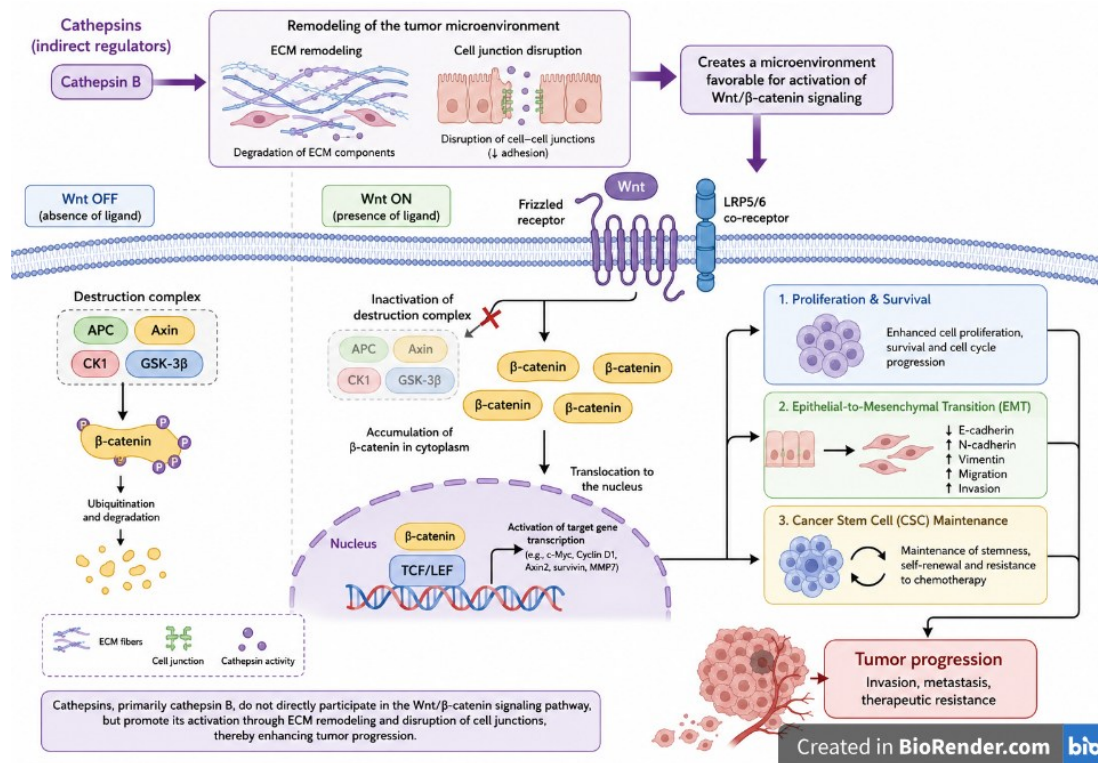


Figure 9. Major mechanisms underlying the interaction between cathepsins and the Wnt/ β -catenin signaling pathway

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Cathepsins indirectly promote Wnt/ β -catenin signaling by remodeling the extracellular matrix and disrupting cell–cell junctions, thereby creating a microenvironment favorable for pathway activation. Sustained Wnt/ β -catenin signaling enhances tumor cell proliferation, epithelial–mesenchymal transition, maintenance of cancer stem cells, and tumor progression.

Collectively, current experimental evidence indicates that cathepsins should not be considered direct components of the Wnt/ β -catenin signaling machinery. Instead, these enzymes modulate pathway activity indirectly by remodeling the extracellular matrix, altering cell–cell adhesion, regulating the biochemical characteristics of the tumor microenvironment, and facilitating epithelial-to-mesenchymal transition. Through these complementary

mechanisms, cathepsins establish a microenvironment that supports sustained activation of Wnt-dependent signaling during malignant progression.

Among the members of the cathepsin family, cathepsin B currently provides the most convincing experimental evidence linking proteolytic activity with regulation of the Wnt/ β -catenin pathway. By contrast, the contribution of other cathepsins remains less clearly established and is supported primarily by indirect experimental observations. Consequently, additional mechanistic studies are required to define the specific roles of individual cathepsins in Wnt signaling and to evaluate their potential as molecular targets for future anticancer therapies.

5. Role of Cathepsins in Tumor Invasion and Metastasis

5.1 Extracellular Matrix Degradation

The capacity of malignant cells to infiltrate surrounding tissues is critically dependent on their ability to breach the extracellular matrix (ECM), which serves as the principal structural barrier separating normal tissue compartments. Under physiological conditions, the ECM provides mechanical stability while simultaneously regulating essential cellular functions, including adhesion, migration, proliferation, differentiation, and intercellular communication. This highly organized three-dimensional network is composed mainly of collagens, laminin, fibronectin, elastin, proteoglycans, and numerous additional structural and signaling molecules. Because these components collectively form both the basement membrane and the interstitial matrix, successful local invasion requires their progressive proteolytic degradation, thereby creating pathways through which malignant cells can penetrate adjacent tissues (30, 47).

Destruction of the extracellular matrix is not accomplished by a single protease but instead results from the coordinated activity of several complementary proteolytic systems. Matrix metalloproteinases (MMPs), serine proteases, and cathepsins function cooperatively to remodel the tumor microenvironment and dismantle extracellular barriers that normally restrict tumor expansion. M. M. Mohamed and B. F. Sloane (39) emphasized that cysteine cathepsins contribute to this process through mechanisms extending beyond direct degradation of extracellular matrix proteins. These enzymes also activate additional proteases and establish localized regions of intense pericellular proteolysis. Acting together, these proteolytic systems progressively weaken tissue architecture, disrupt adhesive interactions between cells and the surrounding matrix, and facilitate continuous invasion of malignant cells into neighboring structures.

A distinguishing feature of malignant tissue is the profound alteration in the intracellular localization of cathepsins. In healthy cells, these proteases remain predominantly confined to lysosomes, whereas cancer cells frequently redistribute them to the plasma membrane or actively secrete them into the extracellular space. L. Sevenich and J. A. Joyce (47) demonstrated that extracellular matrix degradation within tumors is not mediated exclusively by malignant cells. Stromal fibroblasts, tumor-associated macrophages, and other infiltrating immune cells likewise secrete cathepsins, thereby making substantial contributions to proteolytic remodeling throughout the tumor microenvironment. Consequently, extracellular proteolysis becomes a

property of the tumor ecosystem as a whole rather than being restricted to the immediate vicinity of individual cancer cells.

Among the various members of the cathepsin family, cathepsin B has been investigated most extensively as a mediator of extracellular matrix degradation. One of the earliest studies establishing this biological role was conducted by M. R. Buck et al. (77), who demonstrated that cathepsin B isolated from both normal and malignant human tissues efficiently hydrolyzes major constituents of the basement membrane, including type IV collagen, laminin, and fibronectin. These observations significantly expanded the understanding of cathepsin B biology by showing that its function extends beyond lysosomal protein degradation and includes direct participation in destruction of the extracellular barriers that normally limit tumor dissemination.

Subsequent investigations further strengthened the evidence implicating cathepsin B in malignant invasion. B. C. Victor et al. (78) demonstrated that, in inflammatory breast cancer, the catalytically active form of cathepsin B accumulates predominantly on the tumor cell surface rather than remaining confined within lysosomes. Pharmacological inhibition of the enzyme markedly reduced extracellular matrix degradation and significantly impaired the invasive capacity of malignant cells. These findings indicate that localization of cathepsin B within the pericellular environment represents an important determinant of the aggressive biological behavior characteristic of inflammatory breast carcinoma.

Although cathepsin B remains the best-characterized member of the family, remodeling of the extracellular matrix depends on the coordinated action of several additional cysteine cathepsins, particularly cathepsins L, S, and K. According to M. Vizovišek et al. (30), these proteases degrade not only structural constituents of the extracellular matrix but also a variety of regulatory proteins involved in cell adhesion, migration, and intercellular communication. Consequently, their biological function extends well beyond simple degradation of extracellular proteins. Proteolytic processing performed by these enzymes modifies the functional properties of matrix-associated molecules, promotes release of growth factors retained within the extracellular matrix, and generates biologically active protein fragments capable of influencing both malignant and stromal cells.

Efficient remodeling of tumor tissue also depends on close cooperation between cathepsins and other proteolytic enzyme systems. L. Sevenich and J. A. Joyce (47) emphasized that cysteine cathepsins interact functionally with matrix metalloproteinases as well as components of the plasminogen activation system. Initial cleavage of extracellular matrix proteins by cathepsins exposes previously inaccessible substrates, thereby facilitating

subsequent degradation by additional proteases. Through this cooperative mechanism, proteolytic activity becomes progressively amplified, allowing the area permissive for tumor invasion to expand continuously during disease progression.

The consequences of extracellular matrix degradation extend far beyond the simple removal of physical barriers. Proteolytic remodeling simultaneously alters the availability of numerous signaling molecules that are normally immobilized within matrix components. M. M. Mohamed and B. F. Sloane (39) demonstrated that cathepsin-dependent proteolysis promotes the release of growth factors, cytokines, and chemokines, effectively transforming the extracellular matrix from a passive structural scaffold into a dynamic signaling platform. As a result, extracellular matrix remodeling not only facilitates migration of malignant cells but also enhances proliferation, promotes cellular survival, stimulates invasive behavior, and strengthens communication between tumor cells and surrounding stromal populations.

Clinical observations provide additional evidence supporting the importance of cathepsin-mediated extracellular matrix degradation during tumor progression. B. Bian et al. (79) demonstrated that elevated cathepsin B activity contributes significantly to the progression of colorectal cancer by promoting both local tissue invasion and metastatic dissemination. Increased expression of the enzyme consistently correlated with a more aggressive tumor phenotype, whereas suppression of cathepsin B activity markedly reduced the invasive behavior of malignant cells. These findings identify cathepsin B as an important molecular determinant of both local tumor invasion and metastatic competence.

The biochemical characteristics of the tumor microenvironment further enhance the extracellular activity of cathepsins. Most members of this protease family exhibit maximal catalytic activity under acidic conditions, and malignant tissues typically develop an acidic extracellular milieu as a consequence of hypoxia together with intensified glycolytic metabolism. J. M. Rothberg et al. (80) demonstrated that acidification of the tumor microenvironment markedly increases cathepsin B-mediated degradation of basement membrane components and significantly enhances the invasive potential of malignant cells. These observations explain how cathepsins retain substantial enzymatic activity after secretion from lysosomes and continue to promote extracellular tissue destruction during tumor progression.

The principal mechanisms through which cathepsins contribute to extracellular matrix degradation during tumor invasion are illustrated schematically in Figure 10.

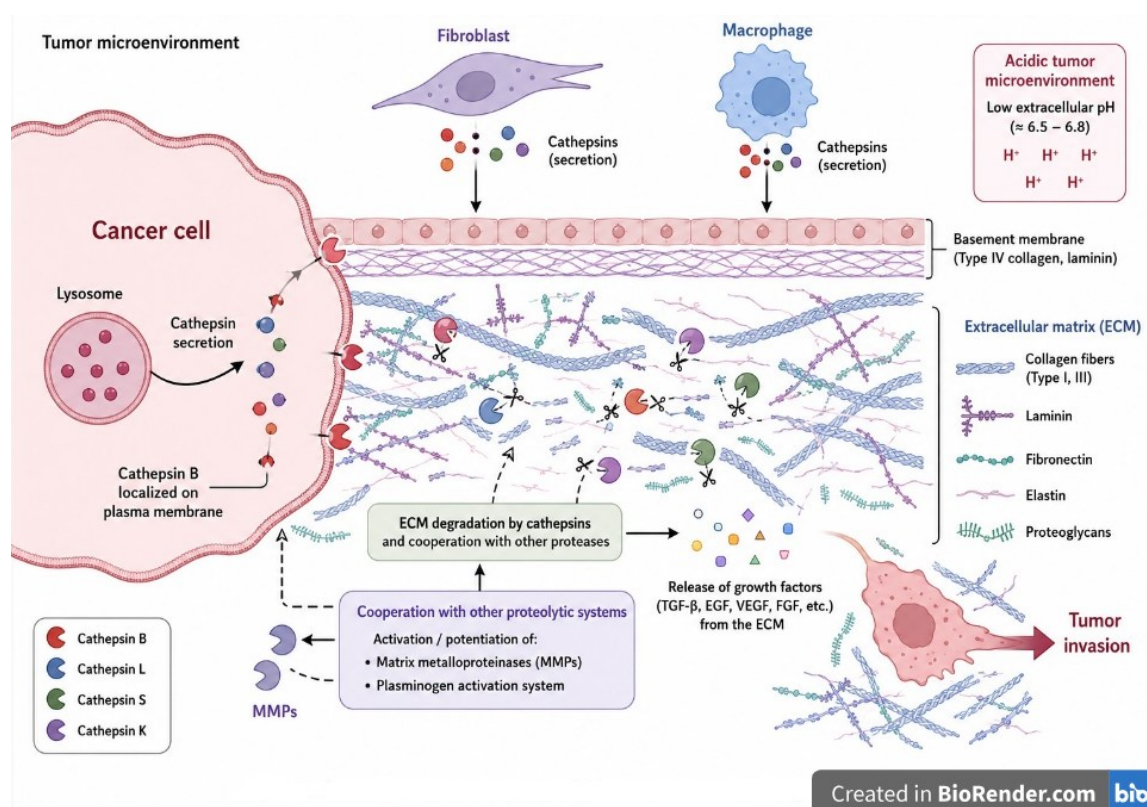


Figure 10. Cathepsin-mediated degradation of the extracellular matrix during tumor invasion

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Cathepsins secreted by tumor cells and stromal cells degrade major components of the extracellular matrix and basement membrane while cooperating with other proteolytic systems. This remodeling promotes the release of matrix-bound growth factors and facilitates tumor cell invasion.

The principal mechanisms by which cathepsins contribute to extracellular matrix (ECM) degradation are summarized in Table 5.

Table 5. Role of cathepsins in extracellular matrix degradation

Cathepsin	Major substrates / targets	Biological effect
Cathepsin B	Type IV collagen, laminin, fibronectin, basement membrane components	Degradation of the basement membrane, promotion of tumor invasion, establishment of pericellular proteolysis
Cathepsin L	Structural and nonstructural extracellular matrix proteins	Extracellular matrix remodeling, promotion of tumor cell migration and invasiveness

Cathepsin S	Extracellular matrix components, regulatory proteins of the tumor microenvironment	Extracellular matrix remodeling, regulation of intercellular signaling and tumor cell–microenvironment interactions
Cathepsin K	Type I collagen and other collagenous structures	Extensive collagen degradation, remodeling of the tissue microenvironment, facilitation of tumor invasion
Cysteine cathepsins (overall)	Extracellular matrix proteins, growth factors, cell adhesion proteins	Extracellular matrix degradation, release of matrix-sequestered growth factors, modulation of cell adhesion, promotion of tumor invasion and metastasis

Source: Compiled by the author based on data from references (30, 39, 47, 77–80).

Current evidence indicates that degradation of the extracellular matrix is not an isolated biochemical event but rather a highly coordinated process integrating structural remodeling with activation of signaling pathways that drive malignant progression. Proteolytic cleavage of extracellular matrix proteins removes the physical barriers limiting tumor expansion while simultaneously altering the biochemical composition of the surrounding microenvironment. These changes promote the release of matrix-bound growth factors, modify adhesive interactions between cells, and create conditions that facilitate continuous migration and invasion of malignant cells (30, 39, 47).

Among all members of the cathepsin family, cathepsin B remains the most extensively investigated enzyme with respect to extracellular matrix degradation. Experimental studies consistently demonstrate that this protease efficiently hydrolyzes major basement membrane constituents, accumulates at the surface of malignant cells, and establishes localized regions of intense pericellular proteolysis that facilitate penetration of tumor cells into adjacent tissues (77, 78). The strong association between elevated cathepsin B activity and aggressive clinical behavior observed in several malignancies further supports its central role in regulating local invasion and metastatic dissemination (79).

Nevertheless, extracellular matrix remodeling cannot be attributed to cathepsin B alone. Cathepsins L, S, and K also make substantial contributions by degrading structural matrix proteins, processing regulatory extracellular molecules, and modifying the biological properties of the tumor microenvironment (30). Acting together, these proteases generate a coordinated proteolytic network that continuously reshapes tumor tissue and enhances communication between malignant cells and their surrounding stromal compartment.

An additional consequence of cathepsin-dependent proteolysis is the regulation of signaling molecules embedded within the extracellular matrix. Degradation of matrix components promotes the liberation of numerous growth factors, cytokines, and chemokines that subsequently activate intracellular signaling pathways responsible for proliferation, migration, survival, and angiogenesis. Consequently, extracellular matrix remodeling functions not only as a mechanism of tissue destruction but also as an important regulator of molecular signaling within the tumor microenvironment (39).

The biological effects of cathepsins are further amplified through functional cooperation with other protease systems. Interactions with matrix metalloproteinases and components of the plasminogen activation system generate sequential proteolytic cascades that progressively increase degradation of extracellular tissues. Such cooperation greatly expands the extent of matrix remodeling and accelerates local tumor invasion, emphasizing that malignant progression results from the integrated activity of multiple proteolytic enzymes rather than from the action of a single protease (47).

Collectively, the available experimental and clinical evidence demonstrates that extracellular matrix degradation represents one of the fundamental mechanisms through which cathepsins promote both local tumor invasion and metastatic spread. Although cathepsin B currently provides the strongest experimental evidence because of its ability to degrade basement membrane components, accumulate at the tumor cell surface, generate zones of intense pericellular proteolysis, and promote an aggressive invasive phenotype, other cysteine cathepsins likewise contribute substantially to this process. Through coordinated remodeling of extracellular tissues, regulation of growth factor availability, and maintenance of a proteolytically active tumor microenvironment, these enzymes collectively establish the biological conditions required for malignant progression. Consequently, cathepsin-mediated extracellular matrix degradation should be viewed not merely as a destructive process but as a complex regulatory mechanism linking structural reorganization of the tumor microenvironment with activation of signaling pathways that govern invasion and metastasis.

5.2 Epithelial–Mesenchymal Transition (EMT)

Epithelial-to-mesenchymal transition (EMT) is a highly coordinated biological program through which epithelial cells lose their characteristic structural organization and acquire features typical of mesenchymal cells. Although this process is indispensable during embryogenesis, organ formation, wound repair, and physiological tissue remodeling, its reactivation in malignant tumors has profound pathological consequences. By enabling cancer

cells to detach from the primary tumor, migrate through surrounding tissues, and ultimately establish metastatic lesions, EMT represents one of the fundamental mechanisms driving cancer progression. R. Kalluri and R. A. Weinberg (81) emphasized that the remarkable cellular plasticity generated during EMT allows malignant cells to adapt efficiently to continuous changes within the tumor microenvironment.

The transition from an epithelial to a mesenchymal phenotype is accompanied by extensive molecular reprogramming. Cells undergoing EMT progressively lose epithelial junction proteins, including E-cadherin, occludin, and claudins, while simultaneously increasing the expression of mesenchymal markers such as N-cadherin, vimentin, and fibronectin. These coordinated alterations are controlled by several transcription factors, including Snail, Slug, Twist, ZEB1, and ZEB2, which regulate cytoskeletal organization, weaken cell–cell adhesion, and enhance the migratory and invasive properties of tumor cells (81, 82).

Increasing experimental evidence indicates that cathepsins actively contribute to EMT through multiple complementary mechanisms. Their involvement extends far beyond lysosomal protein degradation because, in malignant tissues, many cathepsins are secreted into the extracellular compartment, where they remodel the extracellular matrix, regulate the availability of signaling molecules, and modify communication between tumor cells and their surrounding microenvironment. M. M. Mohamed and B. F. Sloane (39) proposed that these proteolytic alterations create conditions that strongly favor activation of EMT-associated signaling pathways and thereby facilitate invasive tumor growth.

Among the members of the cathepsin family, cathepsin B has demonstrated the closest association with acquisition of the mesenchymal phenotype. Z. Yan et al. (65) summarized evidence indicating that increased cathepsin B expression in malignant tumors correlates with suppression of E-cadherin together with elevated expression of N-cadherin, vimentin, and additional molecular markers characteristic of EMT. Moreover, several experimental studies showed that inhibition of cathepsin B partially restores epithelial characteristics while significantly reducing the migratory potential of tumor cells, further supporting its important contribution to EMT-associated malignant progression.

An additional mechanism through which cathepsins facilitate epithelial-to-mesenchymal transition involves regulation of growth factor availability within the extracellular matrix. Proteolytic remodeling of matrix components promotes the release of signaling molecules that are normally retained in an inactive or matrix-bound state, including TGF- β , EGF, FGF, and other potent inducers of EMT. As these factors become available to

neighboring cells, they activate transcriptional programs responsible for mesenchymal differentiation and increased cellular plasticity. Consequently, cathepsins function as indirect regulators of EMT by continuously reshaping the biochemical environment surrounding tumor cells (30, 39).

The surrounding tumor stroma likewise plays an essential role in sustaining epithelial-to-mesenchymal transition. L. Sevenich and J. A. Joyce (47) demonstrated that cathepsins released by tumor-associated macrophages, fibroblasts, and additional stromal cell populations actively participate in remodeling extracellular tissues and regulating communication between malignant and nonmalignant cells. Through these coordinated interactions, the tumor microenvironment becomes increasingly permissive for EMT, indicating that this process is driven not only by intracellular signaling within cancer cells but also by reciprocal communication between tumor cells and their surrounding stromal niche.

The principal mechanisms through which cathepsins contribute to epithelial-to-mesenchymal transition are summarized in Table 6.

Table 6. Major mechanisms by which cathepsins contribute to epithelial-to-mesenchymal transition (EMT)

Cathepsin	Principal mechanism	Biological effect
Cathepsin B	Extracellular matrix (ECM) remodeling and activation of growth factors	Downregulation of E-cadherin, promotion of epithelial-to-mesenchymal transition (EMT), and enhanced tumor invasion
Cathepsin L	Regulation of signaling pathways and interactions with the extracellular matrix	Increased cell migration and enhanced expression of mesenchymal markers
Cathepsin S	Remodeling of the tumor microenvironment	Maintenance of the invasive tumor phenotype
Cysteine cathepsins (overall)	Release of TGF- β and other growth factors, extracellular matrix remodeling	Induction of EMT and enhancement of tumor cell plasticity

Source: Compiled by the author based on data from references (30, 39, 47, 65, 81, 82).

Overall, epithelial-to-mesenchymal transition represents one of the pivotal biological processes through which malignant cells acquire invasive and metastatic capabilities. Current evidence indicates that cathepsins promote EMT predominantly by remodeling the extracellular matrix, increasing the availability of EMT-inducing growth factors, and reshaping the tumor microenvironment. Among the individual family members, cathepsins B and L have demonstrated the strongest experimental association with loss of epithelial characteristics,

acquisition of mesenchymal features, and enhancement of tumor cell invasiveness. These observations identify cathepsin-mediated regulation of EMT as an important molecular mechanism linking extracellular matrix remodeling with subsequent stages of tumor dissemination and metastatic progression.

5.3 Tumor Cell Migration and Invasion

The acquisition of migratory and invasive properties is one of the defining events in malignant tumor progression, enabling cancer cells to disseminate beyond the primary lesion and ultimately establish metastatic colonies in distant organs. Although extracellular matrix degradation provides the structural conditions necessary for invasion, successful dissemination additionally requires profound changes in cellular architecture, adhesion dynamics, and motility. These events are orchestrated through coordinated remodeling of the actin cytoskeleton, modulation of cell–cell and cell–matrix interactions, activation of multiple intracellular signaling pathways, and continuous restructuring of the surrounding extracellular matrix. A growing body of evidence indicates that cathepsins participate in each of these biological processes, thereby functioning as central regulators of tumor cell invasion and migration (55, 83).

Under physiological conditions, cathepsins are predominantly confined to lysosomes, where they participate in intracellular protein turnover and maintenance of cellular homeostasis. During malignant transformation, however, their intracellular trafficking undergoes profound alterations. Instead of remaining restricted to endolysosomal compartments, cathepsins are frequently redistributed to the plasma membrane or secreted into the extracellular environment. This abnormal localization allows proteolytic activity to become concentrated immediately adjacent to the leading edge of migrating tumor cells, creating focal regions of extracellular matrix degradation that facilitate tissue infiltration. Consequently, extracellular accumulation of cathepsins has become recognized as a characteristic molecular feature of highly invasive malignancies.

Among the various members of this protease family, cathepsin B has been most consistently associated with the invasive phenotype of cancer cells. Experimental investigations have demonstrated that this enzyme preferentially accumulates within invadopodia, specialized actin-based membrane protrusions responsible for highly localized proteolysis of the basement membrane and extracellular matrix. Functional studies further revealed that either pharmacological inhibition or genetic suppression of cathepsin B markedly diminishes the capacity of tumor cells to penetrate extracellular matrix barriers, confirming that its activity is

directly required for efficient invasion (84). Comparable observations were reported by B. C. Victor et al. (78), who demonstrated that inhibition of cathepsin B in inflammatory breast cancer cells substantially reduced matrix degradation and significantly impaired their invasive behavior.

The contribution of cathepsin L extends beyond extracellular proteolysis and includes the regulation of cellular motility itself. According to Y. Yang and J. L. Cox (85), elevated expression of cathepsin L in melanoma is strongly correlated with increased migratory and invasive potential, whereas suppression of this enzyme significantly limits the ability of malignant cells to move through surrounding tissues. Importantly, these effects cannot be explained solely by enhanced matrix degradation. The authors demonstrated that cathepsin L also influences the organization of the actin cytoskeleton and regulates cell adhesion dynamics, two interconnected processes that determine directional migration and the mechanical plasticity required for tumor cell movement.

Another member of the cathepsin family that has attracted increasing attention is cathepsin S. In contrast to most cysteine cathepsins, whose activity declines rapidly at neutral pH, cathepsin S preserves substantial catalytic activity under both mildly acidic and nearly physiological extracellular conditions. This biochemical characteristic allows the enzyme to remain functionally active throughout the tumor microenvironment. As reported by S. H. McDowell et al. (83), cathepsin S contributes to degradation of extracellular matrix proteins, regulates interactions between neighboring cells, and supports sustained migratory activity of malignant cells. Moreover, its biological functions are not restricted to tumor cells, as cathepsin S also modulates the behavior of stromal fibroblasts and immune cell populations, thereby indirectly promoting tumor invasion.

The importance of cathepsins during malignant progression is further emphasized by their involvement in communication between tumor cells and the surrounding microenvironment. Experimental studies performed by O. Vasiljeva et al. (48) demonstrated that cathepsin B derived from both malignant epithelial cells and tumor-associated macrophages significantly accelerates tumor progression while facilitating the development of pulmonary metastases in breast cancer models. These observations indicate that the invasive capacity of tumors depends not only on protease production by cancer cells themselves but also on the coordinated contribution of stromal and immune components of the tumor microenvironment.

Cathepsins additionally enhance tumor invasion through extensive functional interactions with other extracellular protease systems. Rather than acting independently, these

enzymes cooperate with matrix metalloproteinases and elements of the plasminogen activation cascade, generating an interconnected proteolytic network capable of efficiently dismantling extracellular matrix structures and basement membranes. Such coordinated enzymatic activity markedly facilitates the movement of malignant cells through surrounding tissues and substantially increases the probability of metastatic dissemination (86).

The principal molecular mechanisms through which cathepsins regulate tumor cell migration and invasion are summarized schematically in Figure 11.

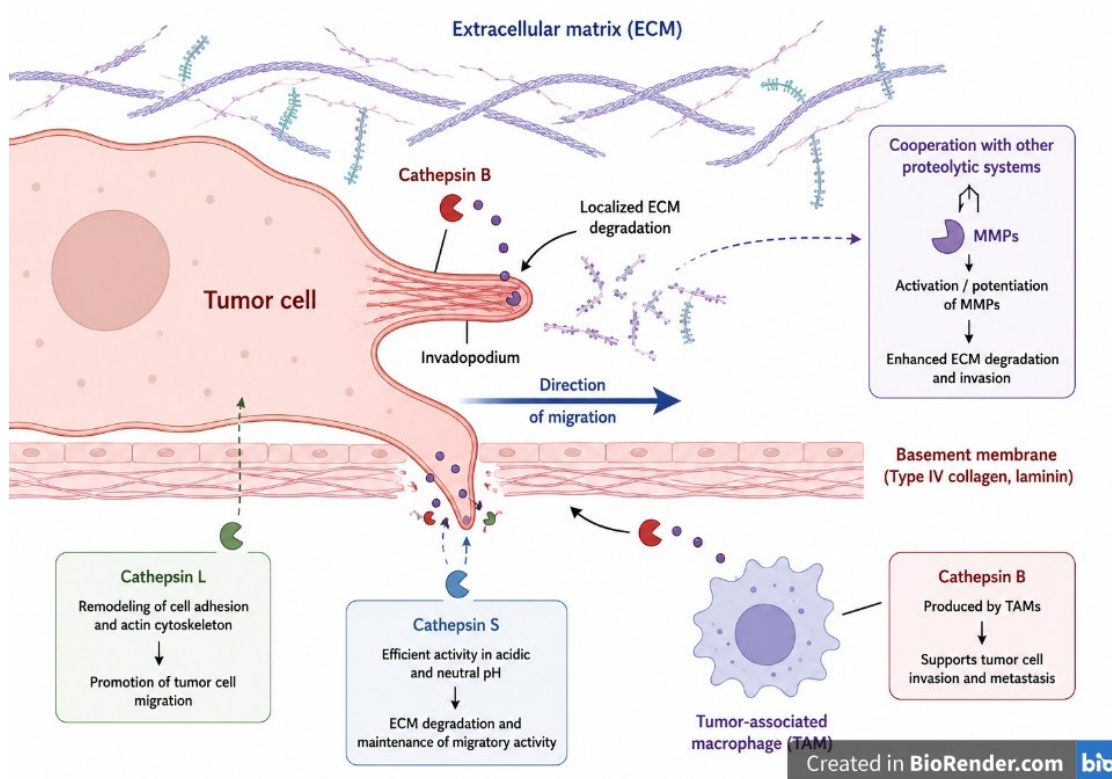


Figure 11. Principal mechanisms of cathepsin involvement in tumor cell migration and invasion

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Cathepsins B, L, and S facilitate tumor cell migration and invasion through localized extracellular matrix degradation, remodeling of cell adhesion, and cooperation with other proteolytic systems. Their coordinated activity promotes directional cell movement, basement membrane penetration, and metastatic dissemination.

Taken together, currently available experimental evidence consistently supports the conclusion that cathepsins function as essential molecular regulators of tumor cell migration and invasion. Among them, cathepsins B, L, and S have been investigated most extensively and have been shown to coordinate multiple stages of the invasive process, including localized

extracellular matrix proteolysis, remodeling of adhesive contacts, formation of invadopodia, and reciprocal interactions between malignant cells and the tumor microenvironment. Through the integration of these complementary mechanisms, cathepsins create a biological environment that favors local tissue infiltration and metastatic dissemination. Their multifaceted contribution to cancer progression therefore supports continued investigation of these proteases as promising molecular targets for the development of next-generation anticancer therapies.

5.4 Formation of Metastatic Niches

The establishment of metastatic niches represents a fundamental event in cancer dissemination because successful metastasis depends not only on the escape of malignant cells from the primary tumor but also on their ability to adapt to and colonize distant tissues. Contemporary concepts of metastasis emphasize that secondary organs are actively conditioned before the arrival of circulating tumor cells. This phenomenon, referred to as pre-metastatic niche formation, involves the release of tumor-derived soluble mediators, extracellular vesicles, cytokines, and growth factors that recruit bone marrow-derived cells, modify immune cell populations, and induce extensive remodeling of the extracellular matrix within target organs (87, 88). These alterations collectively generate a permissive microenvironment that facilitates the attachment, survival, and subsequent expansion of disseminated tumor cells.

Cathepsins have emerged as important mediators of metastatic niche formation because they regulate several biological processes required for the preparation of distant tissues for metastatic colonization. Their functions include degradation and reorganization of extracellular matrix components, modulation of tissue permeability, regulation of stromal and immune cell activity, and maintenance of organ-specific microenvironments that support metastatic growth. Moreover, individual members of the cathepsin family have been associated with preferential metastatic localization. Current evidence links cathepsin B with pulmonary dissemination of mammary tumors, cathepsin S with brain metastasis, cathepsin L with enhanced systemic metastatic competence, and cathepsin K with metastatic lesions in bone tissue (48, 89).

One of the strongest experimental demonstrations of cathepsin involvement in metastatic niche formation was provided by O. Vasiljeva et al. (48). Using a murine model of breast cancer, the authors showed that cathepsin B produced by both malignant cells and tumor-associated macrophages significantly accelerates primary tumor progression while simultaneously promoting the establishment of pulmonary metastases. Importantly, the study revealed that neither tumor-derived nor stromal-derived cathepsin B alone fully accounted for metastatic progression; instead, effective metastatic colonization depended on the combined

contribution of both cellular sources. These findings highlight the cooperative interactions between cancer cells and the surrounding microenvironment during metastatic niche development.

Among all cathepsins, cathepsin S provides one of the clearest examples of organ-specific regulation of metastasis. L. Sevenich et al. (90) demonstrated that elevated expression of cathepsin S in primary breast tumors correlates with a shorter period before the appearance of brain metastases. Mechanistic investigations revealed that cathepsin S, secreted by both tumor cells and macrophages, promotes the passage of malignant cells through the blood–brain barrier by increasing its permeability. Furthermore, inhibition of cathepsin S in only one cellular compartment produced limited effects, whereas simultaneous suppression of the enzyme in both tumor cells and stromal cells markedly reduced brain metastatic burden. These observations indicate that cathepsin S is a critical regulator of the specialized microenvironment required for cerebral metastasis.

Cathepsin L has likewise been implicated in metastatic progression, although its biological effects appear to extend beyond direct degradation of extracellular matrix proteins. According to S. S. Dykes et al. (89), secretion of cathepsin L by both malignant cells and host tissues significantly enhances metastatic dissemination in experimental tumor models. The authors further demonstrated that inhibition of cathepsins L and K decreases the invasive activity of M2-polarized macrophages and suppresses metastatic progression. These findings suggest that cathepsin L contributes to metastasis through dual mechanisms, exerting direct effects on tumor cells while simultaneously modifying the functional phenotype of stromal and immune cells that participate in metastatic niche formation.

A distinct role within the metastatic cascade is performed by cathepsin K, whose biological activity is closely associated with osteoclast-mediated bone remodeling. Owing to its potent collagenolytic properties, cathepsin K plays a particularly important role in skeletal metastasis. I. Podgorski et al. (91) demonstrated that cathepsin K produced by bone marrow-derived cells cleaves SPARC, a matricellular protein involved in regulating bone remodeling and metastatic colonization of the skeleton. Additional experimental studies have shown that pharmacological inhibition of cathepsin K significantly attenuates osteolytic destruction accompanying bone metastases in breast and prostate cancer models (92). These findings identify cathepsin K as one of the principal molecular mediators responsible for establishing and maintaining the bone metastatic niche.

Collectively, current evidence indicates that metastatic niche formation involves a coordinated sequence of molecular and cellular events in which cathepsins function at multiple

stages. Initially, the primary tumor induces systemic changes that alter the biological properties of distant organs. These alterations stimulate stromal and immune cells to increase protease production, including the secretion of multiple cathepsins. Subsequent remodeling of the extracellular matrix and modification of tissue barriers create favorable conditions for circulating tumor cells to adhere, extravasate, and colonize target tissues. Once established within the newly conditioned microenvironment, disseminated tumor cells acquire enhanced capacity for long-term survival, immune evasion, and progressive outgrowth into clinically detectable metastatic lesions. Accordingly, cathepsins should be considered not merely degradative enzymes but essential regulators of the organ-specific microenvironments that determine metastatic success.

The principal mechanisms through which cathepsins contribute to metastatic niche formation are summarized schematically in Figure 12.

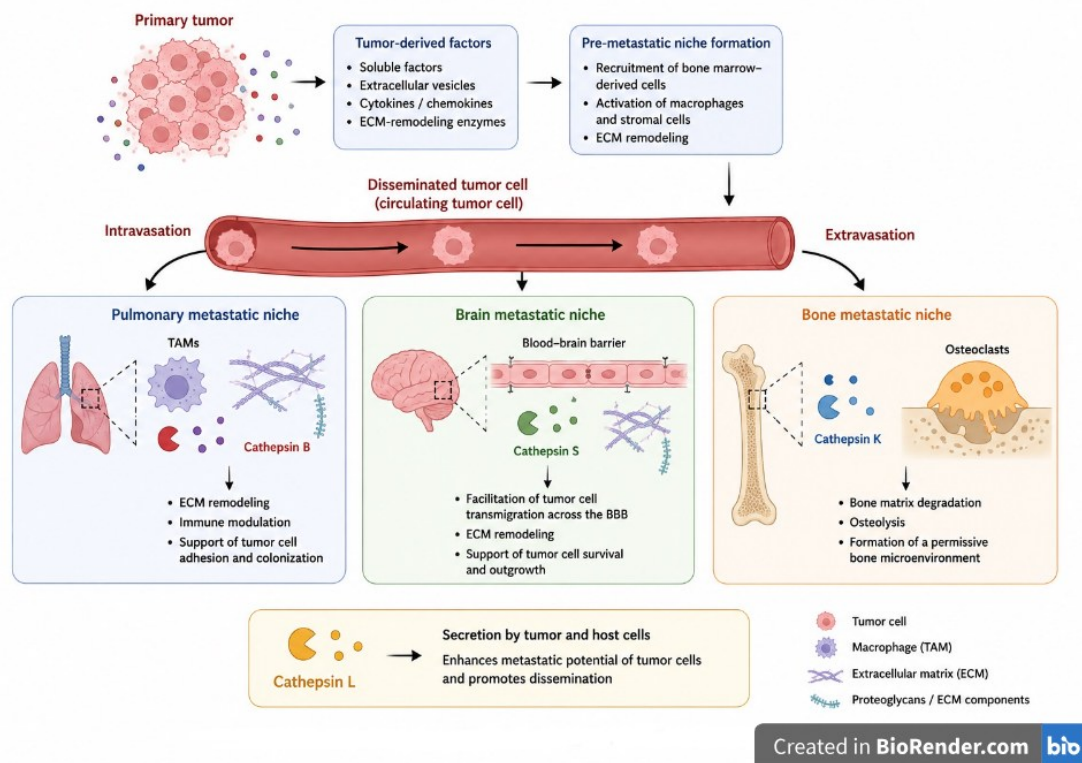


Figure 12. Role of cathepsins in the formation of metastatic niches

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Tumor- and host-derived cathepsins contribute to the formation of pre-metastatic niches by remodeling the extracellular matrix, regulating stromal and immune cell activity, and facilitating colonization of distant organs. Individual cathepsins exhibit organ-specific functions, supporting metastatic progression in the lungs, brain, and bone.

The involvement of individual cathepsins in the formation of metastatic niches is summarized in Table 7.

Table 7. Role of individual cathepsins in the formation of metastatic niches

Cathepsin	Metastatic context	Primary validated effect
Cathepsin B	Pulmonary metastasis of mammary tumors	Promotes tumor progression and pulmonary metastasis; both tumor cell-derived and macrophage-derived cathepsin B contribute to metastatic development
Cathepsin S	Brain metastasis of breast cancer	Facilitates tumor cell transmigration through the blood–brain barrier and is associated with reduced brain metastasis-free survival
Cathepsin L	Metastatic dissemination of malignant tumors	Secretion by both tumor cells and host cells enhances metastatic competence and supports metastatic progression
Cathepsin K	Bone metastasis	Regulates remodeling of the bone microenvironment, promotes osteolysis, and processes proteins involved in skeletal metastatic niche formation

Source: Compiled by the author based on data from references (48, 89–92).

Taken together, available experimental and preclinical studies demonstrate that cathepsins play indispensable roles throughout the establishment of metastatic niches. Their biological activities extend far beyond extracellular matrix degradation and include regulation of tissue permeability, modulation of stromal and immune cell function, and maintenance of organ-specific microenvironments that support metastatic colonization. The most convincing evidence currently supports the involvement of cathepsin B in pulmonary metastasis, cathepsin S in brain metastasis, cathepsin L in enhancing the overall metastatic phenotype, and cathepsin K in skeletal metastasis. Collectively, these findings indicate that cathepsins participate not only in the initial stages of local tumor invasion but also in the subsequent processes governing successful metastatic colonization, highlighting their considerable potential as therapeutic targets for limiting metastatic disease.

6. Therapeutic Potential of Targeting Cathepsins

6.1 Cathepsin Inhibitors

The development of pharmacological agents capable of selectively suppressing cathepsin activity has become one of the major priorities in contemporary molecular oncology. This interest stems from the extensive involvement of cathepsins in virtually every stage of tumor development, including uncontrolled cell proliferation, extracellular matrix degradation, angiogenesis, invasion, metastatic dissemination, and the acquisition of resistance to anticancer therapy. However, because these proteases also perform indispensable physiological functions in protein turnover, immune regulation, tissue remodeling, and cellular homeostasis, successful therapeutic intervention requires inhibitors that preferentially block pathological rather than physiological enzymatic activity. Consequently, the design of highly selective cathepsin inhibitors has become a central objective in the development of targeted anticancer therapies (93, 94).

Considerable progress has been achieved in the identification of compounds capable of interfering with cathepsin function through different molecular mechanisms. Current pharmacological approaches include competitive low-molecular-weight inhibitors, reversible and irreversible covalent inhibitors, naturally occurring inhibitory molecules, and highly selective agents directed against individual members of the cathepsin family. Although these compounds differ in their chemical structures and binding properties, their therapeutic effect ultimately relies on suppression of catalytic activity through interaction with the enzyme active site or stabilization of inactive conformations. In the case of cysteine cathepsins, inhibitor design primarily targets the catalytic cysteine residue responsible for proteolytic activity, whereas inhibition of the aspartic protease cathepsin D requires structurally distinct molecules capable of interacting with its catalytic aspartate residues (93, 94).

The first generation of experimental cathepsin inhibitors consisted largely of broad-spectrum compounds with limited selectivity. One of the earliest and most widely investigated representatives of this group is E-64, an epoxysuccinyl derivative that irreversibly inactivates numerous cysteine cathepsins through covalent modification of their catalytic site. Owing to its potent inhibitory activity, E-64 became an important experimental tool for elucidating the biological functions of lysosomal proteases. Nevertheless, its poor pharmacokinetic characteristics together with insufficient selectivity prevented successful clinical translation. Comparable limitations were observed for other early pan-cathepsin inhibitors, including JPM-

OEt, which demonstrated substantial antitumor activity in preclinical studies but lacked the specificity required for safe therapeutic application (91, 92).

Advances in structural biology and medicinal chemistry subsequently shifted research efforts toward the development of inhibitors exhibiting high specificity for individual cathepsins. Among these, cathepsin K has attracted particular attention because of its critical role in collagen degradation during bone resorption. Odanacatib (MK-0822) represents the best-characterized selective inhibitor within this category. This reversible small-molecule inhibitor demonstrated strong affinity for cathepsin K while exhibiting minimal activity against related cathepsins such as B, L, and S. Clinical investigations confirmed its pronounced ability to suppress bone resorption, reflecting the therapeutic value of selective enzyme inhibition. However, despite these encouraging pharmacological properties, clinical development was ultimately terminated after an increased incidence of adverse cardiovascular events was identified, illustrating the complexity of translating selective cathepsin inhibition into safe clinical practice (95).

In parallel with the development of highly selective inhibitors, considerable attention has been devoted to compounds capable of simultaneously targeting several cathepsins involved in tumor progression. One of the most extensively investigated examples is VBY-825, a reversible inhibitor with activity against cathepsins B, L, S, and V. Preclinical studies demonstrated that administration of VBY-825 suppresses tumor growth, reduces angiogenesis, limits extracellular matrix degradation, and inhibits tumor cell invasion and proliferation. Importantly, the biological effects of this compound extend beyond malignant cells themselves, influencing macrophages and other stromal components of the tumor microenvironment that actively express cathepsins. These findings suggest that simultaneous inhibition of multiple proteases may provide broader therapeutic benefits by disrupting reciprocal interactions between tumor cells and their surrounding microenvironment (96).

Despite these promising advances, increasing experimental evidence indicates that indiscriminate suppression of the entire cathepsin family may produce undesirable biological consequences. Individual cathepsins participate in numerous physiological processes, including autophagic protein degradation, antigen processing and presentation, tissue repair, and maintenance of immune function. Consequently, complete inhibition of multiple family members may interfere with normal cellular physiology and increase the risk of adverse effects. Reflecting these observations, current strategies in anticancer drug development have shifted away from non-selective pan-cathepsin inhibition toward precision approaches based on

selective targeting of individual cathepsins whose expression or activity is specifically associated with particular malignancies or defined stages of tumor progression.

The principal mechanisms through which cathepsin inhibitors exert their biological effects are summarized schematically in Figure 13.

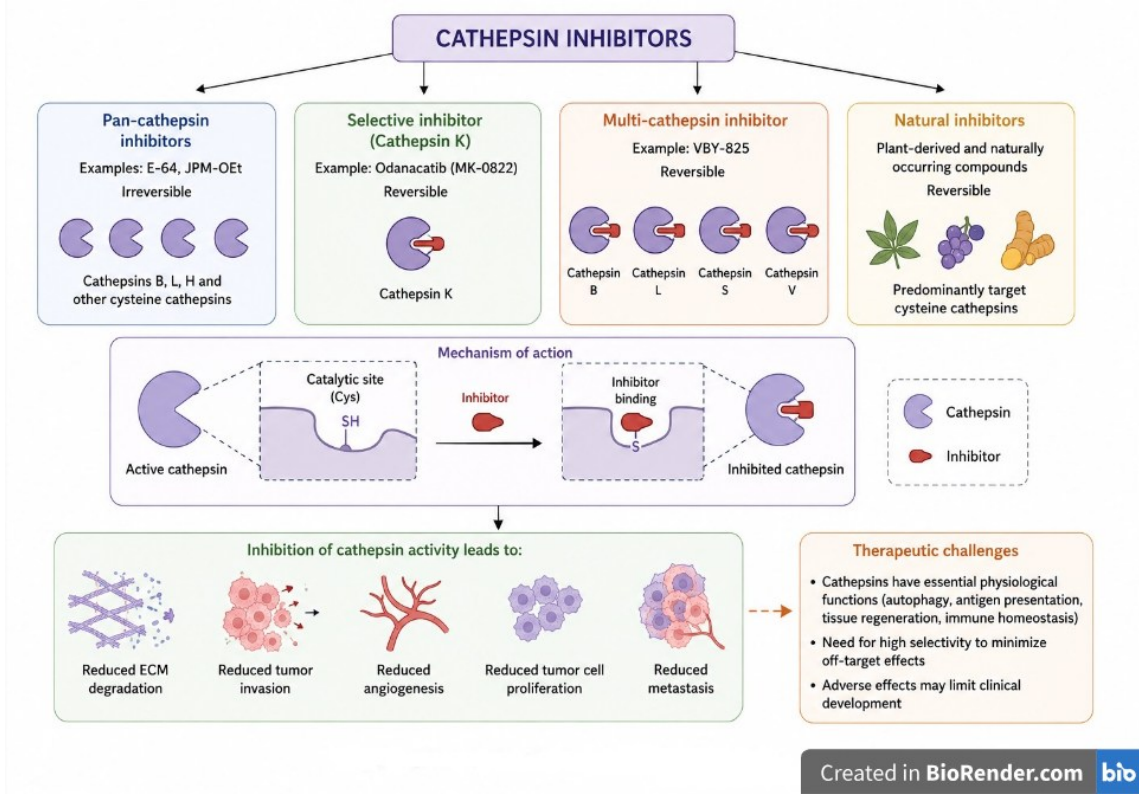


Figure 13. Principal mechanisms of cathepsin inhibition

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The figure presents the main classes of cathepsin inhibitors, their mechanisms of action, and the biological consequences of cathepsin inhibition. It also highlights the therapeutic challenges associated with achieving selective inhibition while preserving the physiological functions of cathepsins.

The principal cathepsin inhibitors and their current stage of development are summarized in Table 8.

Table 8. Principal cathepsin inhibitors and their current status of research

Inhibitor	Primary cathepsin target(s)	Characteristics	Stage of development
E-64	Cathepsins B, L, H, and other cysteine cathepsins	Irreversible broad-spectrum inhibitor used primarily as an experimental research tool	Preclinical studies

JPM-OEt	Cysteine cathepsins	Irreversible experimental inhibitor with broad inhibitory activity	Preclinical studies
Odanacatib (MK-0822)	Cathepsin K	Highly selective reversible inhibitor of cathepsin K	Clinical development discontinued
VBY-825	Cathepsins B, L, S, and V	Reversible multi-cathepsin inhibitor exhibiting antitumor activity	Preclinical studies
Natural inhibitors	Predominantly cysteine cathepsins	Naturally occurring and plant-derived compounds with inhibitory activity toward cathepsins	Preclinical studies

Source: Compiled by the author based on data from references (93–96).

Overall, available experimental and clinical evidence supports the view that cathepsin inhibitors constitute an important and rapidly evolving class of targeted anticancer agents. Significant progress has been achieved through the development of highly selective inhibitors directed against individual cathepsins, as well as multi-target compounds capable of simultaneously modulating tumor cells and the surrounding microenvironment. Nevertheless, clinical experience has also demonstrated that the extensive physiological functions performed by cathepsins require an exceptionally high level of therapeutic precision. For this reason, the future development of cathepsin-directed therapies is expected to focus increasingly on selective molecular targeting strategies that maximize antitumor efficacy while minimizing disruption of normal biological processes.

6.2 Cathepsins as Biomarkers in Oncology

The identification of reliable molecular biomarkers capable of improving cancer diagnosis and patient management remains a major objective of modern oncology. Among the numerous biomolecules currently under investigation, cathepsins have attracted considerable interest because alterations in their expression and enzymatic activity occur early during malignant transformation and become progressively more pronounced as tumors advance. In contrast to many conventional tissue biomarkers, members of the cathepsin family can be detected not only within tumor specimens but also in circulating biological fluids, including plasma, serum, urine, and other body fluids. This characteristic substantially broadens their potential clinical applicability by enabling the development of minimally invasive diagnostic and monitoring strategies (3, 40).

The clinical value of biomarkers is generally classified according to their diagnostic, prognostic, or predictive significance. Diagnostic biomarkers facilitate the identification of malignant disease, prognostic biomarkers provide information regarding the expected clinical outcome independently of treatment, whereas predictive biomarkers estimate the likelihood of response to a particular therapeutic intervention. Current evidence suggests that cathepsins possess the greatest potential as prognostic biomarkers. Numerous clinical investigations have demonstrated significant associations between cathepsin expression and tumor stage, histological grade, invasive behavior, metastatic dissemination, and long-term patient survival, indicating that these proteases reflect the biological aggressiveness of malignant neoplasms.

Among all family members, cathepsin B has been investigated most extensively in the clinical setting. Increased expression of this protease has been documented in a broad spectrum of human malignancies, including colorectal carcinoma, breast cancer, hepatocellular carcinoma, glioblastoma, melanoma, and non-small cell lung cancer. According to M. M. Mohamed and B. F. Sloane (39), elevated cathepsin B expression consistently correlates with enhanced tumor aggressiveness, increased invasive capacity, and unfavorable clinical outcome. Similar conclusions were reported by N. Aggarwal and B. F. Sloane (41), who proposed that cathepsin B expression may serve as an independent prognostic indicator across multiple types of solid tumors.

Another clinically relevant member of the family is cathepsin D. Unlike cysteine cathepsins, cathepsin D belongs to the aspartic protease family and is actively secreted by numerous epithelial malignancies. The strongest evidence supporting its biomarker potential has been obtained in breast cancer, where increased expression is consistently associated with reduced disease-free survival, decreased overall survival, and a higher probability of disease recurrence. Owing to these observations, cathepsin D became one of the earliest cathepsins to be recognized as a clinically relevant prognostic biomarker in oncology (97, 98).

Interest has also grown substantially in cathepsin S because of its unique biochemical characteristics. Unlike most cysteine cathepsins, this enzyme retains considerable catalytic activity under nearly physiological extracellular pH conditions, allowing sustained proteolytic function within the tumor microenvironment. L. Sevenich et al. (90) demonstrated that elevated cathepsin S expression in primary breast tumors is strongly associated with an increased risk of subsequent brain metastasis. These findings suggest that cathepsin S may provide clinically valuable information not only regarding disease prognosis but also concerning the probability of organ-specific metastatic dissemination, thereby extending its potential role beyond that of a conventional prognostic biomarker.

Recent advances in molecular diagnostics have shifted attention from single-marker analysis toward integrated biomarker signatures. Rather than evaluating individual cathepsins in isolation, current research increasingly supports simultaneous assessment of several members of the cathepsin family together with complementary molecular markers. As emphasized by T. Yadati et al. (3), combined analysis of cathepsins B, L, S, K, and D offers a more comprehensive characterization of tumor biology, providing improved assessment of invasive potential, metastatic competence, and the functional state of the tumor microenvironment compared with measurement of a single enzyme alone.

An additional advantage of cathepsins lies in their compatibility with emerging molecular imaging technologies. Unlike conventional biomarker assays that primarily quantify protein abundance, fluorogenic activity-based probes specifically detect enzymatically active cathepsins within living tissues. Preclinical studies have demonstrated that these imaging approaches enable real-time visualization of proteolytic activity in vivo, allowing simultaneous assessment of enzyme localization and function. Such technologies may substantially improve early tumor detection while also providing valuable tools for monitoring therapeutic response and evaluating treatment efficacy during the course of anticancer therapy (99).

The current clinical significance of the principal cathepsins investigated as oncological biomarkers is summarized in Table 9.

Table 9. Cathepsins as potential biomarkers of malignant neoplasms

Cathepsin	Major tumor types	Potential clinical significance
Cathepsin B	Colorectal cancer, breast cancer, glioblastoma, melanoma, non-small cell lung cancer (NSCLC)	Prognostic biomarker associated with tumor invasiveness, metastatic potential, and poor overall survival
Cathepsin D	Breast cancer, ovarian cancer, and other epithelial malignancies	Prognostic biomarker of disease progression, recurrence risk, and unfavorable clinical outcome
Cathepsin S	Breast cancer, colorectal cancer, and other solid tumors	Biomarker of aggressive tumor behavior and increased metastatic risk, particularly for brain metastasis
Cathepsin L	Glioblastoma, pancreatic cancer, and lung cancer	Potential prognostic biomarker reflecting tumor invasiveness and proliferative activity
Cathepsin K	Tumors with bone metastases	Biomarker associated with bone remodeling and osteolytic metastatic disease

Source: Compiled by the author based on data from references (3, 39, 41, 90, 97).

Overall, accumulated clinical evidence indicates that cathepsins possess substantial potential as biomarkers for precision oncology. The most convincing data currently support the clinical relevance of cathepsins B, D, and S, whose expression levels consistently correlate with tumor aggressiveness, metastatic capacity, disease progression, and patient survival. Nevertheless, growing evidence suggests that the highest diagnostic and prognostic accuracy is achieved through integrated biomarker panels that combine multiple cathepsins with other molecular indicators and advanced imaging technologies. Such multimodal approaches are expected to improve individualized risk stratification, facilitate more accurate prediction of disease outcome, and support the selection of personalized therapeutic strategies for patients with malignant neoplasms.

6.3 Current Approaches to Targeted Therapy

The emergence of precision oncology has substantially transformed therapeutic concepts aimed at targeting cathepsins in cancer. Early investigations were focused predominantly on the direct pharmacological inhibition of individual cathepsins in order to suppress their proteolytic activity. More recently, however, research has increasingly shifted toward exploiting the abnormal enzymatic activity of these proteases as a characteristic feature of the tumor microenvironment. Instead of considering cathepsins solely as therapeutic targets, contemporary strategies increasingly utilize them as endogenous biological triggers capable of activating drug delivery systems specifically within malignant tissues. This approach has the potential to enhance therapeutic precision, limit systemic drug exposure, and reduce toxicity affecting healthy organs (100).

One of the most intensively studied strategies is the development of cathepsin B-sensitive prodrugs. In these systems, cytotoxic compounds remain pharmacologically inactive during circulation and are converted into their active forms only after cleavage of a peptide linker by cathepsin B. Because this protease is highly expressed and enzymatically active in numerous solid malignancies, drug activation occurs preferentially within tumor tissue rather than in normal organs. Y. J. Zhong et al. (101) demonstrated that doxorubicin prodrugs containing cathepsin B-cleavable peptide linkers produced selective drug activation at the tumor site while markedly reducing systemic toxicity compared with conventional administration of free doxorubicin. These findings illustrate how enzyme-responsive prodrugs can improve the therapeutic index of established chemotherapeutic agents by increasing tumor selectivity without compromising antitumor efficacy.

The same concept has stimulated the development of nanotechnology-based drug delivery systems that utilize cathepsin activity as an activation mechanism. A representative example is the design of cathepsin B-responsive nanoparticles carrying cytotoxic drugs. J. Kim et al. (102) developed doxorubicin-loaded nanoparticles intended for the treatment of ovarian cancer with peritoneal carcinomatosis, in which drug release occurs only after enzymatic cleavage of a peptide substrate by cathepsin B within the tumor microenvironment. Following preferential accumulation of these nanoparticles inside tumor tissue, localized proteolytic activation enabled efficient release of doxorubicin directly at the disease site. In preclinical models, this strategy achieved superior antitumor activity while simultaneously decreasing systemic adverse effects, highlighting the advantages of combining enzyme-responsive mechanisms with nanoparticle-mediated drug delivery.

Another rapidly advancing field of targeted cancer therapy involves antibody–drug conjugates (ADCs), which combine the selectivity of monoclonal antibodies with highly potent cytotoxic payloads. In these constructs, antibodies recognize tumor-associated antigens and facilitate selective internalization into malignant cells. After trafficking to lysosomes, peptide linkers connecting the antibody and the cytotoxic agent are cleaved by lysosomal proteases—predominantly cathepsins—thereby releasing the active drug intracellularly. S. Balamkundu et al. (103) reported that lysosome-sensitive peptide linkers have become indispensable structural components of numerous ADCs currently approved for clinical application or undergoing clinical evaluation. Their incorporation substantially enhances selective intracellular delivery of cytotoxic agents while minimizing unwanted exposure of healthy tissues.

Consequently, the role of cathepsins in targeted cancer therapy has expanded well beyond direct inhibition of their enzymatic activity. Modern therapeutic approaches increasingly exploit the biochemical characteristics of the tumor microenvironment, particularly its elevated cathepsin activity, as a biological mechanism for selective activation of therapeutic platforms. This principle is now incorporated into diverse technologies, including enzyme-responsive prodrugs, nanoparticle carriers, polymer-based delivery systems, and antibody–drug conjugates. Collectively, these strategies represent an important transition from conventional protease inhibition toward the functional utilization of tumor-associated proteolytic activity to improve treatment specificity and efficacy.

The principal approaches currently employed in cathepsin-associated targeted therapy are summarized in Table 10.

Table 10. Current approaches to cathepsin-associated targeted therapy

Approach	Principle	Examples	Stage of development
Cathepsin inhibitors	Direct pharmacological inhibition of cathepsin enzymatic activity	VBY-825, KGP94, E-64 derivatives	Preclinical studies
Cathepsin B-responsive prodrugs	Activation of inactive therapeutic agents following cathepsin B-mediated cleavage of peptide linkers	Doxorubicin prodrugs	Preclinical studies
Cathepsin B-activated nanoparticles	Localized release of cytotoxic drugs after enzymatic activation within tumor tissue	Doxorubicin-loaded nanoparticles	Preclinical studies
Antibody-drug conjugates (ADCs) with lysosome-cleavable linkers	Intracellular liberation of cytotoxic payloads after cathepsin-dependent cleavage of peptide linkers	ADCs incorporating Val-Cit and related peptide linkers	Preclinical and clinical studies
Cathepsin-activated drug delivery systems	Utilization of elevated tumor-associated cathepsin activity as a biological trigger for targeted drug release	Prodrugs, nanoparticle platforms, polymeric delivery systems	Preclinical studies

Source: Compiled by the author based on data from references (100–103).

Current progress in targeted oncology demonstrates that cathepsins should be regarded not only as molecular targets for pharmacological inhibition but also as valuable biological tools that can be exploited to enhance selective drug delivery. Their increased enzymatic activity within malignant tissues provides an opportunity to activate prodrugs, trigger controlled release of nanoparticle-encapsulated therapeutics, and mediate intracellular payload liberation from antibody–drug conjugates. By restricting activation of anticancer agents predominantly to tumor tissue, these technologies have the potential to improve therapeutic selectivity while reducing systemic toxicity.

Although most cathepsin-responsive therapeutic platforms remain at the preclinical stage of development, the growing body of experimental evidence indicates that they may become an integral component of future precision oncology. Continued optimization of these systems, together with comprehensive evaluation of their long-term safety, pharmacokinetic

characteristics, and clinical effectiveness, will be essential before their widespread implementation in routine cancer treatment.

6.4 Limitations and Future Clinical Perspectives

Despite considerable advances in elucidating the biological functions of cathepsins and exploring their therapeutic potential, the successful incorporation of cathepsin-targeted strategies into routine clinical oncology remains associated with several important challenges. A major limitation is the broad physiological importance of these proteases. In addition to their established involvement in malignant progression, cathepsins are essential for numerous normal biological processes, including intracellular protein turnover, autophagy, antigen processing and presentation, extracellular matrix remodeling, tissue regeneration, and maintenance of immune homeostasis. Consequently, indiscriminate systemic inhibition of cathepsin activity may interfere with essential cellular functions and increase the risk of adverse effects in healthy tissues (3, 40).

Another significant obstacle is the pronounced functional redundancy that exists within the cathepsin family. Many members of this protease group share overlapping substrate specificity and participate in interconnected proteolytic networks, allowing one cathepsin to compensate partially for the inhibition of another. As a result, selective targeting of a single enzyme does not necessarily produce durable antitumor effects because other cathepsins may continue to support extracellular matrix degradation, tumor invasion, and metastatic dissemination. Furthermore, the expression patterns of individual cathepsins vary substantially among different tumor types and often change during disease progression. Their biological activity is also influenced by the cellular composition and biochemical characteristics of the tumor microenvironment, making the development of universally effective therapeutic strategies considerably more complex (40).

An additional challenge arises from the cell-specific functions of cathepsins in different biological contexts. Within malignant cells, increased protease activity generally promotes proliferation, invasion, metastatic spread, and adaptation to the hostile conditions of the tumor microenvironment. In contrast, the same enzymes perform fundamentally different roles in immune cells, where they are indispensable for antigen processing, maturation of dendritic cells, activation of adaptive immune responses, and preservation of effective antitumor immune surveillance. Consequently, excessive pharmacological suppression of selected cathepsins could unintentionally weaken immune mechanisms that contribute to tumor control,

underscoring the importance of achieving an appropriate balance between therapeutic efficacy and preservation of normal immune function (3, 100).

Notwithstanding these limitations, the future clinical outlook for cathepsin-based therapeutic strategies remains highly promising. Current research has increasingly moved away from the concept of complete inhibition of protease activity toward approaches that exploit the unique biological properties of individual cathepsins. These strategies include the development of highly selective inhibitors directed against specific family members, enzyme-responsive drug delivery systems activated selectively within the tumor microenvironment, and therapeutic platforms designed to complement modern immunotherapy and precision oncology. Particular attention has focused on cathepsins B, L, and S, as these enzymes have been consistently implicated in multiple stages of tumor progression while offering opportunities for selective therapeutic intervention with reduced interference in physiological proteolytic processes occurring in normal tissues (100, 104).

Future progress in this field will likely depend increasingly on the implementation of personalized treatment strategies tailored to the molecular characteristics of individual tumors. Combining cathepsin expression profiles with genomic, transcriptomic, and proteomic biomarkers may improve patient stratification and facilitate the selection of the most appropriate targeted therapies. Simultaneously, ongoing advances in nanomedicine, antibody–drug conjugates, and enzyme-responsive drug delivery systems are expected to further improve treatment specificity by restricting pharmacological activation predominantly to malignant tissues and thereby minimizing systemic toxicity.

Overall, the development of cathepsin-directed therapies reflects the broader evolution of modern oncology from non-selective inhibition of cancer-associated pathways toward biologically informed, precision-based therapeutic approaches. Continued progress in molecular oncology, medicinal chemistry, biomarker-guided patient selection, and targeted drug delivery technologies is expected to address many of the current limitations and expand the clinical applicability of cathepsins both as therapeutic targets and as functional components of next-generation anticancer treatment strategies.

7. Discussion

7.1 Summary of Current Scientific Evidence

The analysis of the scientific literature included in this thesis demonstrates that the biological importance of cathepsins in oncology extends far beyond their traditionally recognized lysosomal function. Originally regarded primarily as intracellular proteases responsible for protein degradation and recycling, these enzymes are now understood to participate in a wide range of molecular events that govern both the initiation and subsequent progression of malignant disease. Experimental and clinical evidence accumulated over recent decades indicates that cathepsins act as multifunctional regulators capable of influencing tumor cell behavior, remodeling the surrounding tissue environment, and coordinating communication between malignant cells and multiple components of the tumor microenvironment. Consequently, contemporary molecular oncology considers cathepsins to be integral participants in cancer biology rather than passive degradative enzymes.

Among the numerous members of this protease family, cathepsins B, L, S, and D have been investigated most extensively and currently possess the strongest experimental and clinical support regarding their role in malignant progression. Across a broad spectrum of human cancers, elevated expression or increased enzymatic activity of these proteases has repeatedly been associated with more aggressive biological behavior. Numerous independent studies have linked their dysregulation with accelerated tumor growth, enhanced proliferative capacity, increased invasive and metastatic potential, greater angiogenic activity, and poorer clinical prognosis. Importantly, available evidence suggests that abnormalities in cathepsin expression are detectable during the early stages of carcinogenesis and become progressively more pronounced as tumors evolve, indicating that these enzymes contribute throughout multiple phases of malignant development rather than at a single stage of disease progression.

The literature reviewed in this thesis also demonstrates that cathepsins participate in virtually every key biological process required for successful tumor progression. Their activity contributes to degradation and reorganization of the extracellular matrix, facilitates epithelial–mesenchymal transition, promotes migration and invasion of malignant cells, supports the establishment of pre-metastatic and metastatic niches, and stimulates the formation of new blood vessels within the growing tumor. At the same time, cathepsins interact closely with several major oncogenic signaling pathways, including PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , and Wnt/ β -catenin, thereby influencing cell-cycle regulation, apoptosis, metabolic

adaptation, inflammatory signaling, and communication between tumor cells and their surrounding microenvironment. These interconnected activities position cathepsins as important molecular coordinators linking extracellular proteolysis with intracellular signaling events that collectively determine malignant behavior.

The reviewed evidence likewise highlights the growing clinical relevance of cathepsins. Increasing numbers of experimental and translational studies indicate that altered expression or activity of specific cathepsins may provide clinically valuable information regarding tumor aggressiveness, metastatic potential, patient prognosis, and treatment response. In parallel, advances in precision oncology have expanded therapeutic interest in these enzymes. Beyond conventional enzyme inhibition, novel strategies—including selective cathepsin inhibitors, enzyme-responsive prodrugs, nanoparticle-based delivery systems, antibody–drug conjugates, and other cathepsin-activated therapeutic platforms—demonstrate that tumor-associated proteolytic activity can be exploited to achieve more selective drug delivery while limiting systemic toxicity. These developments illustrate the transition from viewing cathepsins solely as pharmacological targets to recognizing them as functional components of sophisticated precision medicine approaches.

Taken together, the currently available scientific evidence supports the conclusion that cathepsins represent pivotal molecular regulators involved in both the biological evolution of malignant tumors and their clinical behavior. Their influence extends across multiple levels of cancer development, from regulation of intracellular signaling and tissue remodeling to metastatic dissemination and therapeutic responsiveness. Accordingly, continued investigation of cathepsin biology is expected not only to deepen understanding of the molecular mechanisms underlying carcinogenesis but also to facilitate the development of more accurate diagnostic biomarkers, improved prognostic models, and increasingly selective targeted therapies capable of enhancing the clinical management of patients with malignant neoplasms.

7.2 Challenges and Contradictions in Current Research

Although research on cathepsins has advanced considerably over the past two decades, many aspects of their role in cancer remain incompletely understood. Existing experimental and clinical evidence strongly supports the involvement of these proteases in malignant progression; however, important uncertainties persist regarding the precise molecular mechanisms through which individual cathepsins contribute to carcinogenesis and how this knowledge can be translated into clinical practice. These unresolved issues largely arise from the remarkable biological diversity of the cathepsin family, the heterogeneity of malignant

tumors, the complexity of the tumor microenvironment, and substantial methodological differences among published studies. Consequently, investigations addressing similar biological questions sometimes produce inconsistent or even conflicting conclusions.

One of the principal challenges concerns the highly context-dependent behavior of cathepsins. Although elevated expression of many family members is frequently associated with aggressive tumor characteristics and poor clinical outcomes, their biological functions are not universal across all cancer types. The activity of individual cathepsins depends on numerous factors, including tumor origin, histological subtype, disease stage, cellular source of enzyme production, and the molecular composition of the surrounding microenvironment. As a consequence, observations obtained in one malignancy cannot necessarily be generalized to others, highlighting the importance of evaluating cathepsin biology within the specific context of each tumor rather than assuming a common mechanism across all cancers.

Another unresolved issue involves the exact relationship between cathepsins and intracellular signaling pathways. Numerous studies have demonstrated strong associations between cathepsin activity and major oncogenic signaling networks, including PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , and Wnt/ β -catenin. Nevertheless, the underlying molecular basis of these interactions has not yet been fully clarified. It remains uncertain whether cathepsins directly regulate components of these signaling cascades or whether their effects are mediated indirectly through extracellular matrix remodeling, altered release of growth factors and cytokines, or broader changes within the tumor microenvironment. Clarifying these mechanisms is essential for a more complete understanding of how cathepsins contribute to tumor progression and may ultimately facilitate the development of more effective targeted therapies.

Interpretation of experimental data is further complicated by the substantial functional redundancy that characterizes the cathepsin family. Many cathepsins share similar substrate specificity and participate in overlapping proteolytic pathways, enabling related enzymes to compensate when the activity of a single family member is suppressed. Consequently, inhibition of one cathepsin alone does not always result in significant antitumor effects because other proteases may maintain essential biological functions associated with invasion, tissue remodeling, and metastatic spread. Conversely, simultaneous inhibition of multiple cathepsins increases the likelihood of disturbing normal physiological processes, as these enzymes are indispensable for maintaining cellular homeostasis. Achieving an appropriate balance between therapeutic efficacy and preservation of normal biological function therefore remains one of the major obstacles in the development of cathepsin-targeted treatment strategies.

The potential use of cathepsins as clinical biomarkers also continues to present important challenges. Although numerous studies have demonstrated significant associations between cathepsin expression and clinicopathological features of malignant tumors, there is still no universally accepted approach for assessing these enzymes in clinical practice. Differences in laboratory methodologies, analytical techniques, specimen preparation, quantification of protein expression or enzymatic activity, and interpretation of experimental results make direct comparison between studies difficult. This methodological heterogeneity limits the establishment of standardized diagnostic and prognostic criteria that could be reliably implemented in routine oncology.

Another important limitation of the available evidence is the predominance of preclinical investigations over clinical studies. Much of the current understanding of cathepsin biology has been derived from experiments using cultured cancer cell lines, genetically engineered animal models, and other laboratory systems. Although these models have provided valuable mechanistic insights, they cannot fully reproduce the complexity of human malignancies, where tumor heterogeneity, immune responses, and interactions with the surrounding microenvironment play critical roles. Consequently, molecular mechanisms identified under experimental conditions may not always reflect the biological behavior of tumors in patients, emphasizing the need for larger, well-designed clinical studies capable of validating laboratory observations.

Translation of cathepsin-based therapeutic strategies into routine clinical oncology likewise remains limited. A variety of selective inhibitors, enzyme-responsive prodrugs, nanoparticle delivery systems, and other cathepsin-targeted technologies have demonstrated encouraging results in preclinical models; however, only a relatively small number of these approaches have advanced successfully to clinical evaluation. Limited target specificity, compensatory activity among different cathepsins, and concerns regarding disruption of their physiological functions continue to represent significant barriers to clinical implementation. These challenges illustrate the inherent difficulty of selectively inhibiting pathological proteolysis while preserving the normal biological activities that are essential for tissue homeostasis.

Overall, the current literature portrays cathepsin research as a rapidly evolving field that has generated substantial advances while simultaneously revealing numerous unresolved biological and translational questions. Future investigations should focus on defining the precise molecular functions of individual cathepsins, standardizing methods for evaluating their expression and enzymatic activity, and developing highly selective therapeutic strategies

capable of maximizing antitumor efficacy without compromising normal physiological processes. Addressing these challenges will be crucial for translating the expanding knowledge of cathepsin biology into reliable diagnostic tools, prognostic biomarkers, and effective targeted therapies for patients with malignant disease.

7.3 Future Research Perspectives

The growing volume of scientific evidence accumulated in recent years indicates that cathepsins will remain an important focus of molecular oncology research. Although major advances have been made in defining their contribution to tumor development, invasion, and metastasis, many fundamental aspects of their biology have yet to be fully resolved. Future investigations should therefore move beyond the study of individual enzymes and place greater emphasis on understanding the complex regulatory networks that govern cathepsin expression, maturation, activation, intracellular trafficking, substrate selection, and interactions with other proteolytic systems operating within the tumor microenvironment. Elucidating these interconnected mechanisms is expected to provide a more comprehensive understanding of the molecular events that drive carcinogenesis and disease progression.

A particularly important direction for future research involves clarifying the molecular interplay between cathepsins and intracellular signaling pathways that determine malignant cell behavior. Although numerous studies have demonstrated functional links between cathepsins and signaling networks such as PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , and Wnt/ β -catenin, the exact mechanisms underlying these relationships remain only partially understood. Future studies should determine whether cathepsins regulate these pathways through direct interactions with signaling proteins or whether their influence is mediated indirectly by extracellular matrix remodeling, altered growth factor availability, or broader changes within the tumor microenvironment. Resolving this question will be essential for explaining how cathepsins contribute to uncontrolled proliferation, resistance to apoptosis, tumor invasion, and metastatic dissemination.

Another promising area of investigation is the development of more robust biomarker strategies based on comprehensive molecular profiling rather than evaluation of individual enzymes alone. Current evidence suggests that the expression of a single cathepsin rarely reflects the full biological complexity of malignant tumors. Accordingly, future diagnostic approaches should integrate multiple members of the cathepsin family with complementary genomic, transcriptomic, proteomic, and protein-based biomarkers. Such multidimensional molecular profiling may improve the early detection of cancer, increase the accuracy of

prognostic assessment, enable more reliable estimation of metastatic potential, and provide more effective monitoring of treatment response throughout the clinical course of the disease.

Continued progress in targeted therapeutic technologies also represents a key priority for future research. Particular attention should be directed toward the design of highly selective inhibitors capable of suppressing individual cathepsins that play dominant roles in tumor progression while preserving the physiological functions of related family members in healthy tissues. At the same time, further refinement of enzyme-responsive therapeutic platforms—including prodrugs, nanoparticle-based drug delivery systems, polymeric carriers, and other smart delivery technologies—is expected to expand the therapeutic applications of cathepsins. By exploiting the elevated proteolytic activity characteristic of the tumor microenvironment, these approaches may achieve localized activation of anticancer agents, thereby improving therapeutic precision and reducing systemic toxicity.

The relationship between cathepsins and the immune system represents another research area with considerable future potential. The rapid development of cancer immunotherapy has highlighted the importance of understanding how these proteases influence antigen processing, maturation of dendritic cells, activation of T lymphocytes, macrophage polarization, and the establishment of immunosuppressive conditions within the tumor microenvironment. A more detailed understanding of these mechanisms may support the development of novel combination therapies in which cathepsin-targeted approaches are integrated with immune checkpoint inhibitors and other immunotherapeutic strategies to enhance antitumor immune responses and improve clinical outcomes.

Equally important is the expansion of clinical research evaluating cathepsin-directed therapeutic strategies. At present, much of the available knowledge has been generated using cell culture models and experimental animals, whereas clinical validation in human populations remains relatively limited. Future multicenter clinical trials will therefore be essential for assessing the long-term safety, therapeutic effectiveness, and clinical benefits of cathepsin-targeted interventions. Such studies should also help identify patient subgroups most likely to respond to these therapies and define the optimal role of cathepsin-based approaches within personalized oncology.

In summary, further advances in this field will depend on close integration of fundamental molecular research with translational and clinical investigation. Continued exploration of cathepsin biology, improvement of molecular diagnostic technologies, refinement of highly selective therapeutic platforms, and implementation of personalized treatment strategies are expected to substantially broaden the clinical significance of these

proteases. Collectively, these developments may establish cathepsins as integral components of future precision oncology, serving both as informative molecular biomarkers and as valuable therapeutic targets for the diagnosis and treatment of malignant disease.

8. Conclusions

This master's thesis presents a comprehensive analysis of contemporary scientific evidence regarding the biological functions of cathepsins and their involvement in the molecular mechanisms that govern the development and progression of malignant tumors. A critical evaluation of current national and international literature enabled the integration of existing knowledge on the classification of cathepsins, their structural and biochemical properties, regulatory mechanisms controlling their activity, and their physiological functions, with particular attention given to their role in cancer-associated signaling pathways and tumor biology.

The reviewed literature clearly demonstrates that the biological significance of cathepsins extends far beyond their classical function as lysosomal proteases involved in intracellular protein degradation. These enzymes are now recognized as multifunctional regulators that participate in numerous physiological processes, including maintenance of proteostasis, autophagy, antigen processing, extracellular matrix turnover, regulation of intercellular communication, apoptosis, and preservation of tissue homeostasis. Alterations in their expression, intracellular localization, or enzymatic activity contribute directly to the initiation and progression of malignant disease.

The analysis performed in this thesis further indicates that cathepsins are involved in virtually all major stages of cancer development. They participate in regulation of tumor cell proliferation, remodeling of the tumor microenvironment, angiogenesis, epithelial–mesenchymal transition, degradation of extracellular matrix components, migration and invasion of malignant cells, and formation of metastatic niches. Among the members of this enzyme family, cathepsins B, L, S, K, and D currently possess the strongest experimental and clinical evidence supporting their contribution to tumor progression and metastatic dissemination.

The available scientific evidence also confirms that cathepsins are closely integrated with several key oncogenic signaling pathways, including PI3K/Akt, MAPK/ERK, NF- κ B, TGF- β , and Wnt/ β -catenin. Through functional interactions with these signaling networks, cathepsins regulate multiple biological processes essential for malignant transformation, including cell-cycle progression, proliferation, apoptosis resistance, migration, invasion, metabolic adaptation, and communication between tumor cells and their surrounding microenvironment.

Another important conclusion arising from the literature analysis is the considerable clinical potential of cathepsins as molecular biomarkers. Numerous studies demonstrate significant associations between increased expression or activity of individual cathepsins and tumor aggressiveness, metastatic potential, disease stage, and patient prognosis. These observations indicate that cathepsins may become valuable components of future diagnostic and prognostic strategies aimed at improving early cancer detection, predicting disease progression, and monitoring therapeutic response.

The reviewed evidence likewise demonstrates the growing importance of cathepsin-based therapeutic approaches within precision oncology. Current research extends beyond direct inhibition of protease activity and includes the development of highly selective cathepsin inhibitors, enzyme-responsive prodrugs, nanoparticle-mediated drug delivery systems, and antibody–drug conjugates containing lysosome-cleavable peptide linkers. These technologies exploit the increased proteolytic activity characteristic of malignant tissues to achieve localized activation of anticancer agents, thereby enhancing therapeutic selectivity while reducing systemic toxicity.

At the same time, the analysis highlights several important limitations that continue to restrict the clinical implementation of cathepsin-targeted therapies. The broad physiological functions of cathepsins, functional redundancy among different family members, pronounced variability in their expression across tumor types, and the limited amount of large-scale clinical evidence remain major challenges for routine clinical application. Overcoming these limitations will require a deeper understanding of the molecular mechanisms regulating individual cathepsins together with the development of more selective therapeutic strategies and standardized approaches for clinical evaluation.

In conclusion, the findings presented in this thesis demonstrate that cathepsins represent key molecular regulators involved in multiple aspects of malignant transformation, tumor progression, and metastatic dissemination. Continued integration of basic molecular research with translational and clinical investigations is expected to expand current knowledge of cathepsin biology and support the development of novel diagnostic biomarkers, more accurate prognostic tools, and increasingly effective personalized therapeutic strategies for the management of malignant neoplasms.

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