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Cathepsins as Therapeutic targets in skin tumor metastasis.

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Abstract:

Melanoma is considered one of the most aggressive forms of skin cancer, with a high propensity for invasion and metastasis. Lysosomal proteases, particularly cathepsins, play a central role in melanoma biology, influencing tumor cell behaviour and shaping the surrounding microenvironment. These proteases include cysteine cathepsins (such as B, L, and K), aspartyl cathepsins (including D and E), and serine protease cathepsins (such as Cathepsin G), each contributing to extracellular matrix remodeling, facilitation of cellular migration, and modulation of immune cell interactions within the tumor niche. Comparative analyses of benign nevi and malignant melanoma indicate that dysregulation of these cathepsins is associated with the transition from growth-arrested melanocytes to proliferative and invasive tumor cells. Cathepsins are produced not only by melanoma cells but also by stromal and immune cells, contributing to immune suppression and establishing a pro-tumorigenic microenvironment. Certain cathepsin isoforms have been linked to higher metastatic potential and poor clinical outcomes, emphasizing their relevance in disease progression. Understanding the mechanism of cysteine, aspartyl, and Serine Cathepsins in melanoma may reveal novel biomarkers and therapeutic targets. Targeting these proteases represents a promising strategy to limit tumor growth, reduce metastasis, and enhance anti-tumor immune responses, highlighting their effective role in melanoma pathogenesis and precision oncology.

Keywords**:** Tumor, cancer, Cathepsin, melanoma, UV radiation, Nevi, Cystein cathepsin, Aspartyl Cathepsin, serine protease.

Zusammenfassung

Diese Masterarbeit befasst sich mit den Cathepsinen als therapeutisches Ziel bei Hauttumor-Metastasen. Das maligne Melanom ist eine aggressive Form von Hautkrebs mit hoher Invasions- und Metastasierungsneigung. Lysosomale Proteasen, insbesondere Cathepsine, beeinflussen Tumorzellen, das Tumormikromilieu und die Immunantwort. Eine Dysregulation dieser Enzyme fördert die Zellproliferation, Invasion und Metastasenbildung. Das Verständnis ihrer Mechanismen kann neue Biomarker und therapeutische Ansätze eröffnen, um das Tumorwachstum zu begrenzen und die Wirksamkeit antitumoraler Immunreaktionen zu steigern.

Contents:

List of Abbreviations:

1.BCC: Basal cell carcinoma.

2.SCC: Squamous cell carcinoma.

3.UVR: Ultraviolet radiation.

4.FAB-α: Fatty acid binding protein.

5.ECM: Extracellular matrix.

6.TGF-β1: Transforming growth factor beta 1.

7.LMM: Lentigo malignant melanoma.

8.NF1: Neurofibromin.

9.BRAF: B-Rapidly accelerated fibrosarcoma.

10.V600K: Valine 600- Lysine K.

11.Non-V600E: Not Valine 600 E.

12.NRAS3: Neuroblastoma Rat sarcoma.

13.KIT: v-Kit Hardy- Zuckerman 4 feline sarcoma.

14.MAPK: Mitogen-Activated protein kinase.

15.DM: Desmoplastic Melanoma.

16.AM: Acral melanoma.

17.CCND1: Cyclin D1.

18. CDK4: Cyclin-Dependent Kinase 4.

19. CDKN2A: Cyclin-Dependent Kinase Inhibitor 2A.

20.TERT: Telomerase Reverse Transcriptase.

21. SM: Spitz Melanoma.

22.AST: Atypical Spitz Melanoma.

23.ALK: Anaplastic Lymphoma Kinase.

24. NTRK1: Neurotrophic Tyrosine Receptor Kinase1.

25. ROS1: c-ros oncogene 1.

26.RET: REarranged during Transfection.

27.HRAS: Harvey rat sarcoma.

28.MAPK: Mitogen-Activated protein kinase.

29.PI3K-AKT: Phosphoinositide 3- Kinase

30. MM: Mucosal Melanoma.

31. SF3B1: Splicing Factor 3b subunit 1.

32. TP53: Tumor Protein 53.

33.CDKN2A: Cyclin-Dependent kinase inhibitor 2A.

34.MART: Melanoma antigen recognised by T-cells.

35.NY-ESO-1: New York oesophageal squamous cell carcinoma-1.

36.gp-100: Glycoprotein 100.

37. CD8+: Cluster of differentiation 8 positive.

38.CD4+: Cluster of differentiation 4 positive.

39.HLA: Human leukocyte antigen.

40.T-cell: T-lymphocyte.

41.GILT: Gamma-Interferon-Inducible Lysosomal Thiol reductase.

42. APCS: Antigen-Presenting cells.

43. CLIP: Class II -associated invariant chain peptide.

44. ADCS: Antibody-drug conjugates.

45.LC3-II: Microtubule-Associated protein 1 light chain 3, form II.

46. MR: Mannose receptor.

47. GAGS: Glycosaminoglycans.

48. ER: Endoplasmic reticulum.

49. M6P: Mannose-6-Phosphate.

50.TGN: Trans-Golgi network.

51. MCOLN1\TRPML1: Transient receptor potential mucolipin 1.

52. TFEB: Transcription factor EP:

53. IGF-1: Insulin- like growth factor-1.

54. CDP\Cux: Cut-Like homebox.

55. TNF: Tumor Necrosis factor.

56. IL-1-α: Interleukin-1 Alpha.

57. IL-β1: Interleukin-1 Beta.

57. IFNY: Interferon gamma.

58. P2X7: Purinergic receptor P2X, ligand-gated ion channel, 7.

59.STAT: Signal transducer and activator of transcription.

60.mTOR: mechanistic Target of Rapmycin.

61. Bid: BH3 Interacting-Domain Death agonist.

62. Bcl-2: B-Cell lymphoma 2.

63. Bcl-xl: B-cell lymphoma-extra-large.

64. p27.Kip1: Cyclin-dependent kinase inhibitor 1B(Kip1).

65.uPA: Urokinase-type Plasminogen activator.

66. MMP-9: Matrix Metalloproteinase 9.

67. TIMPs: Tissue Inhibitors of Metalloproteinase.

68. Rip1-Tag2: Rat Insulin Promoter 1-T Antigen 2.

69.PaNET: Pancreatic Neuroendocrine Tumor.

70.MMTV-PyMT: Mouse Mammary tumor virus- Polyoma middle T antigen.

71. K14- HPV16: Keratin 14-Human Papillomavirus type 16.

72.Rab7: Ras-related protein.

73. ScFv: Single-chain variable fragment.

74. HIF-1: Hypoxia-inducible factor-1

75. Cat L: Cathepsin L.

76.Cat K: Cathepsin K.

77. Cat S: Cathepsin S.

78. BLCA: Bladder urothelial carcinoma.

79. SKCM: Skin cutaneous melanoma.

80.LGG: Lower-grade glioma.

81.UVM: Uveal Melanoma.

82.CEP: Cepharanthine.

83. Cat C: Cathepsins C.

84. NSPS: Neutrophile serine proteases.

85. NETS: Neutrophile extracellular traps.

86. Cat H: Cathepsin H.

87. ELISA: Enzyme linked immunosorbent.

88. Cat V: Cathepsin V.

89. Cat W: Cathepsin W.

90.NK: Natural killer.

91.Cat F: cathepsin F.

92. Cat O: Cathepsin O.

93: Cat X: Cathepsin X.

94.Cat D: Cathepsin D.

95. PCD: Pro cathepsin D.

96.HNmMM: Head and neck malignant melanoma.

97. TRAIL: TNF-Related Apoptosis including ligand.

98. TUNEL: Terminal deoxynucleotidyl transferase dUTP Nick-END Labeling.

99. Cat G: Cathepsin G.

100.Cat E: Cathepsin E.

1.Introduction:

1.1. skin tumor

Skin tumor is an abnormal proliferation of skin cells resulting from dysregulation of cellular growth. The normal growth of the skin refers to any changes in the skin anatomy that are harmless, not caused by any disease, such as Nevi(1). This is called non-cancerous change or Benign growth. There is another abnormal growth - Cancerous or Malignant growth(2). Tumor development is generally initiated from a single transformed cell that undergoes uncontrolled divisions, forming a mass of abnormal cells(3). It divides to form 2 and then 4 cells and more(4,5). Benign skin tumors usually grow slowly and do not metastasize. For example: Seborrheic keratosis, Moles *Nevus* and Lipoma(6,7).

Malignant skin tumor - Skin cancer- a dangerous form of skin tumor which grows abnormally and uncontrollably, invades other cells and can metastasize other organs in the body(8). It has many types such as Basal cell carcinoma - BCC-, Squamous cell carcinoma -SCC- and the most aggressive type of Melanoma(5). Comparing gene and protein expression profiles between nevi and melanoma can yield valuable insights into the signalling pathways and molecular factors required for the transition of growth-arrested melanocytes into a (pre)malignant state(9).

The most common reason for this disease is exposure to ultraviolet radiation. Studies demonstrated that UVR induces an upregulation of FAP-α expression in both melanocytes and melanoma cells *in vitro*, thereby promoting extracellular matrix (ECM) degradation(10). Elevated cathepsin expression has been strongly linked to enhanced metastatic potential and poorer clinical outcomes. Studies demonstrated that cathepsin B promotes melanoma invasion by inducing TGF-β1 expression, which in turn activates fibroblasts and enhances their ability to facilitate melanoma cell invasion.(11) (12)Notably, transforming growth factor-β isoforms are constitutively
expressed in both primary and metastatic melanoma cells(11). Furthermore, TGF-β1 secreted by melanoma cells has been shown to stimulate FAP-α expression in surrounding fibroblasts(9). Diagnosis of melanoma is based mainly on its clinical symptoms, and the A-B-C-D rule is useful in identifying pigmented lesions, which are

suspicious of melanoma (Asymmetry, Border irregular, Color inhomogeneous and Diameter more than 5 mm(13). Breslow depth is an important prognostic factor for primary melanoma without metastasis; it is a vertical tumor thickness. Also, invasion level (Clark level) and identification of micrometastases in the regional lymph nodes via sentinel lymph node biopsy are important prognostic factors(14).

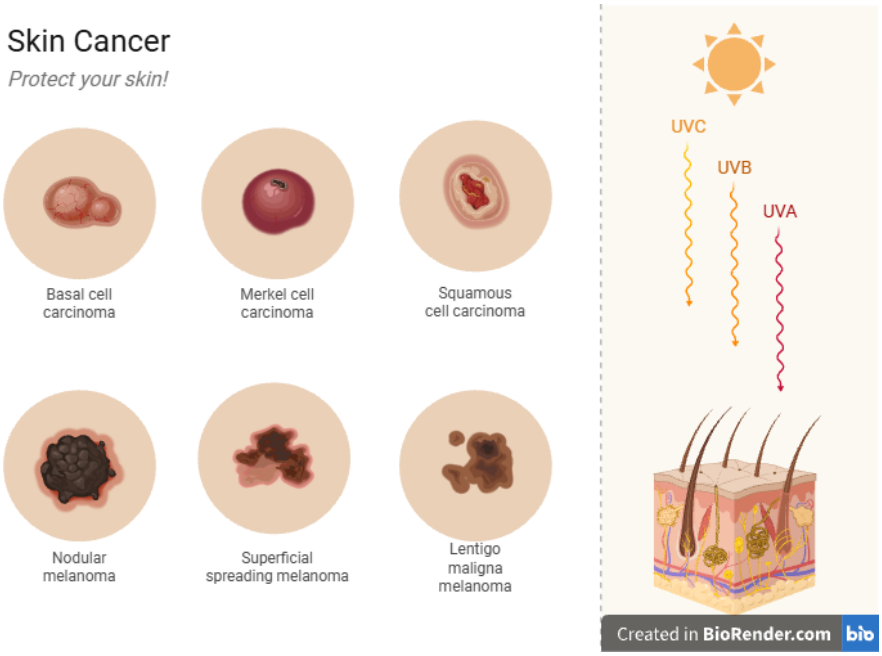

Figure 1, Overview of Skin tumors arise from abnormal proliferation of skin cells due to dysregulation of cellular growth. Benign skin lesions, such as nevi, seborrheic keratosis, and lipomas, are characterized by slow growth and lack of metastatic potential, whereas malignant skin tumors (skin cancers) exhibit uncontrolled growth, tissue invasion, and the ability to metastasize. The major types of skin cancer include basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma, the most aggressive form. Ultraviolet (UV) radiation represents a major etiological factor in melanoma development, as it induces molecular alterations that promote extracellular matrix degradation and tumor progression. Progression from benign nevi to malignant melanoma involves changes in gene and protein expression, including increased activity of proteolytic enzymes such as cathepsins, which enhance melanoma invasion, metastasis, and immune evasion.

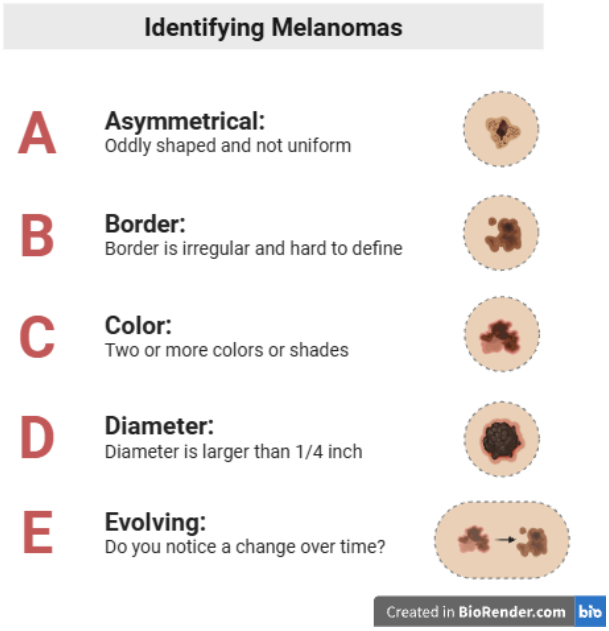

Figure 2, Describe the ABCDE rule serving as a useful tool for identifying suspicious pigmented lesions, including asymmetry, border irregularity, color heterogeneity, and diameter greater than 5 mm. Prognosis of primary melanoma without distant metastasis is strongly influenced by Breslow depth, which reflects vertical tumor thickness. Additional important prognostic factors include the level of tumor invasion (Clark level) and the detection of micrometastases in regional lymph nodes through sentinel lymph node biopsy.

1.2. What is Melanoma:

Melanoma is considered the worst type of skin cancer. In Europe, the rate is about 25 new cases of melanoma per 100,000 populations. It starts with Melanocytes, which are the cells responsible for producing the pigment of skin cells which are known as Melanin(15). The development of normal melanocytes into melanoma cells passes with more than one stage, the first of them is the horizontal growth stage which is followed by the vertical growth stage in which the Metastasis phase starts, and this is the most dangerous part of Melanoma(16).

In Melanoma, Phase 1 and phase 2 are confined to the skin, while phase 3 of melanoma is characterized by regional metastasis, and phase 4 involves distant metastasis. Phase 3 of melanoma patients exhibit a range of 5-year survival rates(17). Metastasis refers to the process by which cancer cells spread from the

skin to other organs of the body. Melanoma metastasis is facilitated by adhesion, motility, proteolytic activities and cell receptors(18). About two-thirds of metastasis are originally confined to the drain age area of regional lymph nodes. Regional metastasis can appear up to 2 cm from the primary tumor, as intransit metastases in the skin between the site of the primary tumor and the first lymph node and as regional lymph node metastasis(19). In the regional metastasis phase, the differentiation between micrometastasis and macrometastasis and the number of lymph nodes involved are crucial. As soon as distant metastasis develops, prognosis depends on the site of the metastasis and on the lactate dehydrogenase levels in the blood(20). The activity of many proteolytic enzymes was shown to be an important point in promoting melanoma cell metastasis(7). These proteolytic enzymes reform the extracellular matrix and release active factors and cell receptors, thereby facilitating communication between melanoma cells and their microenvironment. This result in forming a favorite environment for melanoma progression and metastasis(9).

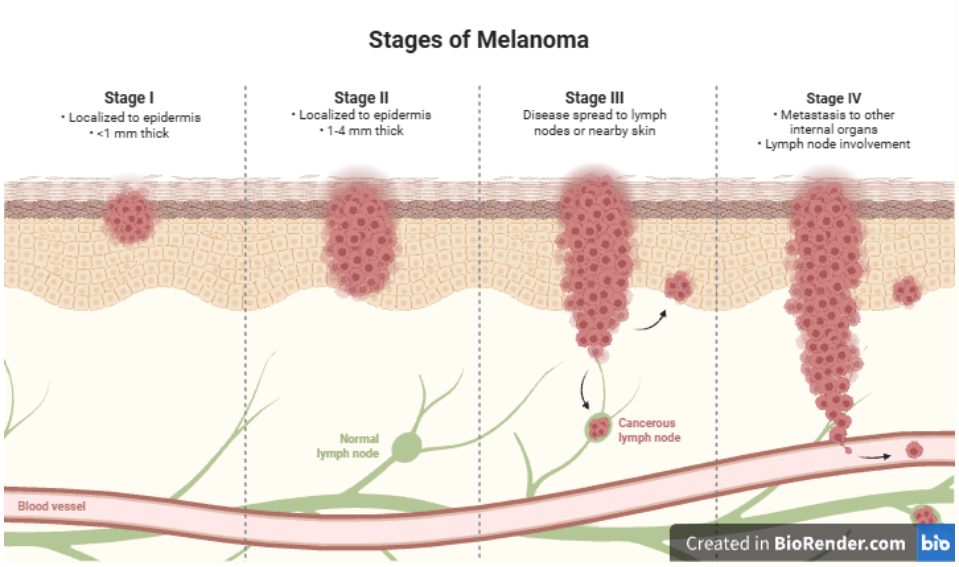

Figure 3, Melanoma progression occurs through distinct stages. Stages I and II are confined to the skin, whereas stage III is characterized by regional metastasis, and stage IV involves distant metastasis. Metastasis refers to the spread of melanoma cells from the primary skin tumor to other organs. This process is facilitated by increased cell adhesion, motility, proteolytic enzyme activity, and the interaction of cell surface receptors. During metastasis, melanoma cells invade blood and lymphatic vessels, with approximately two-thirds of metastatic cases initially involving regional lymph nodes. Regional metastases may appear as in-transit metastases in the skin between the primary tumor and the first draining lymph node or as lymph node metastases.

1.3. Melanoma subtypes:

Melanoma is classified into several subtypes based on anatomical origin, sun exposure, histopathological features, and genetic alterations. These subtypes exhibit distinct biological behaviors, mutation profiles, and clinical outcomes(21).

1-Lentigo Malignant Melanoma (LMM):

LMM is the most common form of melanoma in situ, typically occurring in sun-exposed areas such as the head and neck, primarily in older adults(22). It is characterized by the radial proliferation of atypical melanocytes along the epidermal junction and occasionally infiltrating hair follicles and sweat ducts(23). Although LMM grows slowly, it can progress to invasive melanoma. Genetically, LMM frequently harbors mutations in NF1, BRAF (V600K/non-V600E), NRAS, and, less commonly, KIT, which activate the MAPK pathway and influence tumor proliferation(22). High mutational burden in LMM often correlates with enhanced responsiveness to immunotherapy(24).

2-Desmoplastic Melanoma (DM):

DM is a rare subtype typically arising in chronically sun-exposed skin, particularly in the head and neck region. It is histologically defined by spindle-shaped melanocytes within a dense collagenous stroma(25). DM exhibits fewer BRAF and NRAS mutations but shows frequent NF1 and TP53 alterations, contributing to genomic instability and MAPK pathway activation. DM often demonstrates local invasiveness and may respond to targeted therapies alongside conventional surgical excision and radiotherapy(22,24).

3-Acral Melanoma (AM):

AM arises on non-hair-bearing, sun-protected skin, such as the palms, soles, and subungual regions. Unlike other melanomas, AM is generally unrelated to UV exposure and rarely carries BRAF or NRAS mutations(25). Instead, it frequently harbors KIT mutations and exhibits structural genomic alterations involving CCND1, CDK4, CDKN2A, and TERT, which contribute to aberrant cell cycle regulation. Diagnosis is often delayed due to clinical resemblance to benign Lesions, leading to advanced-stage presentation(22,24).

4-Spitz Melanoma (SM) and Atypical Spitz Tumors (AST):

SM is the malignant counterpart of Spitz nevi, occurring more often in children and young adults, though ASTs may appear in older individuals. These tumors are characterized by cytological atypia, mitotic activity, and variable histology(22). Many harbor BRAF V600E mutations and fusions involving ALK, NTRK1, ROS1, RET, or mutations in HRAS, activating MAPK and PI3K-AKT pathways. SMs generally exhibit rapid growth but lower metastatic potential compared to conventional melanomas(24).

5-Mucosal Melanoma (MM):

MM originates from melanocytes in mucous membranes, including oral, genital, nasal, and conjunctival sites. It is unrelated to sun exposure and shows a different mutation spectrum, with more frequent alterations in KIT and SF3B1, and fewer BRAF or NRAS mutations(26). MM depends on MAPK pathway activation and presents challenges in early detection due to anatomical constraints. Targeted therapies are under investigation based on these molecular characteristics(24).

6-Melanoma Arising in Congenital Nevi:

Melanomas developing within congenital nevi are rare but carry a higher lifetime risk in large or giant nevi. They often harbor activating NRAS mutations and additional alterations in TP53 and CDKN2A, driving MAPK pathway activation and malignant transformation(27). Epigenetic regulation, such as TERT promoter methylation, may also contribute to tumor progression. Early monitoring and intervention are essential due to the potential for rapid malignant evolution(24).

-Table 1: Melanoma subtypes:

Melanoma Subtype	Common Site	UV Exposure	Histopathology / Features	Key Mutations / Alterations	Signaling Pathways	Clinical Notes
1.Lentigo Malignant Melanoma (LMM)	Head & neck	High	Radial growth along epidermal junction may infiltrate hair follicles	NF1, BRAF (V600K/ non-V600E), NRAS, KIT	MAPK	Slow growing, can become invasive; high mutational burden → better immunotherapy response
2.Desmoplastic Melanoma (DM)	Head & neck	Chronic sun exposure	Spindle-shaped melanocytes, dense collagenous stroma	NF1, TP53	MAPK	Locally invasive; responds to surgery + radiotherapy; targeted therapy possible
3. Acral Melanoma (AM)	Palms, soles , subungual	None	May mimic benign Lesions	KIT; structural changes in CCND1, CDK4, CDKN2A, TERT	Cell cycle regulation, MAPK	Often diagnosed late; unrelated to UV
4. spitz Melanoma / AST	Children, young adults	Variable	Cytologic atypia, mitotic activity	BRAF V600E; ALK, NTRK1,	MAPK, PI3K–AKT	Rapid growth but lower metastatic potential

				ROS1, RET fusions; HRAS		
5. Mucosal Melanoma (MM)	Oral, genital, nasal, conjunctival mucosa	None	Arises from mucosal melanocytes	KIT, SF3B1	MAPK	Difficult early detection; targeted therapies under investigation
6. Melanoma in Congenital Nevi	Large or giant nevi	N/A	Arises within congenital nevi	NRAS, TP53, CDKN2A; TERT promoter methylation	MAPK	Rare but higher lifetime risk; early monitoring essential

Table 1: Melanoma subtypes.

1.4. Lysosomes and lysosomal Enzymes:

Lysosomes are acidic intracellular, membrane-bound endocytic organelles, they are present in all eukaryotic cells, except for mature erythrocytes. They play a very important role in the degradation of cellular macromolecules and recycling of their building blocks(28). This degradative function is carried out in the lysosomal lumen by the concerted action of more than 60 hydrolases and accessory proteins(29),(30). Lysosomal enzymes are hydrolytic enzymes at acidic pH, which are localised inside lysosomes, these enzymes break different macromolecules and organelles(30). They are secreted outside the melanoma cells, where they degrade extracellular matrix “ECM” components as collagen, laminin and fibronectin. This damage of the ECM allows cancer cells to invade other tissues and arriving blood or lymphatic vessels and this is their role in metastasis(31). They also have a role in tumor growth and survival by helping in the remodelling of the tumor microenvironment, releasing growth promoting peptides and growth factors. They also help cells adaption to the

stress as hypoxia through autophagy and recycling cellular components for energy and growth(30). Changes in lysosomal PH or membrane protein increases drug resistance by degrading them inside lysosomes after melanoma cells keep them inside and this reduces sensitivity to targeted therapies(32). Given the critical role of hydrolytic enzymes in metastasis, attention has recently shifted to lysosomal enzymes as a potential therapeutic target by inhibiting them so that helps in reducing metastasis, making melanoma cells more sensitive to chemotherapy(33). Lysosomes have different enzymes including peptidase, that together degrades most complex macromolecules. Cathepsins are one of the most important lysosomal enzymes which have a very important role in melanoma and its metastasis(34,35,36).

Lysosomal peptidases constitute a major proteolytic system involved in protein turnover, antigen processing, and regulation of immune responses(37,38). These enzymes are mainly classified according to their catalytic activity into cysteine, serine, and aspartyl peptidases, with the majority functioning as endopeptidases that cleave internal peptide bonds within protein substrates. This endopeptidase activity is essential for initiating protein degradation and shaping immune-related processes within cells(38). Dysregulation of lysosomal endopeptidases has been linked to tumor progression through their impact on antigen presentation, immune cell activation, and modification of the tumor microenvironment(38). Enhanced endopeptidase activity may support tumor survival by facilitating immune evasion, promoting tissue remodeling, and influencing inflammatory signaling pathways. Although these mechanisms have been described in specific tumor models, they represent general processes that are also relevant to highly immunogenic cancers such as melanoma(38).

In contrast, exopeptidases primarily function in the later stages of proteolysis by trimming peptides generated by endopeptidases. Their role is mainly associated with peptide maturation and regulation of intracellular peptide pools rather than direct extracellular matrix degradation(38). Consequently, while both enzyme groups contribute to proteolytic balance, endopeptidases play a dominant role in cancer-associated immune modulation and tumor progression(38).

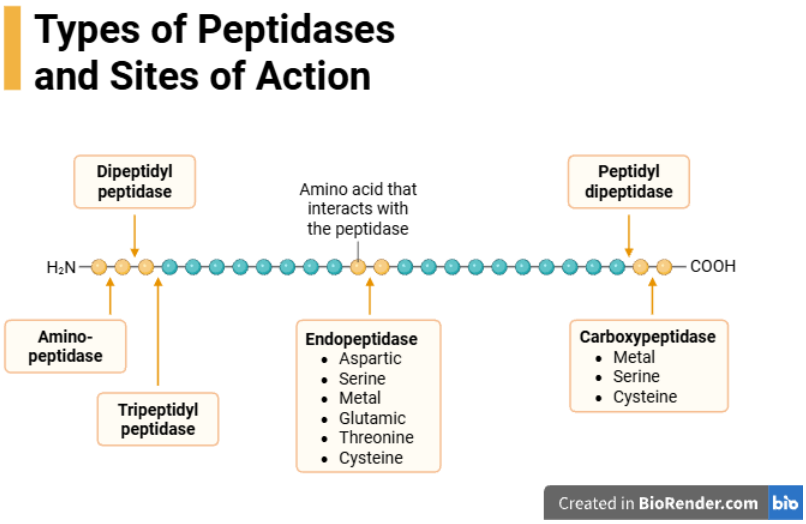

Figure 4: Classification of Peptidases including cysteine, serine, and aspartyl proteases.

1.5. Lysosomal Enzymes in antigen processing and immune evasion:

Melanoma cells commonly express several tumor-associated antigens, including MART-1, NY-ESO-1, gp100, and tyrosinase, all of which can elicit $CD8^+$ cytotoxic responses as well as $CD4^+$ helper T cell activity(39). Effective recognition and elimination of melanoma cells requires that these antigens be processed into peptides and presented on both HLA class I and class II molecules, either directly by tumor cells or indirectly through uptake and processing by professional antigen -presenting cells such as dendritic cells(40). Despite this, melanoma frequently evades immune detection by altering antigen expression or through post-translational modifications that impair epitope generation and T-cell activation. Many melanomas' antigens contain cysteine residues, making them highly susceptible to oxidation, which can significantly limit epitope binding to MHC class II molecules and reduce $CD4^+$ T cell responsiveness(40). The lysosomal reductase GILT plays a critical role in maintaining these antigens in a reduced state, thereby improving the generation of class II- restricted epitopes and enhancing their immunogenicity(41). Although GILT is abundantly expressed in activated dendritic cells, its expression varies widely across melanoma tissues. Notably, GILT has also been shown to influence the expression of lysosomal proteases, suggesting that it may regulate broader aspects of antigen processing machinery within melanoma cells and surrounding immune cells(42).

This highlights the potential significance of GILT as a modulator of antigen presentation and a possible target for improving melanoma immunotherapy strategies(40).

GILT expression can be upregulated in melanoma and various tumor cells following stimulation with IFN-γ. In antigen-presenting cells (APCs), GILT is initially produced as a proenzyme within early endosomes, while the fully mature and enzymatically active form localizes predominantly to late multivesicular endosomes and lysosomes(40). As an enzyme optimized for function in acidic environments, GILT reduces disulfide bonds within internalized proteins and peptides, thereby facilitating their subsequent degradation by lysosomal cathepsins(40). Through this activity, GILT influences both $CD4^+$ and $CD8^+$ T-cell responses, playing roles not only in classical antigen processing but also in cross-presentation pathways utilized by dendritic cells(40). In melanoma, however, the contribution of GILT to HLA class II–restricted antigen presentation remains less clearly defined compared with professional APCs(40). Normally, HLA class II molecules assemble with the invariant chain (Ii) in the endoplasmic reticulum, and Ii is progressively degraded by endolysosomal proteases, leaving CLIP within the peptide-binding groove. Concurrently, antigens undergo processing by acidic proteases and GILT within the endocytic pathway(40). The peptide editor HLA-DM then removes CLIP and assists the loading of stably processed peptides onto class II molecules for presentation to $CD4^+$ T cells. Given that GILT expression varies substantially among melanoma cells and infiltrating APC populations, clarifying how GILT regulates class II antigen processing specifically in melanoma is of considerable interest(40). A deeper understanding of this mechanism may inform the design of improved dendritic cell–based vaccines and other immunotherapeutic strategies for melanoma and additional solid tumors(40).

1.6. Current therapeutic strategies:

Combination therapy, such as chemotherapy combined with surgery or radiotherapy, remains one of the most common treatment strategies(43). Chemotherapeutic agents vary depending on the type and stage of cancer; however, most tumors eventually develop resistance through mechanisms like apoptosis evasion, adaptation to cellular stress, and enhanced DNA repair, leading

to treatment failure(44). In contrast, targeted therapy represents a modern approach that specifically inhibits key molecules or signaling pathways responsible for cancer cell growth and proliferation, thereby limiting tumor progression(45). Targeted therapy represents a modern cancer treatment strategy that uses drugs designed to specifically interfere with key proteins essential for cancer cell growth and survival, therefore, inhibiting these proteins could inhibit cancer progression(46). Targeted delivery strategies offer a promising solution, such as antibody–drug conjugates (ADCs) that selectively direct drugs to cancer cells or the tumor microenvironment, minimizing off-target effects(47). Lessons from past clinical trials, even those halted due to adverse effects, provide critical insights for refining inhibitor design and optimizing therapeutic applications in melanoma treatment(48). In recent years, autophagy has emerged as a potential therapeutic target in melanoma treatment. This catabolic process involves the sequestration of cytoplasmic components within double-membrane vesicles and their subsequent degradation in lysosomes(49). Autophagy plays a dual role in melanoma, functioning both as a mechanism for cell survival and as a tumor suppressor pathway(50). Evidence suggests that its role may vary depending on disease stage: early-stage cutaneous metastatic melanoma is associated with reduced autophagic activity, as indicated by Beclin 1 downregulation and elevated p62 levels, whereas advanced-stage disease shows increased autophagy, with higher LC3-II expression and decreased p62 levels(51,52).

These findings highlight the complex involvement of autophagy in melanoma progression and suggest that modulation of autophagic pathways could provide therapeutic benefits(18).

2. Cathepsins

2.1. Overview of Cathepsins and their pathological role:

The name *Cathepsin* comes from the Greek *Kathepsein*, translating to “to digest” or “to boil down.” It was originally used to refer to a protease isolated from the gastric mucosa that functions optimally in mildly acidic conditions(54). Cathepsins contain the main family of proteases and are classified to Serine (cathepsin A and G),

Cysteine (cathepsin B, C, F, H, K, L, O, S, V, W, and X) or Aspartyl (cathepsin D and E) proteases, according to the amino acid situated in the active site(55,56). Normally present inside the lysosomes as inactive proenzymes- proforms-, Once released in extracellular space, Cathepsins contribute to metastatic potential by facilitating cell migration and invasiveness through various mechanisms (55,57). These mechanisms involve the degradation of the extracellular matrix and basement membrane, modulation of the tumor microenvironment, regulation of immune cell activity, and enhancement of angiogenesis(58). Each cathepsin plays a distinct role in the progression of skin malignancies, depending on its specific biological characteristics(45). There is a study using Mendelian randomization (MR) to explore the causal relationship between cathepsin and skin cancer(55). This study found that Cathepsins are essential proteolytic enzymes that contribute to a wide range of cellular processes in the skin, including intracellular protein degradation, maintenance of epidermal homeostasis, and regulation of hair follicle development(59). Variations in the structural composition and genetic sequences of different proteases lead to distinct functional roles in the initiation and progression of cutaneous malignancies(55). Cathepsins are frequently localized around the periphery of tumors, as reported in previous studies. Moreover, the enzymatic activity of cathepsins within tumor cells has been shown to correlate positively with pH levels(32). The processes of tumor invasion and metastasis are closely associated with the degradation of the extracellular matrix (ECM)(60).

Cathepsins possess the ability to hydrolyse components of the ECM; however, their precise role in promoting tumor cell proliferation, invasion, and metastasis remains to be fully elucidated(55). Research indicates that cathepsin proteins, such as Cathepsin L2, promote skin cancer, while others, like Cathepsin H, exhibit inhibitory effects on BCC and malignant melanoma(61).

Reverse causal analysis suggests that squamous cell carcinoma may upregulate the expression of cathepsin O, indicating a complex interplay between these proteases and skin cancer development(62). This study refers to the dual roles of cathepsin: some of them facilitate tumor growth and metastasis, while others act as potential biomarkers for early detection or therapeutic targets(63).

Cathepsin are classified as: Cysteine Cathepsins, Aspartyl Cathepsins and serine Cathepsins.

- Aspartyl proteases: They are family of cathepsins. They hydrolyze peptic bonds inside polypeptide chains. It consists of Cathepsin D and Cathepsin E(64).

-Serine protease: Serine protease is also another family of cathepsins. Many immune cells utilize serine proteases in their mechanisms of defense against pathogens, Dysregulation of the serine protease activity from these cells causes different diseases(108). In addition to well-known serine proteases such as Cathepsin G and FAP-α, melanoma progression involves a wider range of serine proteases that influence both tumor behavior and the immune microenvironment. These enzymes contribute to extracellular matrix remodeling, facilitate tumor cell migration, and can modulate immune responses, including leukocyte activation and chemotaxis(21).

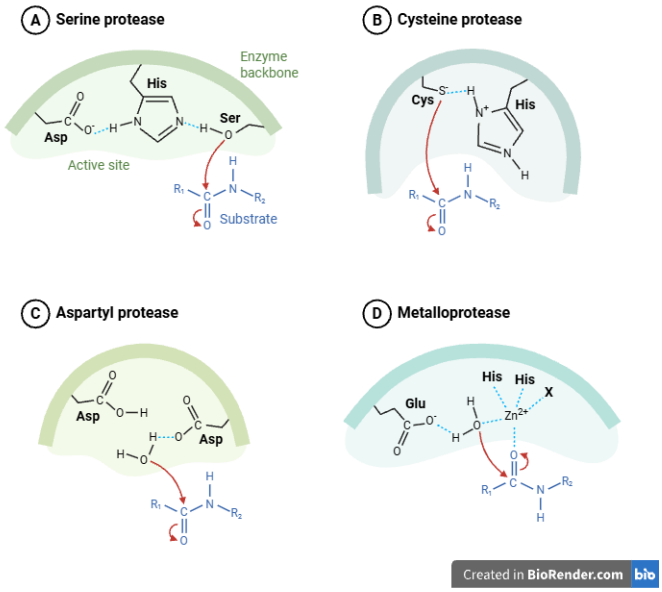

Figure4, Overview of the different classes of cathepsins and their structural characteristics, including cysteine, serine, and aspartyl cathepsins.

2.2. Cysteine cathepsins:

A family of homologous enzymes with a broad spectrum of functions in most tissues and cell types(19). They belong to the papain family of cysteine proteases Cysteine cathepsins also have highly specific and directed proteolytic activity(65).

According to the MEROPS database, the human protease repertoire comprises more than 700 enzymes among which 11 are cysteine cathepsins. These enzymes represent the largest subgroup of cathepsins and belong to the C1a family within the CA clan. The 11 known cysteine cathepsins include B, C (also known as J), F, H, K (or O2), L, O, S, V (or L2), W, and X (also referred to as Z or P(19,20,25). Cysteine cathepsins are predominantly localized within the endolysosomal system, where they act as major proteolytic enzymes. They play crucial roles in various lysosome-associated processes, including protein degradation, lipid metabolism, antigen presentation, autophagy, and lysosome-mediated cell death(67). However, their presence has also been detected in other cellular compartments, such as the cytosol, nucleus, and secretory vesicles(54). Dysregulation of cysteine cathepsins-whether through increased expression or reduced inhibition by endogenous inhibitors-can contribute the onset and progression of numerous pathologies(67). Aberrant activity or mislocalization of these enzymes has been implicated in a wide range of inflammation-associated diseases, including cancer, arthritis and other joint disorders, psoriasis, cardiovascular diseases, bone disorders, and obesity(57). Conversely, loss-of-function mutations in genes encoding specific cysteine cathepsins are linked to several disorders, such as pycnodysostosis, Papillon–Lefèvre syndrome, myopia, and cer tain lysosomal storage diseases(54).

E-64 is a potent and irreversible peptide inhibitor of cysteine proteases, with IC50 values of 2.5, 1.4, and 4.1 nM for recombinant cathepsins L, K, and S, respectively(60). Treatment with E-64 at concentrations ranging from 1 to 20 μM for 24 hours effectively inactivated Cat S(61). However, despite this substantial inhibition, no significant effect on cell migration was observed in these cells. In contrast, there are developed reversible inhibitors, 6n and 6w, at concentrations below 20 μM, not only effectively inactivated Cat S but also reduced cell migration in both tested cancer cell lines(62). These observations suggest that 6n and 6w may be more effective than E-64 in limiting cancer cell migration. In conclusion, α-ketoamide compounds bearing 6n and 6w, with distinct chemical structures on their side chains, have been confirmed to inhibit Cat S activity in cancer cells and potently suppress cell migration in CL1-3, A2058, or both cell types(52). These results underscore the potential of α-ketoamide-based therapy to prevent or delay cancer progression and metastasis(62).

2.2.1 Cysteine cathepsins structure and function:

Structurally, cysteine cathepsins are monomeric proteases with molecular weights ranging from approximately 20 to 35 kDa, except for cathepsin C, which forms a tetramer with an overall mass of about 200 kD. They share the typical papain-like fold(68). Each enzyme consists of two distinct domains-left and right-that together form a catalytic cleft containing a cysteine–histidine catalytic dyad(54,55), most cysteine cathepsins function primarily as endopeptidases; however, cathepsins B, C, H, and X also display exopeptidase activity. Among them, Cathepsins C and X act strictly as exopeptidases-specifically as an aminodipeptidase and a carboxy-monopeptidase, respectively(48).

In contrast, cathepsins B and H exhibit structural versatility that enables them to perform both endo- and exopeptidase functions(54). In cathepsin B, this dual activity is mediated by an occluding loop containing a histidine residue that forms a salt bridge with the enzyme core under acidic conditions(66). This configuration restricts substrate binding to the C-terminal dipeptidyl end. At higher pH levels, histidine deprotonation increases the flexibility of the occluding loop, disrupting the salt bridge and restoring endopeptidase activity(54). For cathepsin H, its aminopeptidase activity arises from steric hindrance caused by a short peptide segment, known as the mini-chain—an octapeptide remnant of the propeptide that remains covalently linked to the enzyme via a disulfide bridge(54).

Functionally, most cysteine cathepsins exhibit optimal activity under slightly acidic conditions and tend to be unstable at neutral pH. An exception is cathepsin S, which remains stable and active even under neutral to mildly alkaline pH. Interestingly, cysteine cathepsins can maintain their structural integrity and catalytic activity near neutral pH when bound to certain polysaccharides(66). For instance, binding of cathepsin B to heparin has been shown to enhance its stability under neutral conditions(54). Beyond heparin, other glycosaminoglycans (GAGs) also modulate cysteine cathepsin function. Chondroitin-4-sulfate, chondroitin-6-sulfate, and keratan sulfate have been reported to enhance the collagenolytic activity of cathepsin K, whereas dermatan sulfate, heparan sulfate, and heparin exert inhibitory effects. Conversely, chondroitin-4-sulfate can reduce the collagen-degrading activity of

cathepsins S and L(54). Moreover, glycosaminoglycans play an essential role in promoting autocatalytic activation of cysteine cathepsins, as demonstrated for cathepsins B, L, and S. This process can occur even at near-neutral pH, which is physiologically significant since many cathepsins are secreted as inactive zymogens before activation(54).

2.2.2. Physiological and pathological roles of Cysteine cathepsins:

In addition to lysosomal functions, cathepsins also have intracellular important roles in the nucleus, plasma membrane, and extracellular roles(69). Intracellular cathepsins degrade proteins that restrict cancer progression, while extracellular cathepsins directly cleave extracellular matrix and activate pro-invasive proteases in the tumor microenvironment(55,57)(28).

-Intracellular Roles: Since cysteine cathepsins exert their primary functions within the Endo lysosomal system, they must first be accurately trafficked to the lysosomes. These enzymes are initially synthesized in the endoplasmic reticulum (ER) as inactive precursors (preprocathepsins) composed of three parts: a signal peptide, a propeptide, and a catalytic domain(66). The signal peptide directs the nascent polypeptide into the ER lumen, where it is glycosylated with mannose residues following removal of the signal peptide. Subsequent phosphorylation of these mannose residues allows the procathepsins to be recognized by mannose-6-phosphate (M6P) receptors in the trans-Golgi network (TGN)(54). From the TGN, procathepsins are transported to lysosomes either directly or indirectly via the plasma membrane. Once in the lysosomal environment, proteolytic cleavage of the N-terminal propeptide converts these precursors into their mature, enzymatically active forms(54). An exception to this classical pathway is cathepsin H, which has been shown to utilize an M6P-independent sorting mechanism mediated by sortilin, another receptor present in the TGN(54). Within lysosomes, cysteine cathepsins not only participate in general protein degradation and turnover, but also perform specialized functions associated with both the innate and adaptive immune systems. Furthermore, they have been implicated in the regulation of autophagy(71).

For instance, cathepsin B has been shown to influence the formation of lysosomes and autophagosomes by cleaving the calcium channel MCOLN1/TRPML1 (transient receptor potential cation channel, mucolipin subfamily, member 1), thereby inhibiting downstream activation of transcription factor EB (TFEB)a key regulator of lysosomal biogenesis and autophagy-related gene expression(54).

Cysteine cathepsins also modulate cellular responses to growth factors. Specifically, cathepsins B and L have been reported to affect the cellular response to insulin-like growth factor-1 (IGF-1), particularly under pathological conditions such as metabolic or proliferative disorders(66).

Interestingly, several cathepsins have also been detected in the cell nucleus, where they participate in regulatory molecular events. In mice, a nuclear isoform of cathepsin L-which lacks the signal peptide can enter the nucleus and regulate the cell cycle by cleaving the transcription factor CDP/Cux (CCAAT-displacement protein/Cut homeobox), a key regulator of the G1/S phase transition(72). Moreover, cathepsin L has been shown to cleave the N-terminal tail of histone H3 during embryonic stem cell differentiation, thus influencing gene expression and chromatin remodeling. In humans, however, this nuclear function appears to be primarily mediated by cathepsin V(54).

- **Extracellular Roles:** Although cysteine cathepsins primarily function intracellularly, they are also secreted into the extracellular space under both physiological and pathological conditions(71). Physiologically, their secretion occurs during processes such as bone remodeling, wound healing, and prohormone processing. Pathologically, extracellular secretion of cysteine cathepsins is more pronounced in conditions such as cancer and inflammation(54). Immune cells are the major source, with macrophages secreting cathepsins at concentrations up to 100 nm. Other cell types, including thyroid cells, keratinocytes, osteoclasts, and smooth muscle cells, also contribute to extracellular cathepsin levels, albeit at lower concentrations(73). Secretion is often driven by overexpression via TFEB or STAT signaling pathways and can be further enhanced by cytokines and interleukins, including TNF, IL-1α, IL-1β, and IFNγ. For instance, IL-1β increases cathepsin K secretion from osteoclasts via LC3-II-mediated mechanisms(54). Extracellular release occurs mainly

through lysosomal exocytosis or alternative secretory pathways. Lysosomes are transported to the cell periphery along macrotubules, dock via SNARE complexes in a calcium-dependent manner, and fuse with the plasma membrane, releasing active cathepsins(74). Additionally, procathepsins can be rerouted to the extracellular space or packaged into secretory vesicles, while purinergic P2X7 receptor activation in hematopoietic cells can trigger cathepsin release in inflammatory conditions(54). Once extracellular, cathepsins remain relatively stable in their inactive zymogen form, which is resistant to neutral pH and oxidative stress. In pathological environments, local acidity favors cathepsin activation and secretion. Some extracellular cathepsins associate with the cell membrane, which protects them and retains activity at the secretion site(75). Cathepsin S co-localizes with integrin αvβ3 on smooth muscle cells, cathepsin X interacts with heparan sulfate proteoglycans and activates β2-integrins to support dendritic and lymphocyte adhesion, and cathepsin B associates with annexin II in caveolae, where it can activate receptor-bound pro-uPA(54).

2.2.3. Cysteine cathepsins in cancer:

In cancer cells, cathepsin protein and mRNA are increased, and over procathepsins are exocytosed resulting in increased localization at the plasma membrane, in endocytic vesicles, and extracellularly(57,76). Cysteine cathepsins have long been recognized as key contributors to cancer progression. Numerous animal models and clinical studies provide strong evidence supporting the critical involvement of several cathepsins in tumor development and metastasis(71). Cysteine cathepsins contribute to multiple aspects of tumorigenesis, including cell transformation, differentiation, motility, adhesion, invasion, angiogenesis, and metastasis, their expression and activity are often elevated in epithelial and mesenchymal cancers, such as breast, lung, brain, colorectal, gastrointestinal, and melanoma(54). As key lysosomal proteases, cysteine cathepsins support cancer cell proliferation and survival by promoting autophagy and protein catabolism, potentially via mTOR signaling(33). Under stress, lysosomal membrane permeabilization can release cathepsins into the cytosol, where they cleave pro- and anti-apoptotic proteins, such as Bid, Bcl-2, and Bcl-xL, thereby modulating apoptosis(54).

Cathepsins B and L contribute to cancer cell proliferation by modulating ERK/MAPK signaling and regulating cell cycle proteins, such as p27^Kip1 and the CDP/Cux transcription factor, with loss of their expression or activity reducing proliferation. During tumor progression, cysteine cathepsins are frequently secreted by tumor and stromal cells, including endothelial cells, fibroblast s, and tumor-associated macrophages(54).

Extracellularly, they degrade ECM components, remodel tissue, and facilitate invasion, while also activating proteolytic cascades and other proteases, such as uPA and MMP-9, by direct cleavage or by inactivating inhibitors like TIMPs. Additionally, they modulate cytokines, chemokines, and growth factors, promoting pro-tumorigenic inflammation(54). The tumor-promoting effects of cysteine cathepsins are highly context-dependent, varying with cell type and tissue.

For example, cathepsin B enhances proliferation, invasion, and angiogenesis in RIP1-Tag2 PanNET and MMTV-PyMT mouse models but shows no significant role in the K14-HPV16 squamous cell carcinoma model(54). Conversely, cathepsin C has little impact on PanNET or mammary tumor development, yet its secretion by stromal cells in SCC contributes to tumor progression and angiogenesis(54). Cathepsin L demonstrates a unique dual role: its overexpression promotes tumorigenesis in Pan-NET, while its loss in K14-HPV16 mice accelerates carcinogenesis, highlighting its context- dependent capacity to either enhance or suppress cancer development(54).

2.2.4. Cysteine cathepsins in Melanoma:

In melanoma, elevated cathepsin levels drive the transition from non-metastatic to highly aggressive, metastatic tumors(66). Rab7, a key regulator of endosome maturation and intracellular cathepsin activation, plays a critical role in this phenotypic switch: its induction promotes proliferation in early melanoma, whereas reduced Rab7 in later stages enhances cathepsin secretion, facilitating invasion and metastasis(57). Notably, cathepsin B secretion correlates with poor patient survival. Despite their potential as therapeutic targets due to their dysregulation in cancer and association with treatment resistance, few cell-permeable cathepsin inhibitors have reached clinical use because of toxicity, limited efficacy, and compensatory upregulation of other cathepsins(54). Consequently, strategies aimed at modulating cathepsin expression or activity, rather than direct inhibition, may offer more effective

anti-cancer approaches(57). Several types of cysteine cathepsins play crucial roles in the development and metastasis of melanoma(54).

2.2.5. Cathepsin L:

Cathepsin L is a papain-type cysteine protease encoded on human chromosome 9q21–22. It is synthesized as pre-procathepsin L, processed through the Golgi apparatus as procathepsin L, and stored in lysosomes as the mature enzyme(77). Cat L functions primarily as an endopeptidase, degrading a broad range of intracellular cytoplasmic and nuclear proteins(78). In addition, procathepsin L is secreted by normal and transformed cells, where it modulates extracellular proteins, such as complement component C3, thereby reducing complement-mediated cytotoxicity, and generating endostatin from collagen XVIII, suggesting a role in angiogenesis(79). Overexpression and secretion of procathepsin L are associated with tumor progression and metastasis(77). In human melanoma models, antisense inhibition or treatment with anti-cathepsin L antibodies signifi-cantly reduced tumor growth and metastatic potential, whereas increased secretion promoted a transition from non-metastatic to highly metastatic phenotypes(79). These findings highlight the importance of regulating cathepsin L expression and secretion in cancer.

Regulatory studies identified critical promoter regions between –1489 and –1646 bp, including two GC boxes and a CCAAT motif, which interact with transcription factors Sp1, Sp3, and NF-Y, essential for Cat L transcription in melanoma cells.

Targeting cathepsin L secretion using molecular tools, such as single-chain variable fragment (ScFv) antibodies, represents a promising strategy to inhibit tumor growth and metastasis(80). Studies demonstrate for the first time that anti-cathepsin L single-chain variable fragment (ScFv) strongly inhibits procathepsin L secretion in human melanoma cells(80).

Recent studies have identified Cat L as a downstream target of hypoxia-inducible factor-1 (HIF-1α) in melanocytes. Under low-oxygen conditions, HIF-1α upregulates Cat L expression, promoting lysosomal degradation of melanosomes through autophagy-related pathways. This process ensures the turnover of pigment-containing organelles and regulates intracellular protein homeostasis(81).

Experimental inhibition of Cat L with 3D8 leads to accumulation of melanosomal proteins and markers of autophagic flux, indicating that proper Cat L activity is essential for melanosome clearance(78). These findings highlight a previously underappreciated intracellular role of Cathepsin L in melanocytes, linking hypoxic signaling to organelle degradation, and suggest that dysregulation of this pathway could impact melanoma biology by altering intracellular proteostasis and potentially affecting tumor progression(81). Cathepsin L represents a promising biomarker and potential therapeutic target. Future studies focusing on selective modulation of its activity or secretion may provide novel strategies to limit tumor growth, enhance treatment efficacy, and improve patient outcomes in melanoma.

2.2.6. Cathepsin K:

Cathepsin K (Cat K) is a lysosomal cysteine protease with potent collagenolytic and elastolytic activity, traditionally associated with bone resorption by osteoclasts. Recent evidence indicates that Cat K is also expressed in skin fibroblasts, suggesting a broader role in extracellular matrix (ECM) turnover(82). Given the importance of matrix degradation in tumor invasion and metastasis and investigated Cat K in melanoma. Immunohistochemical analysis revealed moderate to strong cytoplasmic Cat K expression in most primary cutaneous melanomas, including in situ lesions, thin invasive melanomas (Clark II), and thick invasive melanomas (Clark IV)(83). Strong staining was observed in both the dermal and epidermal compartments, with melanoma cells infiltrating the epidermis (pagetoid spread) showing positivity, whereas overlying keratinocytes remained negative. Some peritumoral stromal fibroblasts also expressed Cat K. All examined cutaneous melanoma metastasis (n=6) exhibited uniformly strong Cat K expression(83). Melanocytic nevi expressed Cat K as well, but at lower levels than melanomas. Functionally, melanoma cells express both pro- and active forms of Cat K and internalize extracellular collagen into lysosomes(83). Inhibition of Cat K significantly reduced melanoma cell invasion through Matrigel and increased detection of internalized collagen, suggesting that Cat K contributes to melanoma invasion and metastasis by mediating intracellular degradation of phagocytosed matrix

proteins(84). In contrast, non-melanoma skin cancers, such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), showed little to no Cat K expression in tumor cells, although stromal fibroblasts often stained strongly. These findings highlight a potential unique role of Cat K in melanoma progression(83).

In invasion assays using MeWo and MMAN melanoma cells, treatment with a cathepsin K inhibitor markedly reduced cell invasion through Matrigel-coated membranes. In contrast, migration through uncoated control membranes was unaffected, indicating that the inhibitor was not cytotoxic and did not impair cell motility(83). With diluent treatment, 56.9% of MeWo cells and 50.4% of MMAN cells invaded through Matrigel, corresponding to 0.6% and 0.3% of plated cells, respectively(83). Treatment with the highest concentration of the Cat K inhibitor reduced invasion by 89% for MeWo and 98% for MMAN cells, with a clear dose-dependent effect observed. LIBR cells did not migrate through the uncoated membranes and were therefore excluded from invasion analyses(83,84).

Cat K is not only involved in ECM degradation but also exhibits diagnostic and potential therapeutic relevance in melanoma(85). Immunohistochemical studies show that Cat K is frequently expressed in primary and metastatic melanomas, including cases where conventional melanocytic markers such as HMB45 or Melan-A are negative(83). This suggests that Cat K could serve as an additional immunohistochemical marker to support melanoma diagnosis. Although Cat K is also present in benign melanocytic nevi, its expression is generally lower compared to malignant lesions, highlighting its relative specificity for melanoma progression(86). From a therapeutic perspective, targeting Cat K may offer opportunities to inhibit melanoma invasion and metastasis. Experimental evidence indicates that pharmacologic inhibition of Cat K or modulation of upstream pathways, such as mTOR signaling, could potentially reduce its activity in tumor cells(83). These findings position Cat K not only as a marker of melanoma aggressiveness but also as a candidate for novel treatment strategies aimed at limiting tumor spread(86).

2.2.7. Cathepsin S:

Cathepsin S (Cat S) is a single-chain, non-glycosylated cysteine protease uniquely active at neutral pH among the 11 cathepsin family members. It shows the highest sequence similarity and identity with Cathepsins K and L, with which it shares a conserved cysteine–histidine catalytic site(87). Cat S

contributes to tumor invasion and metastasis by promoting angiogenesis and degrading the extracellular matrix. However, its prognostic value and role in modulating the tumor microenvironment and immune infiltration across cancers remain unclear(58). Studies showed that comprehensive bioinformatic analyses revealed aberrant Cat S expression and distinct genomic alterations in multiple cancer types(88). Cat S expression correlated significantly with patient prognosis, showing that high Cat S levels were associated with improved overall and disease-specific survival in bladder urothelial carcinoma (BLCA) and skin cutaneous melanoma (SKCM), but with poorer outcomes in brain lower-grade glioma (LGG) and uveal melanoma (UVM)(88). In the previous studies and experiments, Cat S inhibitors were shown to effectively reduce intracellular Cat S activity and cell migration into cancer cells. In cells, 6n and 6w at 5 μM inhibited over 50% of Cat S activity and significantly decreased cell migration(89). The findings suggest that Cathepsin S is associated with cancer cell migration and invasion and may serve as a promising therapeutic target to inhibit these processes(87).

2.2.8. Cathepsin B:

Cathepsin B is a cysteine protease that normally functions as an endopeptidase within the endolysosomal compartments of healthy cells(75). However, during tumor development, its regulatory mechanisms can become dysregulated, leading to increased expression and secretion of the enzyme into the extracellular environment. This dysregulation suggests that Cathepsin B may play a significant role in the molecular alterations associated with cancer progression(90). Cat B has been implicated in promoting tumor growth and enhancing the metastatic potential of human melanoma(91). It was demonstrated that the specific cathepsin B inhibitor CA-074-two cathepsin B inhibitors: CA074 (cell unpermeant) and CA-074Me (cell permeant) (both given at a concentration of 10 μM, as well as antibodies targeting cathepsin B, could independently- without the need for any additional therapeutic agents- exert strong anti-invasive effects by interfering with the mechanisms responsible for metastatic cell dissemination(92). Inhibiting cathepsin B may alter the tumor microenvironment, reduce cancer cell survival and dissemination, and consequently impair metastatic initiation and progression(92,93).

Cathepsin B is increasingly recognized for its role in cancer diagnosis, prognosis, and therapy. Its abnormal expression is associated with tumor aggressiveness and metastatic behavior across multiple cancer types(93). The enzyme can degrade key components of the extracellular matrix and basement membrane, including laminin, fibronectin, and type IV collagen, thereby facilitating tumor invasion and distant dissemination(53). In addition, Cat B can indirectly enhance the activity of other proteases, further promoting tumor progression. Experimental approaches that reduce Cathepsin B expression, such as RNA interference, have been shown to decrease tumor invasiveness(94). While considerable progress has been made in exploring Cat B as a target in combination with conventional chemotherapies, its precise contributions to the proliferation and biological behavior of primary human cutaneous melanoma remain to be fully clarified(53).

Cepharanthine (CEP) is a natural alkaloid with multiple pharmacological properties, including anti-cancer, anti-inflammatory, and immunomodulatory effects. Recent studies suggest that CEP may inhibit primary cutaneous melanoma by targeting Cathepsin B and modulating autophagy(53). Cathepsin B, highly expressed in melanoma, promotes tumor invasion, metastasis, and fibroblast activation, and is linked to autophagy regulation and malignant transformation of melanocytes. CEP has been shown to inhibit Cathepsin B maturation, block autophagosome-lysosome fusion, and induce apoptosis, cell cycle arrest, and autophagy-related cell death in various cancer cells(53). In melanoma models, CEP downregulates Cathepsin B and autophagy-related proteins (LC3-I/II) while increasing tumor suppressor proteins such as p53, p21, and p16, resulting in reduced tumor growth. Moreover, CEP demonstrates good safety in topical or intra-tumoral application, with minimal skin irritation(53). While monotherapy effects in vivo may be limited, combining CEP with chemotherapeutic agents or integrating it into systemic therapy could enhance its anti-melanoma efficacy. These findings highlight CEP as a promising candidate for regional therapeutic strategies against primary cutaneous melanoma, although further studies on molecular mechanisms and biosafety are needed(53).

2.2.9. Cathepsin C:

Cathepsin C, a cysteine protease encoded by the Cat C gene, is an important enzyme that participates in controlling the development and behavior of different tumor types(95). Cat C is a lysosomal cysteine protease that plays a crucial role in immune regulation and inflammation. It is responsible for activating several serine proteases involved in antimicrobial defense, inflammatory responses, and programmed cell death. Through these functions, Cat C contributes to maintaining immune balance and tissue homeostasis(96). Dysregulation of its activity can lead to excessive inflammation and tissue damage, making it a potential therapeutic target for various immune and inflammatory conditions. Additionally, genetic alterations in Cat C have been linked to inherited disorders affecting immune and oral health functions(96). Chronic inflammation is linked to increased cancer risk, making protease inhibition a potential therapeutic strategy. Cathepsin C activates neutrophil serine proteases (NSPs), which contribute to inflammatory processes and the formation of neutrophil extracellular traps (NETs)(97). Elevated Cat C expression has been observed in various tumors and is associated with metastasis and poor patient outcomes(98). Tumor-derived Cat C promotes neutrophil recruitment in the tumor microenvironment, enhancing inflammation and supporting tumor progression. Pharmacological inhibition of Cat C, such as with brensocatib, may reduce NSP activity safely, offering a potential approach for cancer therapy beyond its anti-inflammatory effects(99).

To date, no studies have directly demonstrated a specific role of Cathepsin C in the development or progression of cutaneous melanoma. Although Cat C has been implicated in promoting tumor progression, inflammation-driven carcinogenesis, and metastatic behavior in several other cancer types, its functional involvement in melanoma biology remains largely unexplored(100). This absence of direct evidence highlights a significant gap in the current literature and underscores the need for further investigation into the potential contribution of Cat C to melanoma pathogenesis(100).

2.2.10. Cathepsin H:

Cathepsin H (Cat H) is a lysosomal cysteine protease that was first identified in rat liver lysosomes in 1976 and later isolated from human liver. This enzyme belongs to a family comprising eleven cysteine proteases(101). Although Cat H shares some aminopeptidase and endopeptidase activities with other cysteine proteases, it has recently attracted increasing scientific attention due to its involvement in a wide range of physiological and pathological processes(102). These include tumor progression and metastasis, chemoresistance, inflammatory responses, atherogenesis, type 1 diabetes mellitus, and neurotransmitter production(103). A neutral pH level is perfect for Cat H activity, so it is expected to be active in the extra-lysosomal and extracellular space(104). In a clinical study investigating the role of lysosomal proteases in melanoma progression, the serum levels of Cat H were measured in 43 patients with metastatic melanoma, 54 patients with treated cutaneous melanoma without metastasis, and 30 healthy controls, using quantitative ELISA assays(105). The results demonstrated a significant elevation of Cat H levels in patients with metastatic melanoma compared to healthy individuals, with median concentrations of 13.7 ng/ml versus 4.9 ng/ml ($P < 0.0001$). Interestingly, Cat H was also significantly elevated in melanoma patients without metastasis, with a median level of 9.6 ng/ml, suggesting that Cat H upregulation may occur early in melanoma progression. Furthermore, patients who did not respond to chemoimmunotherapy exhibited notably higher serum Cat H levels compared to those who responded to treatment, indicating a potential association between elevated Cat H and therapeutic resistance(105). Importantly, metastatic melanoma patients with high Cat H concentrations had a significantly shorter overall survival than those with lower Cat H levels ($P < 0.006$; relative risk = 2.4, using the median as a cutoff). Collectively, these findings indicate that serum Cat H levels may serve as a prognostic biomarker for disease progression, treatment response, and overall survival in patients with advanced melanoma(105). However, despite the strong association, the molecular mechanisms underlying Cat H elevation in early melanoma remain poorly defined.

2.2.11. Cathepsin V:

Cathepsin V (Cat V) is a recently identified human cysteine protease closely related to Cathepsin L, sharing approximately 80% sequence identity. Unlike the ubiquitously expressed Cat L, Cat V expression is highly tissue-specific, being primarily localized to the thymus and testis(106). Phylogenetic analyses suggest that Cat V arose from a relatively recent gene duplication event of the Cat L gene, and that the murine Cat L may represent the ancestral form of both human Cat L and Cat V(107). There is currently no clear evidence demonstrating a direct role of Cathepsin V in melanoma. Due to its high structural and functional similarity to Cathepsin L - which has been implicated in melanoma progression and metastasis-it is possible that Cathepsin V may exert similar effects in skin cancer development(106)4. However,
experimental validation is needed to determine whether Cat V contributes to melanoma progression.

2.2.12. Cathepsin W:

Cathepsin W (Cat W) is primarily expressed in cytotoxic immune cells, including $CD8^+$ T lymphocytes and natural killer (NK) cells, where it is thought to contribute to immune-mediated cytotoxicity rather than routine proteolytic processes(108). Recent pan-cancer analyses have indicated that Cat W expression varies across tumor types and is frequently associated with improved patient outcomes. For example, higher Cat W levels in tumor-infiltrating lymphocytes have been correlated with enhanced anti-tumor immune activity and better prognosis in cancers such as skin cutaneous melanoma, breast cancer, endometrial carcinoma, and pancreatic ductal adenocarcinoma(108). These findings suggest that Cat W may act as an indicator of an active immune response within the microenvironment of the tumor rather than as a direct driver of tumorigenesis(108). Overall, Cat W appears to function as potential biomarker of favorable prognosis, reflecting immune surveillance and cytotoxic activity, with its tumor-related effects largely mediated through the immune context rather than intrinsic tumor cell expression(108). However, the mechanistic contribution of Cat W to anti-tumor immunity in melanoma remains to be fully elucidated.

2.2.13. Cathepsin F:

Although several cathepsins have been genetically associated with skin cancers, the available data on Cathepsin F in melanoma is extremely limited. Recent Mendelian randomization study indicated that higher Cat F is correlated with increased risk of basal cell carcinoma, but no clear causal link with melanoma has yet been established. Thus, while Cathepsin F remains a protease of interest, further research is needed to clarify its role in melanoma development or progression(108,109). This highlights a significant gap in the current literature, underscoring the need for experimental studies to elucidate Cat F's potential role in melanoma pathogenesis(108).

2.2.15. Cathepsin X, O:

At present, there is no robust evidence linking Cathepsin O and Cathepsin X directly to melanoma development, progression, or metastasis. Although Cat O has been implicated in other cancers (for example influencing therapy response in breast cancer), its potential role in melanoma remains unclear and unexplored. This highlights a clear knowledge gap, suggesting the need for focused studies to elucidate whether Cat X and Cat O might have functional roles in melanoma biology.

2.3. Aspartyl proteases:

2.3.1. Cathepsin D:

Cathepsin D is a glycoprotein, member of the aspartyl proteases family, which has an important role in regulating apoptotic pathways in cells(110). Cathepsin D (Cat D) is a lysosomal aspartic protease produced as a 52-kDa proenzyme (procathepsin D, pCD) that normally matures inside endosomes and lysosomes through a series of acidic, enzyme-dependent processing steps(111). Although Cat D is primarily confined to intracellular degradative pathways, many tumors show a disruption of its normal trafficking, resulting in abnormal secretion of both pCD and its active forms(112). High levels of secreted Cat D are characteristic of various cancers, where the enzyme can be re-internalized by tumor or

stromal cells and further activated, thereby supporting processes linked to tumor growth, invasion, and remodeling of the extracellular environment(74). In melanoma, Cat D overexpression and secretion are frequently observed and are associated with enhanced tumor aggressiveness and poorer clinical outcomes. These features highlight Cat D as both a functional contributor to malignant progression and a potential biomarker with diagnostic and therapeutic relevance(112). The studies demonstrate that melanoma cells show markedly elevated levels of cathepsin D expression. The exceptionally high cathepsin D levels observed in two of the patients who later experienced disease progression during a 42-month follow-up period suggest that tissue concentrations of this enzyme may be linked to the prognosis of primary cutaneous malignant melanoma(113). The study demonstrates that Cathepsin D is consistently expressed and enzymatically active in head and neck metastatic malignant melanoma (HNmMM). Cathepsin D was detected at the protein and transcript levels in all tumor tissue samples and was also expressed by melanoma cancer stem cells, as confirmed by immunofluorescence(114). Its presence and activity within these CSC populations suggest that Cathepsin D may contribute to melanoma progression by supporting CSC survival, maintenance, or interaction with the surrounding tumor microenvironment(114,115). These findings point to a potential role for Cathepsin D as part of alternative pathways within the renin–angiotensin system that may influence melanoma behavior and could represent a target for therapeutic intervention(116).

2.3.2. Cathepsin E:

Cathepsin E is an aspartic protease mainly expressed in immune cells and released by activated phagocytes, and accumulating evidence highlights its role in anti-tumor immunity(117). In melanoma, Cathepsin E shows a clear tumor-suppressive effect mediated through TRAIL-dependent apoptosis and enhanced macrophage activity(115). Comparative studies demonstrate that Cathepsin E possesses a unique apoptotic capability: unlike cathepsins B and D, which increased tumor cell viability, or cathepsin L, which induced necrosis, Cat E consistently triggered concentration-dependent apoptosis, confirmed by annexin V and TUNEL staining(115). Although these findings were observed in prostate carcinoma cells, the mechanism aligns with its tumor-suppressive behavior previously demonstrated in B16 melanoma models, providing a rationale for the increased melanoma growth and metastasis seen in Cathepsin E–deficient mice(115). Given the toxicity

associated with many anticancer therapies, targeting apoptosis selectively in tumor cells is highly desirable. Cathepsin E therefore represents a promising therapeutic candidate due to its ability to induce apoptosis specifically in malignant cells without harming normal tissues(115). Enhancing Cathepsin E activity, either directly or by increasing its endogenous expression, may offer a targeted approach for melanoma treatment, with potential application alone or in combination with conventional chemotherapies(115).

2.4. Serine protease:

2.4.1 Cathepsin G:

Cathepsin G is part of a specialized lysosomal serine protease family-alongside elastase and proteinase 3- that is predominantly expressed in neutrophils(118). These serine proteases, characterized by a reactive serine residue, contribute to essential immune functions and can transmit outside-in activation signals that shape leukocyte responses, including cytotoxicity, chemotaxis, and proliferation. Cat G therefore plays a key role in modulating neutrophil-driven immune activity(119). Importantly, serine proteases in melanoma can be therapeutically targeted: plant- derived or synthetic serine protease inhibitors have shown potential in preclinical studies to reduce melanoma cell invasiveness limit metastasis and enhance the efficacy of conventional therapies. Furthermore, environmental factors, particularly ultraviolet (UV) radiation, can upregulate certain serine proteases, which may promote microenvironmental changes that favor tumor progression(118). These observations highlight the dual role of serine proteases in melanoma-as contributors to malignancy and as promising targets for novel therapeutic strategies(24).

-Table2 : cathepsins which have roles in melanoma:

Cathepsin	Family	Role in Melanoma
1-Cathepsin L {CTSL}	Cysteine	-Highly expressed in Melanoma , promotes tumor invasion and metastasis through extracellular matrix -ECM- degradation. - inhibition of CTSL reduces tumor growth and cell migration.
2- Cathepsin K {CTSK}	Cysteine	-Strongly upregulated in primary and metastatic melanoma. contributes to ECM breakdown and enhances invasive capacity. Considered a potential diagnostic and therapeutic marker.
3- Cathepsin S {CTSS}	Cysteine	-Expression levels correlate with patient prognosis in several cancers including melanoma. - CTSS inhibitors have been shown to reduce cell invasion.
4-Cathepsin H {CTSH}	Cysteine	-Implicated in ECM remodelling and may support tumor cell survival and adaptation under stress conditions.
5-Cathepsin C {CTSC}	Cysteine	-Participates in inflammatory pathways and may indirectly influence melanoma microenvironment.

6-Cathepsin W {CTSW}	Cysteine	-Mainly expressed in cytotoxic immune cells, potential role in anti tumor immunity rather than tumor promotion.
7-Cathepsin B {CTSB}	Cysteine	-Frequently overexpressed in melanoma, facilitates invasion by degrading basement membrane components. Linked to tumor progression and poor prognosis.
8- Cathepsin D {CTSD}	Aspartyl	-Secreted protease associated with melanoma proliferation, angiogenesis, and metastatic potential. Elevated CTSD levels correlate with advanced disease stages.
9- Cathepsin E {CTSE}	Aspartyl	-Suppresses melanoma growth and metastasis by inducing TRAIL-mediated apoptosis and enhancing macrophage-driven anti-tumor activity. Its selective pro-apoptotic effect on cancer cells highlights its potential as a targeted therapeutic strategy alone or in combination with conventional treatments.

Table 2:Cathepsins which have role in Melanoma.

3. Future Perspectives:

Lysosomal cathepsins represent a promising therapeutic target in melanoma due to their critical roles in tumor progression, invasion, and metastasis. Future research should focus on developing highly specific inhibitors that selectively target tumor-associated cathepsins while minimizing systemic toxicity. Advanced drug-delivery systems, such as nanoparticles or tumor-targeted conjugates, could enhance efficacy and precision. Expanding preclinical findings into well-designed clinical trials is essential to evaluate safety, optimal dosing, and therapeutic outcomes. Additionally, combining Cathepsin inhibitors with existing therapies, including immunotherapy or targeted BRAF/MEK inhibitors, may overcome resistance mechanisms and improve patient responses. Investigating cathepsins as predictive biomarkers for therapy response or disease progression could further support personalized treatment strategies. Finally, a deeper understanding of cathepsin-mediated interactions within the tumor microenvironment will inform the design of next-generation therapies to more effectively disrupt melanoma growth and metastasis.

4.Conclusion:

In conclusion, lysosomal and membrane-associated cathepsins- including cysteine, aspartic, and serine proteases-play critical roles in melanoma progression, metastasis, and tumor microenvironment remodeling. Their dysregulated expression not only drives tumor aggressiveness but also presents promising therapeutic opportunities. Targeting cathepsins, either alone or in combination with existing therapies, have shown encouraging preclinical results and potential clinical benefits. Future research should focus on developing highly specific inhibitors, minimizing systemic side effects, and exploring their utility as biomarkers for early detection and therapy response, ultimately advancing precision medicine approaches in melanoma treatment.

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