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Lysosomal cathepsins as therapeutic targets in pancreatic cancer

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Rim Bashir BSc

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Betreut von | Supervisor:	Dr. Mohammed Aufy Privatdoz.
Mitbetreut von | Co-Supervisor:	Ahmed Mohamed Mahmoud Hussein B.Sc. M.SC. PhD PhD

Abstract

Pancreatic cancer remains one of the most lethal malignant tumors worldwide, characterized by late diagnosis, rapid progression, and significant therapeutic resistance. Among its subtypes, pancreatic ductal adenocarcinoma (PDAC) accounts for the majority of cases and exhibits a 5- year survival rate that has remained very low despite advances in cancer genetics and pharmacology. The complexity of PDAC is driven by a combination of oncogenic mutations, such as a dense stroma and substantial reprogramming of proteolytic pathways that enable invasion and metastasis. Increasing evidence highlights the lysosome as a central regulator of tumor cell survival, controlling nutrient recycling and proteolysis under conditions of metabolic and environmental stress.

Within the lysosomes, cathepsins- a diverse family of cysteine, aspartic and serine proteases- play important roles in protein degradation, signaling, extracellular matrix (ECM) remodeling, and regulation of tumor and stroma interactions. Many cathepsins, including cathepsin E, B, L and S are frequently upregulated or displaced in pancreatic cancers, where they contribute to malignant behaviors such as invasion, metastasis and angiogenesis. Their activity is influenced by the acidic tumor microenvironment (TME), disrupted cellular transport pathway, and the high autophagic flux that characterizes PDAC. Because PDAC is particularly dependent on lysosomal function and autophagy for metabolic maintenance, cathepsins have become promising therapeutic targets.

This thesis explores lysosomal cathepsins in pancreatic cancer, focusing on their biological roles, how they are regulated, and why they could be important targets for therapy within the lysosome and autophagy system. Special attention is given to special ways to interfere with cathepsin activity, such as using small molecule inhibitors that block them. By bringing together what is currently about lysosome biology, cancer metabolism, and protease function, this work aims to provide a clear understanding of both the opportunities and challenges in targeting cathepsins in PDAC. Ultimately, it seeks to support the development of new and more effective strategies for treating this highly aggressive and difficult to treat cancer.

Abstract (deutsch)

Pankreaskrebs bleibt einer der tödlichsten Tumoren weltweit, gekennzeichnet durch späte Diagnosestellung, rasche Progression und ausgeprägte therapeutische Resistenz. Unter den verschiedenen Subtypen stellt das pankreatische duktale Adenokarzinom (PDAC) die häufigste Form dar und weist trotz erheblicher Fortschritte in der Krebsgenetik und Pharmakologie weiterhin eine sehr niedrige 5- Jahres Überlebensrate auf. Die hohe Komplexität des PDAC beruht auf einem Zusammenspiel onkogener Mutationen, sowie einem dichten Stroma und einer umfassenden Umprogrammierung proteolytischer Signalwege, die Invasion und Metastasierung begünstigen. Zunehmende wissenschaftliche Erkenntnisse identifizieren das Lysosom als einen zentralen Regulator des Tumorzellüberlebens, da es unter metabolischem und umweltbedingtem Stress wesentlich zur Nährstoffrückgewinnung und Proteolyse beiträgt.

Innerhalb der Lysosomen übernehmen Cathepsine- eine heterogene Familie von Cystein-, Aspartat- und Serinproteasen- zentrale Funktionen beim Proteinabbau, in der Signaltransduktion, beim Umbau der extrazellulären Matrix (ECM) sowie bei der Regulation der Interaktionen zwischen Tumorzellen und dem umgebenden Stroma. Zahlreiche Cathepsine, darunter Cathepsin E, B, L und S, sind im Pankreaskarzinom häufig überexprimiert oder fehlreguliert und tragen wesentlich zu malignen Eigenschaften wie Invasion, Metastasierung und Angiogenese bei. Ihre Aktivität wird durch das saure Tumormilieu, gestörte intrazelluläre Transportprozesse sowie den erhöhten autophagischen Flux beeinflusst, der charakteristisch für PDAC ist. Da PDAC- Zellen in besonderem Maße von lysosomaler Funktion und Autophagie zur Aufrechterhaltung ihres Stoffwechsels abhängig sind, stellen Cathepsine vielversprechende therapeutische Targets dar.

Diese Arbeit untersucht die Rolle lysosomaler Cathepsine im Pankreaskarzinom mit besonderem Fokus auf ihre biologischen Funktionen, ihre regulatorischen Mechanismen sowie ihr Potenzial als therapeutische Angriffspunkte im Kontext des lysosomalen Systems und der Autophagie. Ein besonderer Schwerpunkt liegt auf Strategien zur gezielten Hemmung der Cathepsinaktivität, insbesondere durch den Einsatz niedermolekularer Inhibitoren. Durch die Zusammenführung aktueller Erkenntnisse aus der Lysosomenbiologie, dem Tumormetabolismus und der Proteaseforschung soll diese Arbeit zu einem vertieften Verständnis der Chancen und Herausforderungen beitragen, die mit der therapeutischen Nutzung von Cathepsinen im PDAC verbunden sind. Langfristig soll sie damit die Entwicklung neuer und wirksamer Behandlungsstrategien für diese hochaggressive und therapeutisch schwer behandelbare Tumorerkrankung unterstützen.

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Introduction

Cancer represents a major health burden, responsible for millions of deaths annually across the world. It describes a group of diseases that are all defined by the uncontrolled growth, survival and spread of abnormal cells [1]. Although individual cancer types differ in their behavior, they share several biological characteristics. These include the ability to grow without normal signals, avoid growth suppression, resist cell death, induce the formation of new blood vessels, invade surrounding tissue and spread to distant organs [2], [3], [4]. Despite remarkable progress in cancer research, many cancers- especially those detected late- remain difficult to treat. Among them pancreatic cancer stands out as one of the most aggressive and treatment resistant forms [5].

Pancreatic ductal adenocarcinoma is the most common type of pancreatic cancer, and is characterized by an exceptionally poor prognosis, as most patients are diagnosed at an advanced stage when surgery is no longer possible [6]. This is due to the lack of early symptoms and the deep anatomical position of the pancreas, which makes detection challenging [7]. On a molecular level, PDAC is driven by many mutations. Activating mutations in KRAS occur in most cases, leading to constant stimulation of pathways that support cell growth and survival [8].

Lysosomes themselves are dynamic organelles responsible for degrading and recycling proteins, lipids, nucleic acids and damaged cellular structures [9]. In cancer, lysosomal systems are often reprogrammed to help tumor cells adapt to difficult conditions. Increased autophagic activity and changes in lysosomal pH contribute to tumor progression [10]. Among the enzymes involved, lysosomal cathepsins play a central role in degrading extracellular matrix components, activating growth factors, and influencing immune responses [11].

Cathepsins – a family of proteases- are mainly found in acidic environment of the lysosomes. They include cysteine, aspartic and serine proteases [12], which are important for protein degradation, antigen processing, and tissue remodeling. In cancer, this regulation often breaks down, cathepsins become overproduced, released into extracellular space or located to cell surface [11].

In PDAC, several cathepsins- such as B, L and S- have been strongly linked to disease progression. They contribute to invasion and metastasis by breaking down extracellular matrix components and influence interaction between tumor cells and the surrounding tissue [13], [14], [15].

Because PDAC is dependent on lysosomal activity and because cathepsins play such important roles in its progression, these proteases have attracted interest as therapeutic

targets. Many strategies have been developed to block their activity [14]. These include small molecule inhibitors that bind directly to the active site of specific cathepsins, or treatments that interfere with lysosomal pH [16], [17], [18].

Although challenges remain- such as achieving specificity [19], overcoming functional overlaps between different cathepsins [20]- the growing understanding of lysosomal and cathepsin biology in PDAC provides a strong basis for further developing therapies that focus on cathepsins and underlines the importance of exploring cathepsin-targeted approaches to improve treatment outcomes [15].

1. Cancer

A century ago, cancer used to be rare. Over the past few decades, the number of cases has increased significantly, likely because of changes in lifestyle and environment [21]. Cancer is now a leading cause of death worldwide [22] and is one of the most serious diseases, with the number of cases rising in the 21st century. Today, 1 out of 4 people faces the risk of developing cancer during their lifetime [21]. Early detection and treatment of cancer improve therapy and decrease both mortality and morbidity. For most cancer types, the 5-year survival rate is about 89% when the tumor is localized but falls to 21% after metastasis. In pancreatic cancer, patients with stage I disease have a 5-year survival of 85%, while it drops to under 10% in advanced stage diagnosis [23].

1.1. Cancer development

Cancer develops when cells start to grow uncontrollably. This often occurs because growth-promoting genes (oncogenes) are activated and tumor suppressing genes are inactivated [1]. Most patients with cancer die due to metastasis rather than from the original tumor itself [24]. For cancer to spread to other parts of the body (metastasis), the cells must loosen their connections to nearby cells and increase their movement and invasion into other tissues [1]. For that to happen, they rely on several processes. They move through the surrounding tissue (stroma), avoid being destroyed by the immune system, adapt to and change the environment around them and develop resistance to treatments [25].

Cancer cells release signals that reshape the stroma. They attract immune cells, stimulate angiogenesis (formation of new blood vessels) and activate cancer-associated fibroblasts (CAFs) [2]. CAFs can support cancer cells by providing nutrients such as lipids, amino acids and signaling molecules. They also weaken the body's immune defense by blocking T cell entry in the tumor. New findings show that CAFs may even present antigens, offering another pathway for influencing immune function [26]. These changes support tumor growth and progression within the tumor's microenvironment (TME) [2]. The TME includes many immune cells that constantly interact with cancer cells. These cells either protect the body or support tumor growth [24]. Tumors can grow their blood vessels in many ways, depending on the type of cancer and where it is located in the body. Different signals, cell types and sometimes even cells that are not normal blood vessel cells, like stem cells, help build the vessel [3]. According to the cancer stem cell (CSC) model, tumors are built in a structured hierarchy, just like healthy tissues. At the top are CSCs, which are able to maintain the tumor growth and to produce many different cell types found in the tumor [27].

1.2. Reactive Oxygen Species (ROS)

ROS are molecules that contain oxygen and are very reactive but do not last long in cells [28]. They are naturally produced during normal cell activity and help regulate signaling processes. In most cases, ROS come from the mitochondrial respiratory chain [29]. Reactive oxygen species have dual functions in cancer. Their effect depends on their levels, distribution and how long it stays inside the cell. They can contribute to cancer growth and metastasis [30]. High levels of ROS can damage cells and increase the risk of cancer [31], by breaking down proteins, cell membranes and chromosomes, leading to cell death [30]. However, in low amounts, ROS are necessary for maintaining homeostasis and help regulate normal cell functions [31].

Oxidative stress occurs when there is an imbalance between ROS production and antioxidant protection. This condition plays a key role in many diseases, including cancer [29]. Oxidative stress influences many signaling pathways that regulate cell growth, including the epidermal growth factor pathway [32]. Studies on how ROS influence cancer believe that oxidative stress causes DNA damage. This damage was expected to increase the number of mutations in cells, which could lead to the development of cancer [29].

1.3. Apoptosis

A failure in the normal cell death process (apoptosis) contribute to both cancer growth and resistance to treatment. Cancer cells prevent the permeabilization through the mitochondrial membrane, which is necessary for cells to die [4].

Cells can undergo apoptosis in 2 pathways- extrinsic and intrinsic- as illustrated in Figure 1. **The intrinsic form** depends on the balance between proteins that prevent cell death- *Bcl-2* (B cell lymphoma 2)- and proteins that promote it- *BAX* (Bcl-2 associated X-protein) [1]. In **the extrinsic form**, signals from outside the cell bind to death receptors on its surface. These signals generally come from the TNF-alpha group. Avoiding apoptosis is essential for cells to successfully become cancerous. By resisting apoptosis, cells with DNA damage continue to multiply, leading to tumor growth. This resistance also helps them survive stressful conditions, such as low oxygen or oxidative stress. It allows cancer cells to stay alive, even when they lack nutrients or survival signals [33].

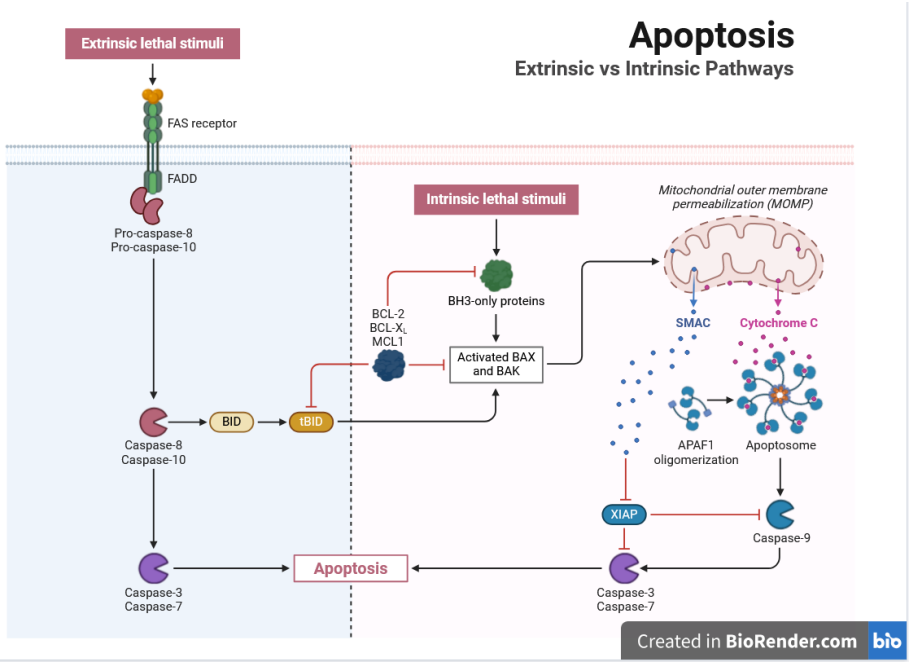

Figure 1 Overview of extrinsic and intrinsic apoptosis pathways. Extrinsic signals activate death receptors and caspase-8/10, while intrinsic stress signals induce mitochondrial outer membrane permeabilization and apoptosome formation. Both pathways converge on caspase-3/7, leading to apoptosis. Created by BioRender.com (21.11. 2025). Template from BioRender, © BioRender- licensed for personal and academic use.

1.4. Autophagy

In addition to pro-death signaling pathways, cells have evolved protective mechanism that counteract cellular stress. One of the most important of these mechanisms is autophagy, a lysosome dependent process [34]. Autophagy represents an conserved recycling system in which double-membrane autophagosomes form around cellular components and carry them to lysosomes for degradation [35]. To eliminate old proteins and defective organelles, cells activate autophagy. This pathway is important for normal cell growth and plays a central part in cancer. Under normal conditions, autophagy helps keep the cell stable by removing damaged components [36]. Autophagy is regulated by complex signaling cascades and specific proteins that coordinate the process [37]. Stress or nutrient deprivation strongly boosts autophagy. By doing so, it recycles nutrients, prevents the accumulation of misfolded proteins, reduces oxidative stress, and regulates signaling pathways- a set of mechanism that help the cell stay alive [36]. The formation and breakdown of the phagosome rely on a group of autophagy-related (ATG) genes produced through several steps: initiation, creating the early autophagosome, growing and extending its membrane, merging it with the lysosome, and finally degradation of the enclosed material- as shown in Figure 2 [35].

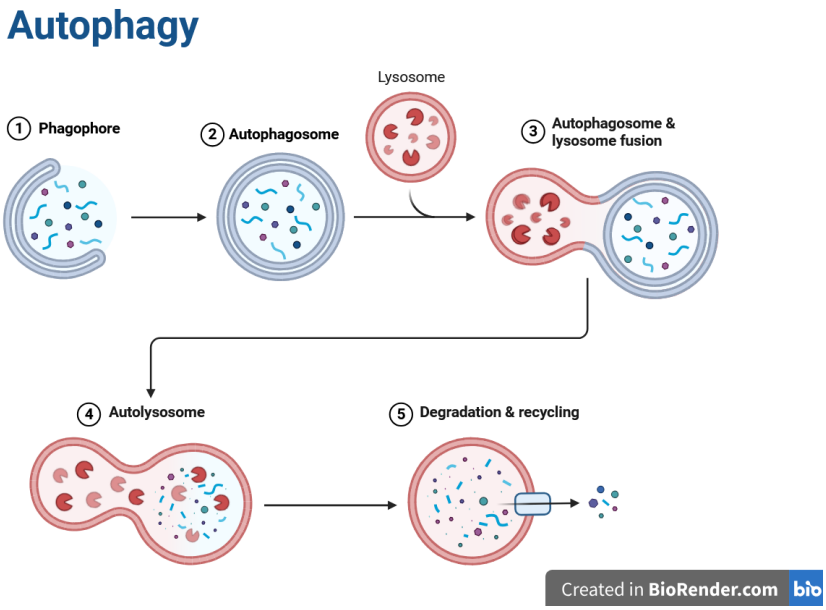

Figure 2 Stages of the autophagic pathway. Cellular material is enclosed by a phagophore to form an autophagosome, which then fuses with a lysosome. The resulting autolysosome degrades the contents, allowing recycled molecules to be reused. Created by BioRender.com (24.11. 2025). Template from BioRender, © BioRender- licensed for personal and academic use.

Research showed that autophagy can both support and suppress cancer, and current studies continue to reveal how this process affects the beginning and development of cancer [38]. On one side, autophagy reduces cell stress, prevents tissue injury and limits inflammation. It also plays a key role in keeping cells healthy by removing damaged components. On the opposite side, in advanced tumors autophagy helps cells survive harmful conditions by preventing genetic damage and helping cancer cells resist stress. This supports cancer development, growth, and progression [39].

2. Pancreatic Carcinoma (PC)

2.1. Pancreas anatomy and physiology

The adult human pancreas is a parenchymal organ weighing around 100 grams and is about 14 to 25 centimeters in length. It has a long and lobular form [7] and is separated into 3 parts: the head, the body and the tail [40].

The pancreatic function is divided into two main parts:

The **exocrine pancreas** is made up of ductal and acinar cells and is responsible for producing, storing and secreting digestive enzymes such as trypsinogen, lipases and amylases [41]. Acinar cells release these enzymes into the duct. Once they reach the small intestine, these enzymes help degrade proteins, fats and carbohydrates [42]. The **endocrine pancreas** consists of the islets of Langerhans- as illustrated in Figure 3-, which are responsible for producing hormones like insulin (beta-cells), glucagon (alpha-cells), somatostatin (delta-cells), ghrelin (epsilon-cells), pancreatic polypeptides [43] and influence many metabolic pathways throughout the body [44]. The islets of Langerhans are small groups of cells located between the acinar cells. There are about one million of them in the pancreas. They develop from endodermal tissue and are found in both the dorsal and ventral regions of the pancreas [7]. The maintenance of normal blood glucose levels depends largely on pancreatic beta-cells. When blood glucose levels increase, beta-cells release insulin, a peptide hormone that facilitates glucose absorption by the liver, muscle and fat [45]. Although the endocrine islets represent less than 5% of the weight of the pancreas, they contain over a billion individual cells in humans [43]. Around 85% of the pancreas consists of acinar cells and are organized into small groups called acini. Each group is linked to the system of pancreatic duct [7].

Both endocrine and exocrine systems work together to ensure proper digestion and cellular homeostasis [44]. Autophagy plays a crucial role in stabilizing cell homeostasis. Blocking autophagy has been shown to induce apoptosis in pancreatic beta-cells, indicating that autophagy functions as a protection for beta-cell survival [46]. After a tumor is fully developed, blocking autophagy often leads to a disease with reduced aggressiveness. Pancreatic tumors show elevated autophagy activity- also referred to as autophagic flux- that supports their expansion. If these autophagy-associated genes are mutated, the tumor can no longer grow or spread efficiently, which leads to cell death or growth arrest [47].

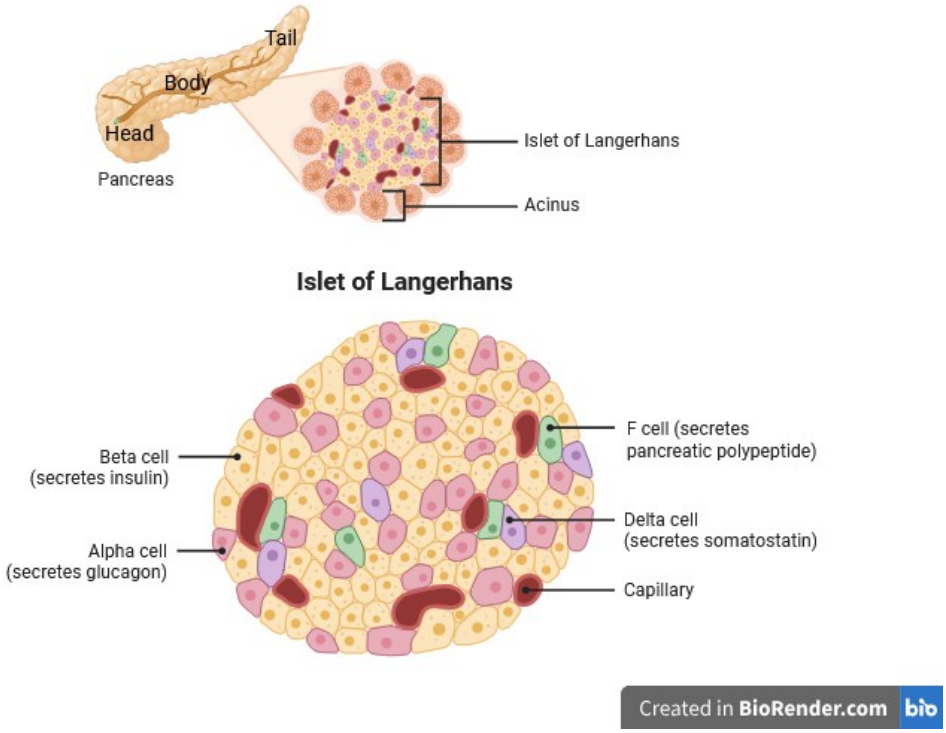

Figure 3 Structure and location of the islet of Langerhans and acinar cells within the pancreas. Created by BioRender.com (06.11. 2025) Template from BioRender, © BioRender- licensed for personal and academic use.

Different diseases target the exocrine and endocrine parts of the pancreas. Disorders such as pancreatitis and pancreatic cancer, most commonly pancreatic ductal adenoma carcinoma, develop from the exocrine pancreas, while endocrine disorders like diabetes develop from the islets of Langerhans [43].

2.1.1. Pancreatitis

Acute and chronic pancreatitis are common digestive problems that often need medical attention [48].

Acute pancreatitis

Acute pancreatitis (AP) represent a leading cause in hospitalization that affects not only adults but also children, pregnant women and elderly patients[49]. The leading causes of AP are gallstone disease and heavy alcohol consumption, each responsible for around 30% to 50% of all cases. Other contributing factors include high triglyceride concentrations (10%) and smoking [50]. AP develops when the body's normal cell defenses that stop trypsinogen

from turning into trypsin, or that control its activity, fail to work properly. Once these protections are overwhelmed, the inflammation begins [51].

People who have had acute pancreatitis are more likely to develop PC especially in the first few years after diagnosis (hazard ratio: 19.28[14.62; 25.41]). The risk lessens with time and returns to normal after five to ten years. Older age and diabetes make the risk higher [50].

Studies have found that nearly 50% of patients who experienced three or more cases of AP also developed chronic pancreatitis [49].

Chronic pancreatitis

Chronic pancreatitis (CP) is a long-term condition where repeated inflammation of the pancreas causes normal pancreatic tissue to be replaced by thick, fibrous tissue [50]. Although people with certain genetic factors may develop it at an early age, it most appears in adults [52]. The most common confirmed cause is alcohol use. Other causes include smoking, autoimmune disorders, cancer and conditions such as hyperparathyroidism [50].

It has been shown that people with chronic pancreatitis have a higher risk of developing pancreatic cancer. This increased risk is connected to ongoing inflammation which causes pancreatic stellate cells to grow and multiply [52].

2.2. Pancreatic ductal adenoma carcinoma (PDAC)

The incidence of pancreatic cancer has doubled over the past two decades. Most cases are diagnosed at an advanced stage, resulting in low survival rates and resistant to available treatments [5], [14]. Only around 20% of patients are diagnosed at an early stage [5].By the time it is diagnosed, the disease is often either locally advanced or has already spread to other parts of the body [53]. Diseases such as diabetes, pancreatitis and cancer can arise from pancreatic dysfunction. Pancreatic cancer is the most fatal among them. The average survival rate after diagnosis is under 6 months and only around 8% of patients survive for 5 years. This poor outcome is mainly because of delayed diagnosis, as symptoms often do not appear until the cancer has spread [42].

Most pancreatic cancer cases are pancreatic ductal adenoma carcinoma (PDAC) [54]. In the USA, PDAC ranks as the third most deadly cancer. Even with improved knowledge of its pathogenesis, most patients die within five years of diagnosis [55]. Because PDAC is usually discovered after it has grown or spread, only less than 20% of patients can be treated with surgery [6]. Several other types of pancreatic cancer exist, including tumors that develop

from acinar cells. These cancers are undifferentiated and can resemble liver cancer in appearance, but they are very uncommon [42].

Around 60-70% of PDAC begins in the head of the pancreas, while around 15% occurs in the body and another 15% in the tail [56]. The disease causes the growth of thick tissue made of fibroblasts and inflammatory cells, called desmoplasia. In a healthy pancreas there are cells called pancreatic stellate cells that act as fibroblasts. When the pancreas is damaged these cells become active and may help form this thick tissue in cancer [57]. The tumor microenvironment of PDAC also include cancer-associated fibroblasts (CAFs), which produce and remodel the stroma along with different immune cells- as illustrated in Figure 4 [58].

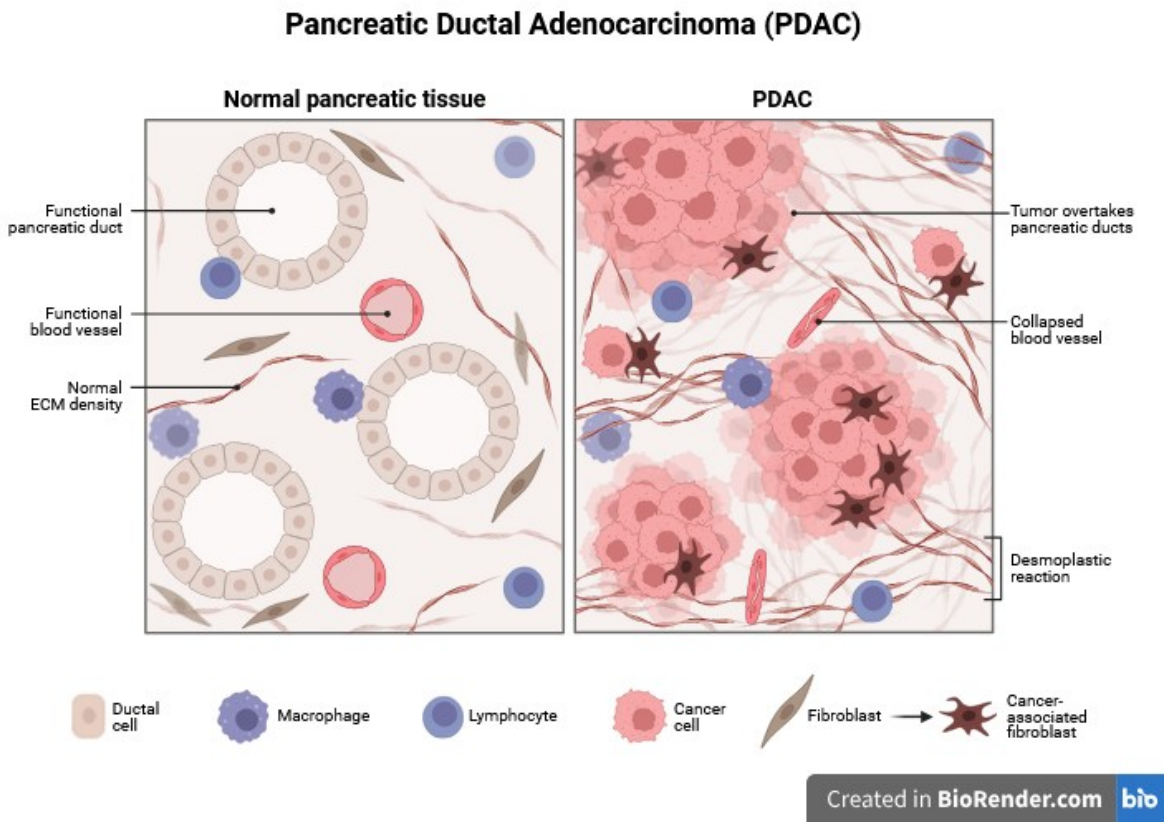

Figure 4 Comparison of normal pancreatic tissue and PDAC. Normal tissue shows organized structures, functional vasculature and balanced extracellular matrix density. In PDAC, cancer cells replace normal ductal cells, blood vessels collapse, and extensive fibrosis occurs, accompanied by cancer-associated fibroblasts. Created by BioRender.com (06.11. 2025) Template from BioRender, © BioRender- licensed for personal and academic use.

The most common early tumor forming changes that can lead to pancreatic adenocarcinoma is called pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN) [42]. PanINs are very small (< 5mm) and can't be

directly seen on pancreatic imaging. They may contain the same genetic mutations found in invasive pancreatic cancers and the number of these mutation increases as abnormality of the cells rises [59]. IPMN appear like finger-shaped-structures, called papillae that extend to the pancreatic duct [42].

Mucinous tumors represent the second most common form of pancreatic cancer making up <10% of all cases. Compared to PDAC they tend to be less aggressive and are associated with a reduced risk of mortality. These tumors also develop from the ductal cells but are known for producing mucin [42].

2.3. Biomarkers

Many different biomarkers have been studied to help detect pancreatic diseases, especially pancreatic cancer [60].

Computer tomography (CT) scans of the pancreas with blood vessels imaging, along with chest and pelvis scans, are used at diagnosis to check the stage of pancreatic cancer [61]. They have revealed small signs that could signal pancreatic cancer, even when advanced imaging technology is not used [60]. Tumors can be classified based on their relationship which blood vessels as not touching, touching or surrounding them. PC usually spreads to the liver, lungs, lymph nodes and bones [61].

Magnetic resonance imaging (MRI) of the pancreas with contrast is also a useful method for checking the stage of the disease when patients are first diagnosed [62]. MRI can examine the pancreatic duct, surrounding tissue and blood vessels without requiring an invasive procedure [63]. It does not use radiation and gives clearer pictures of soft tissues than CT scans. On the other hand, it can be affected by patients movement, is more expensive than CT, imaging methods are not standardized and it can cause claustrophobia in patients [62]. For many clinical cases, CT and MRI perform similarly. Still, MRI shows clearer benefits when visualization of the pancreatic duct, very small changes in the pancreatic tissue or cystic lesions inside or around the pancreas [64].

Endoscopic ultrasound (EUS) is the most accurate method for early detecting pancreatic tumors. Studies comparing imaging techniques found that EUS detect abnormalities in 43% of high-risk cancer patients, while MRI detected 33% and CT only 11% [62]. In addition, EUS can detect small irregularities such as uneven texture, which were first observed in cases with chronic pancreatitis. Although these changes are not specific to cancer, they may indicate tissue near regions with a higher number of precancerous lesions [60].

2.4. Risk factors

Since pancreatic cancer is often diagnosed at an advanced stage, identifying risk factors plays an important role in early detection and in the development of preventive strategies. Risk factors can be categorized into non modifiable like (sex, age, diabetes) and modifiable (smoking, obesity, pancreatitis, infection) [65]. Nowadays, the number of men with pancreatic cancer appears to be higher than that of women, notably in those under the age of 75 [5].

Diabetes mellitus

Diabetes has been shown to be associated with a significantly higher risk of PC. Patients with type 1 diabetes and who have had the disease for more than ten years face a 5 to 10-fold higher risk, while those with a history exceeding 20 years are at even greater risk [65]. Diabetes mellitus functions both as a risk factor and as a potential consequence of the disease [56]. Newly diagnosed patients report the onset of diabetes, while those with pre-existing diabetes often experience a worsening of their condition [5]. Pancreatic cancer is associated with increased HbA1c levels, [65] serving as a key parameter in the diagnosis of diabetes [66] and a possible biomarker for PC [56].

Cigarette Smoking

One of the most important factors is smoking [67] . A study showed that smokers have a 74% increased risk of pancreatic cancer. It demonstrated that cigarette smoke can promote stem cell traits in pancreatic cells. This stem cell like behavior enables the cancer cells to regenerate themselves and develop into multiple cell types, contributing to tumor progression [65]. In PDAC, smoking might increase the risk by causing fibrosis in the pancreas. This process is typical in chronic pancreatitis, which raises the risk of pancreatic cancer [68]. Long-term cessation of smoking is associated with a progressive decline in pancreatic cancer risk, with former smokers approaching the risk level of never smokers within 10-20 years after quitting [5]. Smoking might also indirectly increase the risk of PDAC by disturbing the pancreas's hormone function. Smoking reduces insulin secretion while raising glucagon levels, leading to diabetic conditions which may contribute to cancer growth [68].

Obesity

The growth of pancreatic cancer cells depends strongly on lipid metabolism, with both lipid oxidation and synthesis being essential. Unlike normal tissue, fat reserves improve cancer cell survival [65]. It is also important to note that a lot of weight loss is linked to a higher risk of pancreatic cancer. Individuals losing more than 8 pounds showed nearly double the risk

with a hazard ratio of 1.92 (95% Cl 1.58-2.32), and this relation was even stronger when weight loss occurred alongside newly diagnosed diabetes [5].

Alcohol

Heavy alcohol consumption is increasingly connected to pancreatic cancer risk, especially since it can cause pancreatitis, a recognized risk factor for the disease [67].

Allergy

Growing interest has been shown toward the influence of the immune system in pancreatic cancer. People with allergies appear to be protected, possibly because heightened immune activity enhances antitumor responses. Studies support this association, showing reduced cancer risk and improved survival among allergic patients [5].

Family history

Pancreatic cancer is strongly influenced by family history. Around 7-10% of patients have relatives with the disease. It is called familial pancreatic cancer when two or more first-degree relatives are affected. First degree relatives have been shown to have about 9 times the usual risk, increasing to 32 times higher when several close family members are affected [59].

Pancreatitis

Chronic pancreatitis is a significant risk factor for PDAC. The cancer slowly develops over time, usually around 10 to 20 years [62]. Inflammation and cellular injury caused by pancreatitis can promote PC development. However, pancreatitis can also occur as a manifestation of an existing tumor [5]. By interfering with endoplasmic reticulum, mitochondria and lysosomal autophagy systems in pancreatic cells, pancreatitis may result in oncogenic signaling, genetic damage and chromosomal alterations [65].

3. Lysosomes and Cathepsins

3.1. Lysosomal function

Lysosomes are single-membrane organelles that degrade cellular waste, recycle cell components through autophagy and control important processes such as calcium balance and nutrient signaling. Because of these roles, their proper function is essential for cell stability [9].

Lysosomes contain more than 60 enzymes [10]- such as lipases, proteases and glycosidases-[11] that digest and recycle cellular components. They are essential for maintaining normal cell function and their malfunction is linked to several diseases [10]. Lysosomal enzymes are produced in the endoplasmic reticulum after which they are packaged into vesicles and delivered to the lysosomes through the Golgi apparatus [69].

In cancer cells, the systems responsible for breaking down cellular components do not work properly. This leads to changes in the structure and stability of lysosomal membranes, leaving the cells more sensitive to internal or external signals [70]. Under stress cells may experience lysosomal membrane permeabilization (LMP) leading to the release of lysosomal enzymes- such as cathepsins- into the cytoplasm. These enzymes activate lysosomal-dependent cell death (LDCD). This process can be suppressed by inhibiting cathepsins [9]. On the other hand, LDCD offers a method to destroy tumor cells. It remains effective against cancer cells that do not respond to apoptosis [70]. Cathepsin activity requires an acidic environment, which is maintained by the V-ATPase (H+ ATPase) proton pump in the lysosomal membrane. Through fusion with other vesicles like endosomes or phagosomes, lysosomes digest proteins, lipids and sugars [11].

In comparison, cancer cells enhance their metabolism to promote tumor growth and survival, by changing lysosomal function and composition. Changes in lysosomal activity and secretion also help them invade and spread to other tissues. In addition, increased autophagy allows cancer cells to resist chemo- and radiotherapy [10].

3.2. Cathepsin classification and characteristics

The term cathepsins, meaning “to digest”, refers to a family of proteolytic enzymes classified by structure and catalytic mechanism into three main groups- aspartic cathepsins (D, E), serine cathepsins (A, G) and cysteine cathepsins. The cysteine cathepsins, the most studied group, include 11 members: B, C, F, H, L, S, K, O, V, W and X [12]. Around 31% of proteases

in the human body are serine proteases, followed by cysteine proteases (25%) and aspartic proteases (4%).

Cathepsins are key regulators of cellular processes such as protein degradation, immune system regulation, metabolic balance and apoptosis. Their activity is important to maintain homeostasis. Dysregulated activity, such as loss of activity, uncontrolled proteolysis or dysregulated signaling, can trigger cancer [71]. Cathepsins have become an important topic in cancer research, particularly to understand how they are regulated and how they might be targeted therapeutically. Each cathepsin has its own role in supporting tumor growth, depending on the localization and its expression levels [15]. Autophagy, as part of the lysosomal proteolytic system, is essential for controlling cell death and can directly eliminate cancer cells. In contrast, its dysfunction is strongly associated with tumor growth. Stress- induced destabilization of the lysosomal membrane permits the cytosolic release of hydrolytic enzymes, triggering apoptosis as well as apoptosis-like forms of cell death [72].

Cathepsins are most active in the acidic pH of lysosomes but can also function at slightly higher levels. For instance, cathepsin S remains active and stable around pH 6.5, while cathepsin D works best at pH 4 [11]. Although most cathepsins lose stability at neutral pH, molecules like catalase and heparin can help maintain their structure and activity. They are produced in an inactive form and gain activity once the N-terminal propeptide is removed. This activation can happen either through autolysis in lysosomes or through other proteases [73].

Even outside the cell, cysteine cathepsins remain active, showing that factors other than pH influence their function. Some cathepsins are capable of degrading collagen and elastin, leading to reshaping the extracellular matrix (ECM). This can lead to the development of different diseases [74]. For example, cathepsins support cancer spread by degrading the ECM and cell connections, which allows cancer cells to move more easily. They also contribute to tumor growth, invasion and the development to treatment resistance [11]. The extracellular matrix is made of proteins like collagen, elastin and proteoglycans, all of which can be broken down by cathepsins [74].

3.2.1. Aspartic cathepsins (D, E)

Cathepsin D

Cathepsin D degrades structural proteins such as fibronectin and laminin and has been identified as a biomarker in breast cancer [20]. It is typically found inside acidic areas of lysosomes and desmosomes. Cathepsin D functions as a housekeeping gene that maintains basic cellular activity in most genes [75].

Cathepsin E

Cathepsin E (CTSE) contributes to immune regulation by participating in antigen presentation via the MHC class II pathway [20].

Cathepsin E is expressed in multiple cell types, such as immune cells, gastrointestinal cells and various lymphoid cells [6]. Its localization differs by cell type. In dendritic cells, microglia and macrophages CTSE localizes in endosomal compartments in its active form [72], whereas in erythrocytes and gastric cells it is located in the plasma membrane [6]. In certain cancer types, endolysosomal proteases relocate from their usual compartments to extracellular regions. As a result, the functions of Cathepsin E changes due to this shift in localization [72].

3.2.2. Serine cathepsins (A, G)

Cathepsin A

Within lysosomes, cathepsin A (CTSA) serves as a multifunctional enzyme that not only degrades proteins through carboxypeptidase activity but also interacts with glycosidases under acidic conditions. It remains active at neutral pH, where it performs amidase and esterase functions, showing its adaptability to different cellular pH levels [76]. CTSA is present in many tissues, showing the highest levels in kidney tubule cells, liver tissue, hepatocytes and major neuronal populations [77].

Cathepsin *G*

Produced by activated neutrophils, Cathepsin G contributes to inflammation by degrading various proteins, such as components of the ECM. It also supports the antibacterial defense of neutrophils. Because neutrophils often infiltrate tumors, Cathepsin G may influence tumor cell behavior, such as growth and movement, although its specific role in cancer progression remains unclear [78].

3.2.3. Cysteine cathepsins (B, C, F, H, L, S, K, O, V, W, X)

Cathepsin B

Cathepsin B, a member of the papain family, acts as both an endo- and exopeptidase. It is expressed in many tissues, including macrophages, epithelial cells, pancreases, kidneys and hepatocytes. Its main function is intracellular protein degradation, but it also contributes to immune responses and bone remodeling [79]. Cathepsin B works best in

acidic conditions, showing the highest gelatinase activity at about pH 5.6 [20]. When keratinocytes secret cathepsin B, it binds to the cell surface and supports migration by breaking down parts of the extracellular matrix during for example wound repair [11].

Cathepsin C

Cathepsin C is an enzyme that removes dipeptides from proteins and helps activate other proteases in the body. It can break down different peptide molecules and acts as a transpeptidase [79]. It also helps regulate the activity of glycosidases [76]. This enzyme is important for brain function and plays a role in inflammation in the nervous system [79].

Cathepsin F

Cathepsin F (CTSF) is primarily expressed in skeletal muscle and testicular tissue, whereas its absent in leukocytes and the thymus. In addition, elevated levels of CTSF have been observed in various cancer types, indicating a possible connection to cancer development [80]. Recent studies have identified cathepsin F as a potential marker in cervical and gastric cancer cells. It has been shown to lower the levels of the apoptosis- regulating protein Bcl-2 and thereby promoting cell death [81].

Cathepsin H

Cathepsin H (CTSH) is present in almost all tissues and mainly acts as an aminopeptidase, removing amino acids from the ends of proteins, but it also has limited endopeptidase activity [79]. CTSH also functions as an endopeptidase that can degrade many different proteins [71].

Cathepsin L

Cathepsin L can break down transcription factors, recycle proteins within the cell and assist in immune processes like antigen presentation [79]. It plays a major role in producing various neuropeptides such as enkephalin and beta-endorphin. Its absence can disrupt chromatin organization because of its connection to histone regulation [20]. In the thymus, it supports natural killer cell development and contributes to neuron growth in the brain [79]. By triggering their glycoprotein activation, cathepsins L and B help viruses such as Ebola virus and paramyxovirus to enter cells [20].

Cathepsin S

Cathepsin S helps remove damaged proteins in lysosomes and is essential for immune function through MHC class II antigen processing [82]. Unlike other cathepsins, it is active at a neutral pH. Dysregulation of cathepsin S can promote several diseases, including cancer, cardiovascular disorders and arthritis by acting on proteins outside the cell [83].

Cathepsin S plays a dual role in cancer and immune regulation. It supports anticancer immunity through MHC class II, but it also promotes tumor growth by supporting tumor-supportive immune-cells polarization and proliferation. It enhances both tumor invasion and angiogenesis [84].

In cancer, tumor cells and immune cells such as macrophages release cathepsin S and L into the surrounding tissue. These enzymes help tumors grow by degrading the extracellular matrix and turning on other enzymes, such as matrix metalloproteinases (MMPs) that allow cancer cells to move and invade [85].

Cathepsin K

Cathepsin K (CatK) is highly expressed in bone cells called osteoclasts. It is also present in various tissues such as heart, placenta, ovary, lung and skeletal muscle [79]. It plays a major role in breaking down bone matrix components such as collagen [20]. Because cathepsin K plays a central role in breaking down collagen, it has been identified as an important therapeutic target for treating osteoporosis [79].

Cathepsin O

The function of cathepsin O is still largely unknown. The roles of cathepsins, in general, are continually being discovered, with new functions identified and overlapping activities observed among different family members [20].

Cathepsin V

Although the exact role of cathepsin V in cancer remains unclear, recent studies report higher levels of this enzyme in several tumor types, suggesting a potential role in cancer progression. Moreover, cathepsin V appears to suppress immune defense against cancer by activating cystatin F, which blocks the activity of cathepsin C, H and L [84].

Cathepsin W

Cathepsin W is a cysteine protease that belongs to the papain-related group [86]. Higher levels of cathepsin W expression have been linked to improved survival in endometrial cancer and is associated with higher infiltration of immune cells, including B cells, dendritic cells and macrophages [84]. Cathepsins are mainly present in antigen-presenting cells. Cathepsin W has been detected specifically in natural killer cells and CD8+ T-cells [86].

Cathepsin X

Cathepsin X (CatX) has been found to enhance cancer cell invasiveness and degrade the tumor suppressive protein profilin-1. Additionally, it contributes to programmed cell death and performs significant activities in the immune cells [84]. CatX is another member of the

family that relocates to the cell surface, where it contributes to cell attachments and intracellular communication [11].

While the dysregulation of cathepsins and lysosomal activity promotes tumor growth, invasion, and therapy resistance in various cancers [11], [70], [84], [85], specific genetic mutations also play a crucial role in the development and progression of pancreatic cancer [26], [87].

4. Important mutations in pancreatic cancer

Subtypes of pancreatic cancer that can benefit from targeted treatments are often linked to defined genetic mutations. About 10-15% of cases are related to inherited mutations. These genetic changes disrupt normal process such as DNA repair, cell growth and gene regulation involved in cancer treatment [26].

4.1. BRCA1/BRCA2 mutations

BRCA1 and BRCA2 (breast cancer type 1/2) are important strands using a repair method called homologous recombination. If these genes are damaged, cells cannot repair DNA properly, increasing cancer risk [26]. These mutations were first found in breast cancers and later identified in pancreatic cancer [87].

The National Comprehensive Cancer Network (NCCN) guidelines suggest that patients be treated with a platinum-based chemotherapy, such as FOLFIRINOX or gemcitabine combined with cisplatin [88].

Poly (ADP-ribose) polymerase (PARP) inhibitors work by targeting cancer cells that have a defect in homologous recombination repair (HRR) [89]. PARP-1 protein helps repair DNA damage by participating in the base excision repair (BER) process, which is responsible for fixing single-strand breaks in DNA [90]. PARP inhibitors block the PARP-1 protein at points where single-strand DNA is damaged, stopping it from completing its repair process, which leads to severe double-strand DNA breaks when the cell tries to copy its DNA [89]. Therefore, using PARP inhibitors in cells lacking functional BRCA genes results in severe damage that leads to cell death [90].

4.2. KRAS mutations

Pancreatic cancer is strongly associated with mutations in the KRAS (Kirsten rat sarcoma viral oncogene homolog) gene. KRAS plays an important role in starting and sustaining tumor growth. Therefore, its signaling pathway is a major target for therapy [8]. More than 90% of PC carry KRAS mutations, most commonly at the G12D, G12V and G12R positions [91]. KRAS is responsible for producing a Ras- family protein that functions as a GTP-binding enzyme [8]. These proteins act as molecular switches that shift between an active, GTP-bound state and an inactive, GDP-bound state [92]. These mutations keep KRAS active in its GTP- bound form, leading to stimulating tumor growth downstream signaling routes like the MAPK (mitogen-activated protein kinase) and PI3K (phosphatidylinositol 3-kinase) pathways [91].

In PDAC, KRAS^G12D and G12V account for most cases [8]. Recent studies have shown that KRAS^G12R mutations are more common in early stages of the disease and often received neoadjuvant chemotherapy treatment [93]. Despite extensive efforts to target KRAS mutations, their extremely strong binding to GTP and GDP has made it difficult to develop small molecules that can successfully inhibit their binding pocket [26].

For RAS to work, it needs several chemical changes after translation including a step called farnesylation. Trying to inhibit farnesylation has not been effective, because KRAS can use a different group - geranylyeranyl- instead. Once produced, RAS has to move to the cell membrane, a step that depends on the protein PDE-delta. The drug Deltarasin blocks PDE-delta, stopping RAS from reaching the membrane. As a result, KRAS-dependant PC cells lose their ability to grow and survive [42].

Conversely, inhibitors for KRAS^G12D have been developed using alternative strategies, as the mutant aspartate at position 12, which is less reactive and is difficult to target directly [94]. MRTX1133, discovered through structure-based design, is a selective and strong noncovalent KRAS^G12D inhibitor. It suppresses KRAS^G12D signaling in cells and in vivo and demonstrates antitumor effects in mice [95]. MRTX1133 targets the switch II pocket, preventing the interactions between proteins required for activating the KRAS pathway [96]. Several other KRAS^G12D inhibitors, such as HRS-4642, are currently in development, with some undergoing early phase clinical trials while others still in preclinical testing, such as ERAS-4 [94].

The use of T-cell receptor (TCR)-engineered T cells that specifically target the KRAS^G12V neoantigen represents a potential therapeutic advancement for PC [97]. TCRs reacting to KRAS^G12V, which were taken from patients' tumor infiltrating lymphocytes, have been shown to detect this neoantigen through certain HLA (human leukocyte antigen) molecules and destroy cancer cells in both in vivo and in vitro [98]. HLA-bound neoantigens are promising molecular targets that can be used for CAR-T cell therapy [99]. A special type of CAR known as T-cell receptor mimic CAR (TCRm CAR) is designed with a single-chain variable fragment (scFV) that specifically detect HLA complexes. Recent studies have shown that TCRm CAR modified T cells can trigger strong antitumor immune response by identifying peptides that come from overproduced proteins in tumors [100].

Two inhibitors specifically targeting KRAS^G12C, adagrasib (MRTX849) and sotorasib (AMG510), have progressed to clinical testing and shown promising results in cancers with this mutation [8]. Sotorasib received approval from the FDA in 2021, whereas adagrasib was identified by the FDA as a breakthrough treatment. While KRAS^G12C inhibitors have shown major benefits in cancers like lung cancer, this mutation is uncommon in PC [26].

Taken together, although KRAS mutations represent a central driver of PC and multiple mutation- specific targeting strategies are under active investigation, effective therapeutic approaches remain limited, as many KRAS variants are difficult to inhibit and some clinically targetable mutations occur only rarely in PC [26], [91]. This highlights the need to explore additional mechanisms that support pancreatic tumor growth and survival, such as dependence of PDAC cells on lysosomal function and autophagy [101].

5. Cathepsins in pancreatic cancer

Pancreatic ductal adenocarcinoma is characterized by a continuously elevated lysosomal and autophagic activity, which supports tumor cell survival under metabolic stress and contributes to both tumor initiation and progression [101].

Cathepsin E, B, L and S have been implicated in pancreatic cancer progression, particularly in process such as invasion and metastasis. Consequently, cathepsin inhibitors have been examined as potential therapeutic strategies, primarily in preclinical models [14], [102]. Cathepsins B and L are lysosomal enzymes that stay active at neutral pH and are involved in cell death. They initiate cell death by influencing mitochondrial pathways and caspase activation [103]. Different imaging methods like MRI, PET (Positron emission tomography), SPECT (single- photon emission computed tomography), CT and ultrasound are widely used to find and evaluate cancers without surgery [104].

5.2. Cathepsin inhibition

Inhibiting cathepsin activity with inhibitors can help prevent tumor growth, angiogenesis and cancer cell invasion. These inhibitors may occur naturally in the body -endogenous- or be synthetically produced. By limiting cathepsin activity, they offer potential for therapeutic use in controlling tumor growth [20].

5.3. Cathepsin E as target for Treatment

In PDAC and PanINs, cathepsin E is strongly overexpressed, and its levels increases in parallel with tumor development [102]. Expression of CTSE is also observed in pancreatic diseases, such as chronic pancreatitis and pancreatic cysts, both of which are regarded as risk factors for PDAC [6].

The central function of CTSE is protein degradation by hydrolyzing peptide bonds at defined sequence sites. Its potential has been examined in disease diagnostics, imaging and therapy for cancer such as PDAC [6].

Cathepsin E is considered as a promising biomarker for PDAC, which could help in developing new imaging methods to monitor disease progression [19]. Two special characteristics make CTSE a special imaging target. First it is restricted to intracellular compartments and secondly it remains in an inactive pro-form (procathepsin E) [105]. A minimally invasive approach to identify CTSE in pancreatic tissue is the use of a fluorescent

probe that specifically detects CTSE activity through optical imaging [6]. This approach is realized by a confocal laser endomicroscopy (CLE). A study found that combining CLE with a CTSE-activable probe is a useful tool for tracking pancreatic cancer and detecting PanINs along with early PDAC [102]. The probe becomes active only when lysosomal enzymes cleave it. It carries several Cy5.5 fluorescent dye molecules bound to a copolymer that directs the probe toward tumor areas. When the dyes are close together, the fluorescence remains suppressed. Once the enzymes separate them, the signal becomes strongly amplified, producing a 12 -time stronger signal. This enhanced signal enables sensitive in vivo visualization of pancreatic tumors [6]. The intracellular localization of CTSE and its persistence as an inactive pro-form make it particularly suitable for enzyme-activatable imaging approaches, highlighting its translational relevance for early diagnosis and disease monitoring in PDAC [6], [102]. It has been already demonstrated that Cathepsin E is remarkably increased in PDAC compared with normal pancreatic tissue [105]. Moreover, pancreatic juice from PDAC patients contained significantly greater amounts of CTSE than in those with chronic pancreatitis [102]. Taken together, the strong overexpression of cathepsin E in PanINs and PDAC, as well as its increased levels in pancreatic juice, indicate that CTSE is closely associated with early tumor development and disease progression rather than late-stage invasive behavior [102], [105].

The FDA has approved PDT for treating early-stage and advanced cancers. Photodynamic therapy (PDT) works in 2 steps: a photosensitive drug is first applied, and afterward it is triggered by light at a defined wavelength [106]. 5- Aminolevulinic acid (5-ALA) is a widely used photosensitizer to visualize tumors and works well with PDT. Because CTSE is highly expressed in PDAC, a peptide containing 5-ALA was designed so that CTSE can cleave it and release the drug only in CTSE -positive cells. With PDT, this prodrug induces pancreatic cancer cell death both in cell culture and in animal models [6]. However, ensuring that the drug accumulates mainly in cancer cells is still a major challenge. To overcome this issue, scientists are developing new ways to deliver 5-ALA, for example by using nanoparticles. These nanoparticle-based systems can improve the drug's solubility, direct it more precisely to tumor tissue, and increase its biological stability [107]. In addition to its role in tumor therapy, 5-ALA has also been examined for treating autoimmune and inflammatory conditions, as well as for use in transplantation. Its effects are driven by the induction of heme oxygenase-1, an enzyme that breaks down heme into free iron, carbon monoxide and biliverdin. These effects are enhanced when 5-ALA is paired with sodium ferrous citrate, and they disappear when heme oxygenase-1 is inhibited [108]. Fluorescence-guided surgery enables surgeons to clearly visualize cancerous regions, making it possible to remove tumor tissue with high precision. It has been shown that using 5-ALA for this purpose can improve surgical success and increase survival in patients with PC. Beyond its use in imaging, 5-ALA

can promote apoptosis in pancreatic tumor cells. This effect may occur because the compound interferes with enzymes that are essential for mitochondrial energy production, leading to an accumulation of ROS that trigger cell death. Continued research is needed to understand its mechanisms more fully and to optimize its use in pancreatic cancer therapy [107]. In addition, new CTSE inhibitors have been discovered in cyanobacteria, including grassystatin A, which can now be produced synthetically. It was also found that CTSE selectively cleaves peptide substrates that contain alanine at a specific position. This enabled the design of peptide sequences to track CTSE activity and explore treatment options [6].

A major challenge is the lack of inhibitors that specifically block cathepsin E without affecting other aspartic proteases. While pepstatin, an inhibitor of aspartyl protease [19], is widely applied to inhibit CTSE, it is unspecific and also targets cathepsin D and other proteases [6]. Interestingly, the HIV protease inhibitor ritonavir (RIT), FDA approved, has been reported to bind to CTSE with moderate affinity, [105] , but it failed to produce any meaningful reduction in tumor growth. RIT is also a strong inhibitor of the viral aspartyl protease [19]. Its structure could be optimized to develop high-affinity agents with optimized pharmacokinetics [105]. However, the lack of highly specific cathepsin E inhibitors and the limited efficacy observed with unspecific aspartic protease inhibitors illustrate the current challenges in translating CTSE targeting into effective systemic therapies [6].

5.4. Cathepsin B as target for Treatment

In cancer cells, cathepsins shift from their typical intracellular locations to the cell periphery following transit through the trans-Golgi. Recent studies highlight the role of V-ATPases on the cell surface, as well as the acidic environment in regulating the release of cathepsin B [15]. High levels and abnormal release of cathepsin B outside the cell have been linked to several stages of tumor growth and cancer progression [109]. In pancreatic cancer stem-like cells (P-CSLC), the lysosomal protease cathepsin B (CTSB) was strongly increased in comparison to parental cells. Surface expression of CTSB was likewise higher in P-CSLCs and these cells also released higher levels of CTSB [110].

Within normal tissues, cathepsin B is largely restricted to lysosomes in its precursor state. In contrast, in cancer cells, activity has also been observed at the plasma membrane, where the enzyme appears in its mature form [111]. These observations indicate that CTSB may act as a prognostic biomarker of poor prognosis and highlight its potential as a therapeutic target, particularly in relation to P-CSLCs [112].

In PDAC, glycolysis and autophagy were found to be highly active and functionally linked. Cystatin B promotes autophagic flux through competition with Cystatin C for cathepsin B binding and inhibition, thereby maintaining its proteolytic function [113]. Cystatin B is a strong, reversible blocker of cathepsins B, L, and H. Cystatin C is present in every type of body fluid and functions as the main inhibitor of cathepsin B outside the cell. The inhibition prevents these digestive enzymes from escaping into the cytoplasm [114]. As a result, the heightened autophagy supplies essential substrates that reinforce biosynthesis and promote glycolytic metabolism. After cathepsin B was suppressed, Cystatin B could no longer enhance autophagic activity. Blocking CTSB also stopped Cystatin B from promoting glycolysis and the growth of pancreatic cancer cells [113]. The enrichment of cathepsin B in pancreatic cancer stem-like cells and its role in sustaining autophagy-dependent glycolytic metabolism suggest a contribution to intrinsic therapy resistance in PDAC [99], [100].

It has been demonstrated that Arg and Abl, which are non-receptor tyrosine kinases, increase the release of cathepsin B through the secretion of transcription factors involved in epithelial- mesenchymal transition (EMT), tumor cell invasion and resistance in treatment [115]. Increased cathepsin B expression in cancer is also linked to high levels of Sp1 (specificity protein) and Sp3 within tumor cells and tissues [116]. Sp1 and Sp3 belong to a family of GC-rich promoter- binding transcription factors that control the expression of genes involved in cell growth, survival and invasive behavior and are frequently elevated in cancer cells [117]. Cathepsin B transcription is regulated by Ets1 (E-twenty-six), Sp1 and Sp3. Among them, Ets1 is recognized as a proto-oncogene that promotes the invasive behavior of carcinoma and endothelial [116] .

Suppressing the activity of CTSB with inhibitors might be an effective way to slow or treat cancer. In a pancreatic islet cancer mouse model (Rip1-Tag2), the broad cathepsin inhibitor JPM-OEt showed strong anti-tumor activity in prevention, intervention and regression trials- each targeting distinct stages of tumor growth [109]. A study showed that JPM-OEt, a epoxide inhibitor that binds cathepsins irreversibly, effectively reduced the progression of Rip1-Tag2 tumors [118]. When combined with chemotherapy, it led to stronger tumor reduction, reduced invasiveness and improved survival [109]. Research demonstrated that JPM-OEt reduces cathepsin activity most effectively in organs located near to where it is injected, like pancreas, kidney and liver. In contrast the effect is weaker in organs farther away such as the lungs [118].

The proteasome inhibitor Bortezomib (PS-341) promotes the death of pancreatic cancer cells by increasing oxidative stress, which makes lysosomes release cathepsin B into the cytosol and activate caspase-2 leading to mitochondria damage which leads to apoptosis.

Overall PS-341 kills cancer cells by connecting lysosomal and mitochondrial pathways that control cell death [119]. However, the basic processes behind this remain unclear [120].

CA-074 acts as a specific inhibitor of cathepsin B [16]. It is known to be very potent and works by irreversibly attaching to Cys29 (cysteine at the 29th amino acid position in cathepsin B), which completely inactivates the enzyme [121]. During cancer, CTSB relocates from lysosomes with acidic pH of 4.6 to neutral regions (pH 7.2) like the cytosol and extracellular regions. It has been shown that CA-074 inhibits cathepsin B with maximal activity under acidic conditions, which is 100 times stronger than at neutral pH. The strong pH dependency of CA-074 may limit its efficacy in extracellular compartments of the tumor microenviroment [16]. Although CA-074 is mainly a strong inhibitor of cathepsin B, it can also suppress cathepsin H and L. Its effect on these enzymes is much weaker than its effect on cathepsin B [121]. Research using mouse models with human metastatic melanoma in the lungs found that CA-074 reduced tumor size and caused the tumors to grow more slowly [122]. CA-074me, the methylated form of CA-074, is used as a selective inhibitor of CTSB. The methyl-ester modification makes the inhibitor more lipophilic and helps passing through cell membranes more easily. Inside the cell, it turns into CA-074 and inactivates cathepsin B showing good treatment effects [123]. In mouse models, this treatment greatly reduced pancreatic swelling, inflammation and damage to acinar cells [124].

Cystatin A (CSTA) showed a strong reduction in cancer growth and improved survival time [125]. CSTA was mainly detected in immune cells that had infiltrated pancreatic tumor tissue, especially in neutrophiles, while cathepsin B was found in both tumor and infiltrating immune cells, mainly macrophages. CSTA belongs to the cystatin family, which acts as cytoplasmic inhibitors of cysteine proteases [126]. It has been shown that patients with PC express higher levels of cystatin A in CD4+ T cells- which are immune cells- compared to healthy individuals. CSTA has also been found in multiple tumor tissues and identified as a diagnostic biomarker [125]. A reduction in cystatin A levels can disturb the normal balance of cysteine proteases in the cell. This imbalance has been linked to the appearance of more invasive tumors. It is also seen in tumors that return after treatment or show rapid and aggressive growth [114]. Higher CSTA levels in CD4+ T cells and in the blood may indicate changes driven by the pancreatic cancer environment, which involves cathepsin B and cystatin A. More research is needed to understand these CD4+ T cells, as they do not show features of typical tumor fighting Th1 cells or of regulatory T cells that suppress immune activity [126].

Increased cathepsin B can activate fusion of liposomes containing dioleoylphosphatidylethanolamine (DOPE), a lipid that promotes membrane merging. This activation occurs through the cleavage of protective peptide components on the liposome

surface. Once these elements are removed, DOPE is able to adopt its fusogenic form and initiate membrane fusion [127]. Lipids like DOPE are incorporated into polymer delivery structures to help dissolve the drug better and to promote fusion of the produced nanoparticles with the cancer cell membranes, thereby improving intracellular drug delivery and potentially increasing antitumor activity[128]. As cathepsin B exists mostly in acidic lysosomes, dual sensitive liposomes have been designed to respond to both low pH and this enzyme for targeted delivery of medicines inside cells [127]. Liposomes made from DOPE are widely recognized as highly efficient carriers for delivering small interfering RNA to cancer cells. Despite their benefits, they are poorly suited for circulation in the bloodstream because they form large clusters with serum proteins, which reduces their stability and deliver efficiency. By adding a polyethylene glycol (PEG) coating, this issue can be minimized. The coated liposomes remain stable for a longer time in the blood and show improved accumulation in tumors relative to the uncoated versions [129].

Although preclinical studies demonstrates that pharmacological inhibition of cathepsin B can reduce tumor growth and enhance chemotherapeutic efficacy, factors such as pH-dependent inhibitor activity, off-target effects and limited tissue distribution represent major challenges for clinical translation [16], [121].

5.5. Cathepsin L as target for Treatment

During pancreatic cancer, cathepsin L is produced and released into the cancer tissue by several cells, including cancer cells, endothelial cells, fibroblast and immune cells that infiltrate the tumor. Among these, macrophages are recognized as the main producer of cancer related enzymes [130].

Uncontrolled expressions of cathepsin L and its increased presence in the extracellular matrix are frequently observed in PC. Suppression of this enzyme may offer a potential avenue for cancer therapy [13]. The acidic surroundings around the tumor increases extracellular cathepsin L activity, which promotes the process of invasion and metastasis [131]. Collectively, the increased expression acidic and extracellular activity of cathepsin L within the acidic tumor microenvironment contribute to PDAC progression by promoting invasion, metastasis and angiogenesis [13], [131]. In pancreatic cancer mouse models, elevated cathepsin L activity has been linked to tumor development [13] High cathepsin L expression has also been associated with poor patient survival in clinical studies [131]. Deletion of the cathepsin L gene in mice resulted in smaller tumor size. Similarly, covalent inhibitors of cathepsin L have been found to limit the growth of pancreatic cancer [13].

Since the pancreas is located deep in the body, collecting tissue samples is often difficult. Therefore, researchers have focused on identifying blood-derived biomarkers as tools for prognosis. Among these, cathepsin L levels combined with disease stage have been recognized as an independent indicator for pancreatic cancer [131]. This association underscores the translational relevance of cathepsin L not only as driver of tumor progression but also as a prognostic biomarker with potential clinical applicability [13], [131].

Molecular docking and enzyme activity tests demonstrated that ASPER-29 attaches to cathepsin L through a conventional binding mechanism, leading to suppression of its activity. ASPER-29 also showed the same inhibitory effect for cathepsin B [17]. By blocking both targets at once, the compound significantly limited the ability of BxPC-3 and PANC-1 pancreatic cancer cells to spread, in both experimental systems in vivo/ in vitro [132]. Due to their opposing characteristics- PANC-1 being metastatic and KRAS-mutant, and BxPC-3 being non-metastatic- these cell lines serve as complementary PDAC models [133].

Protein disulfide isomerases (PDIs) are endoplasmic reticulum enzymes that assist in protein folding and function as molecular chaperones. They are commonly upregulated in cancers and are considered potential targets for therapy [134]. E64FC26 functions as a newly discovered inhibitor of protein disulfide isomerase [18]. In pancreatic cancer cells, E64FC26 caused an accumulation of immature cathepsin L and a decrease in its mature form, indicating disrupted lysosomal activity and protein stability. The inhibitor appears to reduce cysteine protease function by blocking the formation of disulfide bridges [69].

Another study investigated how cathepsin L affects the formation of new blood vessels in cancer and to test how well KGP94 can inhibit this process. KGP94 is a selective small thiosemicarbazone based molecule that stops cathepsin L activity by targeting the active site [135]. KGP94 can weaken the metastatic potential of cancer cells under both normal and stress related conditions, supporting cathepsin L inhibition as a promising approach to limit cancer spread [136]. Although these preclinical findings support cathepsin L inhibition as a therapeutic strategy, its broad expression and role in essential lysosomal functions may limit the therapeutic window of systemic targeting approaches [135], [136].

5.6. Cathepsin S as target for Treatment

As a cysteine protease, Cathepsin S (CatS) is deeply engaged in tumor progression, supporting invasion, angiogenesis and metastatic spread [137]. It has been shown that blocking Cathepsin S can both slow cancer and make chemotherapy more effective [14].

High levels of cathepsin S are found in tissues of the mononuclear phagocyte system (MPS) [104], particularly in antigen- presenting cells (like monocytes and macrophages) and is active at neutral pH [14]. During cancer, Cathepsin S is relocated from the lysosomes into the extracellular matrix [137]. In pancreatic cancer, it promotes tumor development and is spread by degrading the extracellular matrix [14]. Deficiency of cathepsin S limits angiogenesis and tumor cell growth, showing that Cathepsin S plays an important role in angiogenesis and cancer progression [138]. These findings indicate that cathepsin S contributes to PDAC progression primarily through its activity in immune cells of the tumor microenvironment, where it promotes extracellular matrix remodeling and angiogenesis [14], [138]. Inhibiting Cathepsin S can slow tumor growth and improve the effects of chemotherapy. 3 new alpha- fluorocinnamate derivates (1F, 2F, 3F) were developed as inhibitors of CatS, with 2F showing the best activity against PC cells. Covalent docking showed a strong interaction within the CatS active site, making them promising candidates for further inhibitor development. The observed chemo-sensitizing effects of CatS inhibition further highlight its therapeutic relevance in PDAC treatment strategies [14].

HPMA copolymers help deliver cancer drugs more effectively because of the EPR (enhanced permeability and retention) effect [104]. The EPR effect is thought to offer an effective strategy for transporting macromolecular drugs and nanoparticle-based therapies to tumors. This works because tumor blood vessels have gaps that let big molecules slip through more easily. Once these agents enter the tumor, poor lymphatic drainage causes them to remain there for an extended time, leading to extended drug retention [139]. However, they often accumulate in MPS-related tissues such as the liver and spleen, which limits their use for diagnosis. Current research focuses on improving their removal from these tissues [104]. Adding Cathepsin S- sensitive linkers to HPMA copolymers helps them reduce their buildup in MPS tissues, which makes the imaging clearer [140]. Cathepsin S is an enzyme that naturally exists in high levels within phagocytic cells. The mononuclear phagocytic system plays a major role in removing many drug carriers from the circulation, thereby reducing the effectiveness of targeted therapies [141]. By designing linkers that can be specifically broken down by cathepsin S, studies aim to take advantage of the enzyme's higher presence in these non-target tissues compared to many tumors. This allows the radiolabeled compound to be eliminated more quickly from non- target tissues, while remaining longer in tumor tissues [140]. Thus, beyond direct enzyme inhibition, cathepsin S represents a valuable translational target for improving drug delivery and imaging specificity, although challenges related to accumulation in MPS tissue remain [139], [141].

5.7. YAP1 degradation by cathepsins in pancreatic cancer

Yes- associated protein 1 (YAP1) functions as the primary transducer of the Hippo signaling pathway, which regulates processes such as tumor development, tissue repair and regeneration [142]. It also interacts with other cancer-related signaling pathways.

YAP1 is considered as a new biomarker for PC [143]. The transition from pancreatic intraepithelial neoplasia to pancreatic ductal adenocarcinoma depends on YAP, which can drive either via direct transition of pancreatic epithelial cells or through its effects on pancreatic stellate cells. YAP contributes to fibrosis and increased tissue stiffness through multiple mechanisms [144]. However, how it promotes immune infiltration in PC remains unclear.

YAP/TAZ pathway

Acting downstream of KRAS, the *YAP/TAZ* pathway plays a central role in PDAC progression. YAP and TAZ (= WW-domain-containing Transcriptional co-activator with PDZ- binding motif) are transcriptional co-activators. They act as central points for multiple signaling pathways [54]. These networks control processes including development, metabolism, tissue repair, organ growth and mitosis, which are all key drivers of cancer development. In PDAC, YAP/TAZ can work together with KRAS to initiate tumorigenesis and, in some cases, replace KRAS to maintain survival in highly aggressive tumor subtypes [145].

YAP Regulation

The Hippo signaling pathway, a tumor suppressor cascade, serves as a central regulator of YAP/TAZ activity. Through sequential activation of MST1/2, SAV1, LATS1/2 and MOB1/2, Hippo signaling ultimately phosphorylates YAP and TAZ [54]. This leads to YAP/TAZ attaching to 14-3-3 proteins, which keeps them in the cytoplasm. There, they are further modified by phosphorylation, marked with ubiquitin and finally broken down [146]. In the absence of active Hippo signaling, YAP/TAZ remain unphosphorylated and move into the nucleus, where they bind to TEAD (TEA- domain DNA- binding) transcription factors (TEAD 1-4). These complexes further cooperate with other transcriptional regulators at specific promoters and enhancers [145]. While YAP/TAZ is quickly modulated by upstream regulators including PI3K, actin remodeling, Rho and GPCR signaling, their expression levels are further influenced by other pathways and epigenetic events [54]. YAP and TAZ also partner with SMAD proteins, which are key transcription factors in many cellular processes. When combined, the YAP/TAZ-SMAD complex influences stem cell maintenance and contribute to helping cancer cells avoid immune detection. Additional transcription factors that cooperate with YAP/TAZ include p73, RUNX and many more [146]. Furthermore, RAS signaling stabilizes YAP1 outside

of the Hippo cascade by downregulating SOCS5/6, factors that normally target for degradation [54].

YAP and lysosomal cathepsins

The soft extracellular matrix in pancreatic cancer facilitates YAP1 degradation through the autophagy-lysosome pathway instead of the Hippo signaling pathway. This process is driven by PTEN (phosphatase and tensin homolog) activation which stimulates lysosomal biogenesis and thereby activates cysteine cathepsins, which can destroy YAP1 [144]. In the majority of human PDAC cases, PTEN expression is reduced [147]. Among them, cathepsin L has been shown that it alone, under acidic conditions, can digest YAP1. Inhibition of lysosomes prevent YAP1 degradation, leading to its accumulation and oncogenic transformation. Clinical data demonstrate that reduced lysosomal activity is associated with fibrosis, stroma stiffening, higher YAP1 levels and poor prognosis [144].

KRAS and YAP1

The expression of Yap1 shows a strong association with the expression of multiple proto-oncogenes, including *KRAS* [143]. PDAC cells carrying LOH at KRAS demonstrated elevated nuclear YAP1 activity [55]. The loss of the WT (wild type) KRAS allele, resulting in a shift from heterozygosity to homozygosity and known as loss of heterozygosity (LOH), enhances invasion and migration in PDAC. Furthermore, Kras^G12D-LOH triggers an alternative glutamine metabolic pathway, which supports malignant progression [9]. Studies indicated that WT KRAS normally retrains YAP1 in the cytoplasm, thus limiting its transcriptional effects. Notably, expression of a constitutively active YAP1 mutant was sufficient to promote PDAC cell proliferation even in presence of WT KRAS [55]. Activated YAP1 acts as a strong oncogenic factor associated with the development with various cancer types [142]. Patient tumors with LOH at KRAS showed upregulation of YAP1 activated genes, and nuclear YAP1 localization served as a predictor of poor survival [55].

5.8. MUC1 regulation of cathepsin B in pancreatic cancer

MUC1, an overexpressed transmembrane mucin in pancreatic cancer, appears to help regulate polyamine metabolism. Higher MUC1 expression is associated with increased expression of genes involved in polyamine metabolism [148]. Polyamines are essential for many key steps in cell growth. They support the production of proteins, regulate cell death, help maintain chromatin structure, reduce oxidative damage, and regulate channels that are important for communication between cells [149]. MUC1 is made as one chain that later separates into two connected parts, an outer domain and an inner tail. The outer part

protects the cell surface, while the cytoplasmatic tail (MUC1-c) binds to β-catenin and activates signals that help cancer grow, survive and avoid cell death [103]. Because of its large molecular size, MUC1 functions under normal healthy conditions as a protective layer on the membrane, helping to block pathogens from reaching the cell. In pancreatic cancer, MUC1 is produced in large amounts and shows reduced glycosylation. These changes contribute to the activation of multiple signaling cascades that support cancer development, uncontrolled growth, and tumor spread [149]. Many factors cause cancer cells to resist drugs, such as transporters and enzymes. These changes reduce drug effects and alter metabolism, with MUC1 acting as an important regulator in this process [150]. Blocking or reducing MUC1 increases Cathepsin B activity in the cytosol, a sign that lysosomal membranes became permeable. MUC-1c prevents this process by keeping lysosomes stable and preventing Cathepsin B release, thereby protecting pancreatic cancer cells from dying [103].

The amount of MUC1 in pancreatic cancer cells depends on threonine and on the enzyme threonyl tRNA synthetase (TRS), which is responsible for attaching threonine to tRNA during protein synthesis. TRS increases MUC1 production and supports cancer cell migration. Both TRS and MUC1 show a positive correlation in pancreatic cancer and are connected with patient survival [151]. Reducing the enzyme SAT1 (spermidine/spermine N1-acetyltransferase 1), which is involved in polyamine metabolism, was found to reduce the oncogenic effects of MUC1. MUC1 enhances SAT1 expression through stabilization of HIF-1α, which binds to and activates SAT1 promoter. Blocking MUC1 lowers SAT1 activity and decreases the production of its metabolites [148]. HSP70 interacts with MUC1-c to deliver it to the lysosomes, protecting the cells from damage and helping them survive. Reducing HSP70 levels causes lysosomal damage and leads to the death of pancreatic cancer cells [103].

Multiple sensitive and specific methods have been created to measure MUC1 levels. As these analytical techniques improve, measuring mucin levels in the blood of cancer patients may become increasingly useful for identifying MUC1 expression profile and guiding the use of new MUC1- focused treatments [149].

A variety of vaccine platforms- including DNA vaccines, dendritic cell vaccines and glycopeptide approaches- have been created to specifically target MUC1. Many of these candidates are currently being tested in clinical trials. The Vibrio cholerae toxin B subunit (CTB) has shown strong potential as a carrier for subunit vaccines and has demonstrated significant reductions in tumor growth [149].

Beyond vaccine- based strategies, another promising approach involves chimeric antigen receptor T cells, known as CAR-T cells. These are specially modified receptors that

reprogram T cells so they can recognize and attack cancer cells more effectively. The advantage of CAR-T therapy is that it creates tumor- specific T cells quickly, thereby overcoming the limitations and slower response associated with traditional active immune responses [152]. Recently, studies have developed CAR-T cells that use parts of a new monoclonal IgG1 antibody called TAB004. This antibody binds only to MUC1 found on pancreatic cancer cells. These modified CAR-T cells showed promising ability to destroy cancer cells [149].

Further experiments revealed that GO-201, a peptide specifically designed to inhibit the MUC1-c domain, effectively blocks its oncogenic signaling functions. As a result, this leads to decreased tumor- cell growth and reduced cell survival in both in vivo and in vitro models [103].

6. Current Therapies for PC

Pancreatic ductal adenocarcinoma is mainly treated through surgery and chemotherapy. However, at the time of diagnosis only about 15-20% of patients are suitable candidates for surgery [153]. With the increased use of less invasive surgery and modern surgical aids, both the safety and quality of pancreatic cancer operations have improved. At the same time systemic chemotherapy has progressed, improving patient survival and is now more used before surgery, often together with radiotherapy [154].

Current standard therapy for initial treatment involves FOLFIRONOX- which combines 5-fluorouracil, leucovorin, irinotecan and oxaliplatin- or the use of gemcitabine together with nanoparticle albumin-bound paclitaxel (nab-paclitaxel) [155]. So far, other treatment combinations have not yet produced better major survival improvements than current. However, ongoing studies into the molecular and environmental factors of PC is revealing new therapeutic targets in specific patient groups. Early identification of these patients could allow personalized treatment and better results [26].

6.2. FOLFIRINOX

FOLFIRINOX shows a greater tumor response rate than gemcitabine (32% compared to 9%), but it is linked to increased toxicity. Due to its strong therapeutic effect, FOLFIRINOX has been introduced before surgery as a neoadjuvant therapy. Although it is generally prescribed for patients with overall good condition, there is no evidence that this factor influences treatment sensitivity [156]. FOLFIRINOX is often given in a modified form known as modified FOLFIRINOX (mFOLFIRINOX). In this version the dose of irinotecan is reduced, and the bolus form of fluorouracil may be removed to reduce toxicity [88]. Neoadjuvant therapy has multiple possible advantages compared with chemotherapy given after surgery. Since the tumor still has good blood circulation, the drugs can reach the tissue more effectively and attack small cancer cells. Moreover, it has been shown that patients tolerate it easier than postoperative chemotherapy, leading to more people finishing their treatment [154]. Studies in PDAC indicate that FOLFIRINOX enhances the activity and number of effector T-cells and decreases at the same time immune cells that suppress the immune response [153]. Typical side effects include fever, loss of weight, tiredness or diarrhea. Still, some side effects from FOLFIRINOX are unpredictable, not related to dosage and difficult to manage by simple dose adjustment [157].

Mechanism of action

5-Fluorouracil (5-FU): The main way in which 5-Fluorouracil causes cancer cell destruction is through the inhibition of thymidylate synthase. First, thymidine phosphorylase transforms 5-FU into 5-fluorodeoxyuridine, and then thymidine kinase 1 adds a phosphate group to produce 5-fluorodeoxyuridine monophosphate (FdUMP) [158]. FdUMP binds to the active region of thymidylate synthase and forms a tight complex with both the enzyme and its cofactor. This interaction prevents deoxyuridine monophosphate from binding where it normally would, thereby blocking thymidylate production. As a result, the pool of available thymidylate becomes reduced, which interferes with DNA synthesis and repair [159].

Leucovorin: In treatment, 5-FU is typically combined with folinic acid (leucovorin). The combination- including irinotecan and oxaliplatin- significantly reduces the chance of relapse and increases the overall patient survival [160]. Inside the cell, folinic acid is converted into 5,10-methylenetetrahydrofolate. This form helps stabilize the complex between thymidylate synthase, its cofactor, and the active metabolite of 5-FU, reducing the release of the enzyme. As a result, this stabilization increases the effectiveness of the 5-fluorouracil metabolite in inhibiting thymidylate synthase [159].

Irinotecan: Irinotecan has been commonly used to treat cancer. As a selective inhibitor of topoisomerase I, it works by stabilizing the topoisomerase I- DNA cleavage complexes, making it useful for understanding the cellular mechanisms that repair DNA damage caused by protein-DNA attachments and replication associated stress [161]. Topoisomerases are enzymes found in the cell nucleus that keep DNA in the right shape, while transcription and replication. Type I topoisomerases cut one strand of the DNA while type II topoisomerases create double-strand breaks. These cuts allow the DNA to unwind and loosen, so it can be properly used as a functional template [162]. Irinotecan itself is inactive and must be converted by carboxylesterase enzymes into its active form, SN-38 [161]. During DNA replication, the complex formed by irinotecan prevents the replication fork from moving forward. Through this blockage, pressure builds on the remaining DNA strand. This stress leads to breaks in the DNA, including double-strand breaks, ultimately resulting in cell death [163].

Oxaliplatin: Oxaliplatin is an anticancer drug, that contains platinum [164]. It enters tumor cells primarily through copper transporter 1 (CTR1). Once inside the cell, the platinum compound becomes activated through a process in which its chloride ligands are replaced by water or small sulfhydryl-containing molecules [165]. Oxaliplatin contains a 1,2-diaminocyclohexanxe (DACH) carrier ligand. This ligand is strongly lipophilic, which enhances the drug's ability to cross the cell membrane through passive diffusion and by using transporter proteins other than CTR1 [166]. Platinum interacts with DNA by creating

both intra-strand and inter-strand crosslinks between nucleotides [165]. The interaction allows the amine group of the DACH ligand to bind to the guanine base 3' end of the DNA. The resulting bond changes the normal shape of the DNA. By creating platinum-DNA adducts, the drug damages the DNA, leading to cell death [166].

6.3. Gemcitabine

Gemcitabine is a commonly used chemotherapy drug in the treatment of cancer. For patients with PDAC it is often better tolerated than many other chemotherapy drugs. Its side effects can affect blood cells, leading to anemia or thrombocytopenia and may also include other symptoms including tiredness, vomiting and increased alanine aminotransferase levels [167]. Gemcitabine in combination with nanoparticle albumin-bound (nab-) paclitaxel is regarded as the preferred initial treatment for patients with poor health or limited physical condition [168]. In addition, therapy with gemcitabine resulted in lower pain levels, reduced use of analgesics and better physical condition [169].

Mechanism of action

Gemcitabin: Gemcitabine is a modified form of the natural nucleoside deoxycytidine. It works by interfering with DNA synthesis, thereby preventing cells from moving through the cell cycle, particularly between the G1 and S phase [170]. Gemcitabine must be phosphorylated inside the cell to become active. This process depends on specific enzymes involved in its conversion. Since this drug is hydrophilic, it relies on nucleoside transporters to enter the cell. These include both sodium-dependent concentrative nucleoside transporters (hCNTs) and sodium-independent equilibrative nucleoside transporters (hENTs) [171]. Gemcitabine can be transported into cells through five nucleoside transporters: hCNT1-3 and hENT1-2. However, studies suggest that hENT1 is the main transporter responsible for bringing gemcitabine inside, while hENT2, hCNT1 and 3 assist [170]. After entering the cell, gemcitabine is converted into gemcitabine monophosphate by the enzyme deoxycytidine kinase. This form is further changed into diphosphate and then into gemcitabine triphosphate. These final forms are the ones that cause its cancer killing effects [171]. The triphosphate can be inserted into the replicating DNA strand inducing apoptosis. Through this effect, gemcitabine triphosphate becomes fixed within the DNA. Specifically, once gemcitabine is inserted, the addition of one more nucleotide blocks DNA polymerases from continuing and prevents repair enzymes from removing the gemcitabine. This effectively blocks DNA replication [170]. In addition, a reduction in the amounts of gemcitabine triphosphate and natural deoxycytidine triphosphate inside the cell slows the ability of the enzyme deoxycytidylate deaminase to break down gemcitabine

monophosphate. This enzyme activity depends on a proper amount of deoxycytidine triphosphate to function properly [171].

Nab-Paclitaxel (Abraxane): In nanomedicine albumin is considered an excellent drug carrier because of several reasons. As the most common plasma protein, it provides good biocompatibility, low immune reaction and clinical safety. Its molecular structure enables it to bind a wide variety of drugs, protecting them from rapid clearance and metabolism [172]. Nab-paclitaxel consists of smaller particles, which facilitates the transportation of paclitaxel into cancer cells, resulting in stronger antitumor effects. Compared with standard paclitaxel, it shows a larger distribution in the body and reaches higher plasma concentration levels [173]. Abraxane crosses the blood vessel wall through vesicular pathways, reaches the tumor, and is then held inside cancer cells by binding to the protein SPARC (secreted protein acidic and rich in cysteine). The accumulated drug destroys malignant cells and causes the release of large amounts of tumor antigens, thereby supporting antitumor immune activation [174]. SPARC, also called osteonectin, is a glycoprotein and highly expressed in several cancers, including lung, breast and pancreatic ductal adenocarcinoma. In PDAC, it is present in both the cancer cells and the surrounding stroma [173].

6.4. Chemoresistance

One important characteristic of tumor cells is their potential to evade the effects of chemotherapy. Components of the ECM can contribute to this drug resistance by activating multiple signaling mechanisms. These signals trigger changes in gene activity and protein function that help cancer cells withstand the effects of chemotherapeutic treatment [175]. While FOLFIRINOX and gemcitabine/nab-paclitaxel significantly improve survival in pancreatic cancer, many patients develop resistance to these treatments leading to reduced effectiveness. The cause of this chemoresistance is complex and involves interactions between PC cells, cancer stem cells and the tumor environment [171]. One key reason for this resistance is the ability of pancreatic cancer cells to infiltrate the surrounding tissue and actively exchange nutrients and even genetic material with cells in the TME [58]. Activated cancer-associated fibroblasts stimulate large amounts of ECM components, forming a thick tissue barrier that prevent the drugs from reaching the tumor. PDAC is characterized by having very few blood vessels and the increased pressure inside the tumor caused by the thick matrix, which makes it hard for chemotherapy to be delivered effectively [176].

Studies show that cancer stem cells are strongly involved in cancer returning after chemotherapy. They often carry drug resistant transporters and activate survival pathways, allowing them to avoid apoptosis, which makes them resistant to treatment [177].

A growing focus in understanding treatment resistance in PDAC is the role of cellular senescence. Both radiation and chemotherapy can push tumor into a senescent-like condition instead of killing them. FOLFIRINOX and gemcitabine have shown to cause senescence in cancer cells through increased ROS levels, slowing tumor progression [178]. Findings suggest that irbesartan could serve as a promising anti-cancer agent by improving the effectiveness of gemcitabine therapy and could play an important role in treating PDAC [179]. Though, in gemcitabine resistant cells, senescence can be reversible if key tumor suppressor mechanisms are inactive, allowing the cells to start dividing again. This means that some cancer cells may survive treatment by going into a temporary senescent state and later resistance [178].

Conclusion

Pancreatic ductal adenocarcinoma remains one of the most lethal cancers, marked by late detection, high metastatic potential, and limited treatment options. Throughout this thesis, lysosomes and their associated proteases, the cathepsins, have emerged as important components in the survival and progression of PDAC. Their involvement in autophagy, extracellular matrix remodeling, invasion, immune modulation, and tumor– stroma interactions highlights their essential contribution to the aggressive biology of the disease. Because PDAC depends on lysosomal pathways to withstand stress and maintain rapid growth, lysosomal cathepsins not only represent important biomarkers of cancer activity but also promising therapeutic targets.

The evidence reviewed in this thesis shows that individual cathepsins contribute to PDAC progression through defined yet overlapping functions and that several of them can be targeted in ways that weaken important survival pathways in pancreatic cancer cells. Cathepsin B can be inhibited with compounds such as CA-074 or regulated by endogenous inhibitors like cystatin A, both of which reduce its contribution to tumor invasion and ECM breakdown. Cathepsin E, although less studied, has shown promise as a target in approaches using photodynamic therapy, where its expression pattern may help guide more selective tumor killing. Cathepsin L, an important protease involved in autophagy, can be blocked with molecules such as E64FC26, which has been shown to interfere with tumor cell survival. Cathepsin S, known for its roles in immune modulation and matrix remodeling, can be targeted wit alpha- fluorocinnamate derivates, which reduce its proteolytic activity and may help limit tumor progression. Additional strategies indirectly affect cathepsin-related pathways, such as promoting YAP1 degradation, which reduces tumor growth, or targeting MUC1, a transmembrane protein that interacts with lysosomal and proteolytic signaling to support PDAC aggressiveness.

Together, these examples demonstrate that interfering with cathepsin activity- or with pathways that depend on it- can weaken key characteristics of PDAC, including its high invasiveness, and immune resistance. Although challenges remain, especially concerning specificity and compensatory mechanisms between different cathepsins, the therapeutic concept is strong.

In conclusion, lysosomal cathepsins represent a valuable group of therapeutic targets in PDAC. By combining knowledge from cancer biology, protease research, and emerging therapeutic approaches, this thesis highlights their potential to contribute to more effective and more precise treatment strategies. Continued research into cathepsin function, regulation and inhibition will be essential for translating these insights into clinical benefit,

offering new hope for improving outcomes in a cancer that has long remained one of the most difficult to treat.

Table of Contents

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