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CATHEPSIN D: MOLECULAR, GENETIC AND FUNCTIONAL ASPECTS IN THE TUMOR MICROENVIRONMENT

CATEPSINA D: ASPECTOS MOLECULARES, GENÉTICOS E FUNCIONAIS NO MICROAMBIENTE TUMORAL

CATEPSINA D: ASPECTOS MOLECULARES, GENÉTICOS Y FUNCIONALES EN EL MICROAMBIENTE TUMORAL

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ABSTRACT

Cathepsin D (catD) is an endoprotease acidic lysosomal protein (aspartyl protease) found in virtually all mammalian cells that was initially associated with the degradation of nonspecific polypeptides. Initially, it is formed as inactive proenzymes that are subsequently activated by the proteolytic removal of the N-terminal propeptide. The detailed physiological function of catD is poorly understood. However, it is considered a multifunctional protease responsible for essential biological processes related to the regulation of the cell cycle, differentiation, migration, tissue remodeling, neuronal growth, ovulation and apoptosis. In addition, it has been reported in inflammatory and neurodegenerative processes, as a potential tumor marker and in carcinogenesis. Given its relevance, this study reviewed and characterized integrative data on the molecular and functional aspects of catD associated with the tumor microenvironment. The results provide a scientific overview of cathepsin D and its participation in the tumor microenvironment that can support new studies and prospective trials focused on carcinogenesis.

Keywords: Endoproteases. Cellular Physiology. Tumor Microenvironment. Cancer.

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RESUMO

A catepsina D (catD) é uma endoprotease lisossomal ácida (aspartil protease) encontrada praticamente em todas as células de mamíferos que foi, a princípio, relacionada com a degradação de polipeptídeos não específicos. Inicialmente, forma-se como proenzimas inativas e que, posteriormente, são ativadas pela remoção proteolítica do pró-peptídeo N-terminal. A função fisiológica pormenorizada da catD é pouco esclarecida. Entretanto, é considerada uma protease multifuncional responsável por processos biológicos essenciais relacionados com a regulação do ciclo celular, diferenciação, migração, remodelamento tecidual, crescimento neuronal, ovulação e apoptose. Além disso, tem sido relatada em processos inflamatórios, neurodegenerativos, como um potencial marcador tumoral e na carcinogênese. Diante de sua relevância, este estudo revisou e caracterizou integrativamente dados dos aspectos moleculares e funcionais da catD associados ao microambiente tumoral. Os resultados trazem um panorama científico sobre a catepsina D e sua participação no microambiente tumoral que podem subsidiar novos estudos e ensaios prospectivos voltados à carcinogênese.

Palavras-chave: Endoproteases. Fisiologia Celular. Microambiente Tumoral. Câncer.

RESUMEN

La catepsina D (catD) es una endoproteasa lisosomal ácida (aspartil proteasa) presente en prácticamente todas las células de mamíferos, inicialmente asociada a la degradación de polipéptidos inespecíficos. Inicialmente se forma como proenzimas inactivas que posteriormente se activan mediante la eliminación proteolítica del propéptido N-terminal. La función fisiológica detallada de la catD es poco conocida. Sin embargo, se considera una proteasa multifuncional responsable de procesos biológicos esenciales relacionados con la regulación del ciclo celular, la diferenciación, la migración, la remodelación tisular, el crecimiento neuronal, la ovulación y la apoptosis. Además, se ha descrito su participación en procesos inflamatorios y neurodegenerativos, como posible marcador tumoral y en la carcinogénesis. Dada su relevancia, este estudio revisó y caracterizó de forma integral los datos sobre los aspectos moleculares y funcionales de la catD asociados al microambiente tumoral. Los resultados proporcionan una visión científica general de la catepsina D y su papel en el microambiente tumoral, lo que podría respaldar nuevos estudios y ensayos prospectivos centrados en la carcinogénesis.

Palabras clave: Endoproteasas. Fisiología Celular. Microambiente Tumoral. Cáncer.

INTRODUCTION

Cathepsin D (catD) is an endoprotease acidic lysosomal protein , found in virtually all mammalian cells (Moraes *et al*., 2022; Rochefort, 1990; Saku *et al*., 1991; Větvicka *et al*., 2010) being grouped, due to its chemical characteristics, in the aspartyl protease family, is a molecule

synthesized in the rough endoplasmic reticulum as pre-procathepsin D with 52 kDa that is subsequently converted into an intermediate molecule with a single and active chain, by the removal of the signal peptide, with 48 kDa and, in the intracellular vesicles (endosomes, lysosomes and phagosomes), after this conversion, fully active, it is composed of a heavy chain of 34 kDa and a light chain of 14 kDa . This structure presents up to eight amino acid residues in the binding cleft of the active site, with a preference for hydrophobic residues around the cleaved bond (Merops, n.d.; Aguilera *et al*., 2023; Benes, Větvicka & Fusek, 2008, 2008; Erickson *et al*., 1981; Větvicka *et al*., 2010). Additionally, estrogen is cited as an agent that regulates catD through binding to estrogen response elements (EREs) and modulating the transcription of cathepsin D (Cavailles *et al*., 1988), as well as being associated with breast cancer (Alhudiri *et al*., 2024; Garcia *et al*., 1996; Liaudet-Coopman *et al*., 2006; Masson *et al*., 2011; Ohri *et al*., 2008; Rochefort, 1990; Rochefort & Liaudet-Coopman, 1999; Tandon *et al*., 1990).

The physiological function of catD, although not well elucidated, has been reported to involve the degradation of tissue proteins under both normal and pathological conditions (Conte & Fonseca, 2021), acting by mediating the breakdown of unfolded protein aggregates delivered by endocytosis or autophagy (Li *et al*., 2017), as well as in lipid metabolism (Masson *et al*., 2011; Mutka *et al*., 2010). Furthermore, the autophagy-lysosome pathway is recognized as the primary clearance system for amyloid beta (Aβ) and tau proteins, and the enzymatic function of catD is not restricted solely to the acidic environment of lysosomes. One important consequence of this is its role in the regulation of apoptosis (Benes, Větvicka & Fusek, 2008).

According to Tulone *et al*. (2007), cathepsin D deficiency is associated with a spectrum of pathologies that can lead to death. On the other hand, catD is expressed at high levels in many immune system cells, though its role in immune function is not yet well elucidated. However, catD has been associated with various pathological conditions, such as cancer (Větvicka *et al*., 1994), neurodegenerative diseases like Alzheimer's (Zhou *et al*., 2006), and lipofuscinosis neuronal ceroid - LCN (Siintola *et al*., 2006). Steinfeld *et al*. (2006) cite the increase in catD during ischemic, inflammatory, and regenerative processes, such as coronary heart disease (Vivanco & Martín-Ventura, 2005), inflammatory bowel disease (Hausmann *et al*., 2004), wound healing, and epidermal differentiation (Egberts *et al*., 2004). In addition to cathepsins, D, B, and L are also involved in other inflammatory processes, such as periodontics, rheumatoid arthritis, atherosclerosis, pancreatitis and gastritis (Kuester *et al*., 2008). Moraes *et al*. (2022), using surface plasmon resonance (SPR) and the Biacore T200 system (GE Healthcare), reported the

formation of a molecular complex between human catD and snake venom phospholipase A_2 (PLA_2). In addition, they are related to the remodeling of extracellular matrix (ECM) proteins (Rodríguez-Moreno & Legaz, 2025).

Regarding the involvement of catD in Alzheimer's disease (AD), a neurodegenerative pathology that results in cognitive and neuropsychiatric restrictions which can progressively lead to eventual incapacitation (Sereniki & Vital, 2008; Zhao & Tang, 2002; Janus & Westaway, 2001), genetic factors are cited. According to Smith (1999), among the related genes, ApoE4, α-2-macroglobulin, and cathepsin D (CTSD) stand out as being involved in the metabolism of Aβ and are characterized in all cases as risk factors for the disease.

For Lipofuscinosis Neuronal Ceroid (NCL), Zhang *et al*. (2025) and Monteiro *et al*. (2021) characterize it as a group of neurodegenerative diseases that result in the accumulation of lipid pigment within the lysosomes of neurons and other tissues. Of the different types, NCL10 encodes catD, which aids in neuronal stability and exerts effects in extracellular environments. Likewise, post-mortem diagnoses of the disease revealed massive neuron and cerebral cortex lesions, extensive gliosis, and absence of myelin (Steinfeld *et al*., 2006) and Epithelial ovarian cancer (EOC) (Pranjol *et al*., 2015; Pranjol & Whatmore, 2020).

Regarding cancer (CA), one of the most common diseases that impact health worldwide (Santos *et al*., 2023; Ferlay *et al*., 2013). In Brazil, it is estimated that new cases will exceed 704,000 between 2023 and 2025 (Santos *et al*., 2023). On the other hand, since the end of the 20th century, the disease has become one of the main preventable causes of death (Instituto Nacional de Câncer, 2023).

In this context, in reviewing the role of proteases in cancer, Koblinski *et al*. (2000) mention that local proteolysis is facilitated by proteases outside the tumor cell and describe that they are possibly bound to the cell surface and/or secreted by the tumor cell. Additionally, they infer that proteases inside the tumor cell also participate in local proteolysis, digesting the phagocytosed extracellular matrix. Furthermore, in metastasis, the cell must intravasate - invade other tissues, survive in the circulation, stop, leave the vasculature (extravasation), enter the surrounding tissue and grow.

One of the essential molecules in this process is cathepsin D. In carcinogenesis, catD is associated with DNA stimulation during mitosis and tissue regeneration; due to its proteolytic properties, it can facilitate tumor dissemination by digesting proteoglycans in the interstitial matrix and basement membrane (Eisenberg, Koifman & de Mama, 2001). Vashishta *et al*. (2006)

mention that although invasion and proliferation in vitro and tumor growth in vivo demonstrated that pCD expression potentiates the carcinogenic properties of NCI-H23 cells and that activation of the peptide is essential for these activities.

In this context, this review raises and characterizes, in an integrative manner, the molecular and physiological aspects associated with catD in the tumor microenvironment, as well as some genetic factors linked to cancer. Additionally, it provides an overview of the intra- and extracellular physiological mechanisms, the influence of pH and oxygenation on carcinogenesis and potential relationships between the Wnt signaling pathway and catD. The data may help in understanding the dynamics of the tumor microenvironment and contribute to directing new research on the mechanisms of catD action in carcinogenic processes and potential therapeutic pathways.

TUMOR MICROENVIRONMENT AND catD

The tumor microenvironment (TME) is the cellular environment in which the tumor exists (Hui & Chen, 2015), where multiple functions are performed not only in growth and metastasis but also in cancer metabolism and progression (Wei *et al*., 2020; Truffi *et al*., 2020)), and catD is now known to be a major secreted protein found in the cancer microenvironment (Větvicka *et al*., 2010; Pranjol & Whatmore, 2020). Genetically and morphophysiologically, the processes of dissemination, differentiation, and survival of individual cells in multicellular organisms are carefully regulated to meet the needs of the organism as a whole, characteristics that are lost in cancer cells (Cooper & Adams, 2022). In research, the TME presents remarkable heterogeneity, with substantial variability within and between tumors, thus influencing tumor biology, response to treatment, and prognosis (Deng *et al*., 2025; Visser & Joyce, 2023; Hinshaw & Shevde, 2019).

Functional variations of the TME are characterized by a complex set of factors that act dynamically in the mechanisms of cancer progression, treatment resistance, and metastasis (Tong *et al*., 2022). One relevant aspect of this process is related to the metabolic reprogramming that occurs in cancer cells, as well as in the surrounding stromal and immune cells (Yang *et al*., 2023).

Cui *et al*. (2024) and Long *et al*. (2024) mention an influence of tumor metabolism and immune cells on the occurrence, proliferation, metastasis, and prognosis of cancers. Additionally, they describe tumor tissue as forming a specific tumor microenvironment (TME), where the presence of tumor cells, immune cells, stromal cells, and others is observed. Among these, stem

cells (around 1 to 2%) stand out in the process and infiltrate the tumor mass, possessing the ability to give rise to tumor cells from cancer stem cells (CSC). They are considered to pose a greater risk due to their capacity for self-renewal, tumor *initiation*, and invasion into other tissues. However, tumors can arise from mutations that occur not only in stem cells but also in differentiated cells that acquire characteristics similar to those of stem cells (García-Heredia & Carnero, 2020).

Tumor cells, in order to adapt to their environment, undergo reprogramming of the metabolism of several substances, a fact that can further affect the formation of the tumor microenvironment and the function of various cells, especially immune cells, that eventually promote tumor development (Long *et al*., 2024). According to Hosonuma and Yoshimura (2023), the TME is characterized not only by the presence and interactions of several cells, such as tumor, immune, stromal, and blood vessels mediated by factors such as cytokines, but also by the existence of specific metabolites. In this dynamic complex, it is emphasized that there are variations in oxygenation, pH, circulation, growth, the immune system, cell types, interleukins - IL, signaling, and the canonical transport pathway - Wnt signaling. These relevant factors should be considered in studies aimed at therapeutic prospects.

GENETIC ASPECTS ASSOCIATED WITH CANCER AND catD

Only 5-10% of all cancer cases can be attributed to inherited genetic mutations, while the remaining 90-95% have their roots in environmental and lifestyle factors. These lifestyle factors include smoking, diet (fried foods, red meat), alcohol, sun exposure, environmental pollutants, infections, stress, obesity, and physical inactivity (Anand *et al*., 2008). Thus, cancer results from changes in genes present in DNA that are associated with how cells grow and/or multiply; during this process, some changes may occur as random errors in cell multiplication. These changes can be caused by various carcinogenic agents, as well as errors inherited from our progenitors (INC, 2023).

In the evolutionary process from normal cells to the neoplastic state, certain typical cancer events are acquired. Hanahan and Weinberg (2011) list, as critical characteristics for neoplasia, the maintenance of proliferative signaling, metabolic reprogramming, resistance to programmed cell death, genetic instability and mutations, angiogenesis, tissue invasion and metastasis,

generation of the inflammatory process, unlimited proliferation, evasion of the immune system, and resistance to antiproliferative signals.

In human deoxyribonucleic acid (DNA), genes typically involved in and responsible for cancer progression include, according to Hanif *et al*. (2025), *XRCC1, XRCC4, KRAS, TP53, EGFR, PI3KCA, ERCC2/XPD, MET, ALK1, BRAF, HER2, LTKB-1/STK11, AKT1*, and *MAP2K1*. In addition to these, *HER2, MAP2K1/MEK1, AKT, NRAS,* and several combinations of concomitant mutations were also noted as stimulators of carcinogenic processes. That is, for the authors, these genes impair the efficiency of DNA repair or interact with various signaling pathways that result in cell proliferation, differentiation, angiogenesis, invasion, and metastasis, such as the PI3K/AKT pathway (phosphatidylinositol 3-kinase/protein kinase B), MAPKs pathway (mitogen-activated protein), PKC pathway (protein kinase C), JAK/STAT pathway (Janus kinase/signal transducer and activator of transcription), as well as RAS signaling – mediated by G protein.

Regarding DNA alterations, various agents can damage it and trigger a wide variety of cellular responses, such as cell cycle interruption, upregulation of DNA repair systems, apoptosis, or mutagenesis (Gates, 2009; Hurley, 2002; Zhou & Elledge, 2000). Thus, elucidating and understanding the effects of structural damage and the resulting biological responses are relevant, as they have the potential to generate advances in cell biology, predictive toxicology and therapeutics (GATES, 2009).

Regarding the participation of catD, Eisenberg, Koifman & de Mama (2001) mention that it plays a role in carcinogenesis associated with the stimulation of DNA and mitosis during tissue regeneration. Additionally, through its proteolytic power, it facilitates tumor dissemination. In addition, most of the DNA alterations linked to cancer occur in genes that provide instructions for making proteins or specialized RNA, such as microRNA. Some of these mutations lead to increased levels of proteins that stimulate uncontrolled cell growth, while others reduce protein levels, causing cells to stop growing. Additionally, some mutations halt proteins that signal cells to self-destruct when they are damaged (INC, 2023).

catD is one of the most abundant lysosomal endoproteases (Oberle *et al*., 2010), and the CTSD gene, located in the 11p15.5 region with 9 exons, is responsible for encoding this protein (Mijanovic *et al*., 2021; Lehesjoki & Gardiner, 2010; Garcia *et al*., 1996; Masson *et al*., 2010)). It features a mixed promoter that controls the expression of the catD gene, typically allowing for TATA-independent transcription initiation by specificity binding site protein - gene regulation

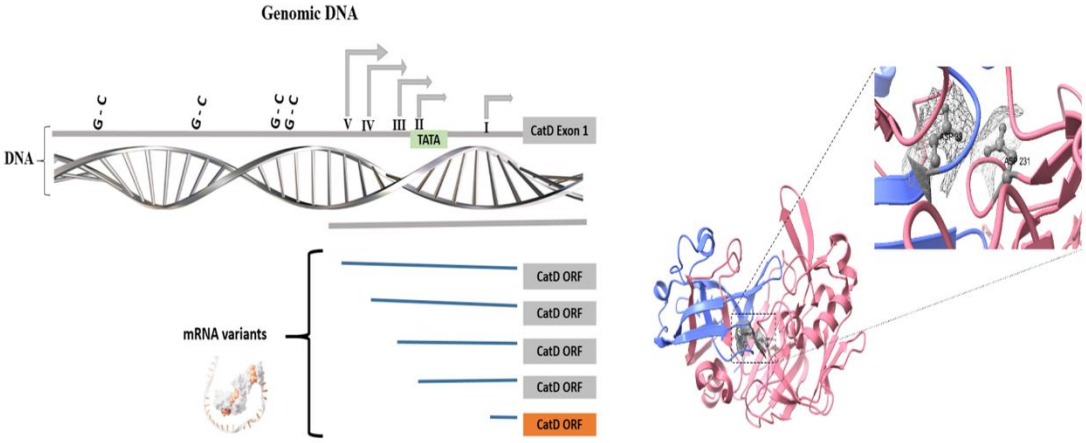
(Sp1) and TATA-dependent initiation. Additionally, there is estrogen-activated transcription (Cavaillès *et al*., 1993; Mijanovic *et al*., 2021). Furthermore, according to Zargaran *et al*. (2016), the expression of this gene is also regulated by steroid hormones, growth factors, tumor necrosis factor alpha, and retinoic acid and is involved, after transcription, in tumor metastasis processes.

According to Tan *et al*. (2013), these molecules were initially characterized by cathepsins E, F, G, and Z, promoting cellular tumorigenesis. Tumor cells are induced by the proliferation of cathepsins B, D, E, and X. Cathepsin X mediates the epithelial-mesenchymal transition (EMT) of tumor cells, while cathepsins A, B, and D induce the dissemination of tumor cells. Cathepsins B, K, L, and Z degrade the extracellular matrix (ECM), and cathepsins B, D, H, L, S, and Z increase the motility and invasion of tumor cells that migrate to surrounding tissues, blood, and lymphatic vessels, metastasizing to distant sites in the body. At metastatic sites, cathepsins B, D, and H mediate the proliferation of tumor cells, while cathepsins B, D, H, and S mediate angiogenesis and metastatic tumor formations (Berchem *et al*., 2002; Tan *et al*., 2013).

catD is involved in different oncogenesis mechanisms and, according to Zaidi *et al*. (2008), the promoter region in the DNA is present at five sites considered major for the initiation of transcription (TSS - I to V). Additionally, the five transcription initiation sites are mapped at −20, −44, −51, −60, and −72 from the first base of the initiation codon. Under basal conditions, the authors state that the expression of human catD is independent of the TATA box and starts at one of several transcription initiation sites (TSS-II to -V), upstream of the TATA box, and is possibly directed by GC boxes and the Sp1 factor (Figure 1). In the case of breast cancer, *op. cit*., they are stimulated by estrogen, and catD is reported to be overexpressed, with transcription dependent on the TATA box, starting at TSS-I. The lower part of the figure illustrates a comparison between the lengths of different catD mRNAs. Furthermore, according to Zaidi *et al*. (2008), the difference in length is only between the 5’ untranslated regions of the mRNA. The first four mRNAs with longer 5’ untranslated regions are present in higher proportion under basal conditions where TATA-independent transcription is dominant. Meanwhile, in breast cancer cells, the proportion of mRNAs with shorter 5’ untranslated regions (orange) is increased.

Figure 1

Illustration of the genomic DNA segment and the promoter region, said to be present, with five sites described as major transcription start sites (TSS - I to V).



Sources: Modified from Zaidi *et al*. (2008), Cavailles *et al*. (1993), using AI Freepik (2025). The image on the right depicts the three-dimensional structure of mature human cathepsin D, comprising 347 residues (PDB 6QCB). Modified to highlight the catalytic residues (Asp33 and Asp231 – catalytic dyad). Source: Protein Data Bank – PDB. (2025), ID: https://www.rcsb.org/.

INTRA AND EXTRACELLULAR PHYSIOLOGICAL MECHANISMS INVOLVING catD

Cathepsins exhibit a diversity of transport pathways to reach various intra- and extracellular sites where proteases can function in a spatially confined and temporally regulated manner (Brix, 2018; Akkari *et al*., 2016; Gocheva & Joyce, 2007). Lysosomes are multifaceted structures that mediate the transport of these biomolecules, participate in molecular degradation, antigen presentation, destruction of intracellular pathogens, plasma membrane repair, exosome release, cell adhesion/migration and apoptosis (Figure 2), in addition to being closely associated with cancer cell proliferation, invasion and metastasis, as well as immune escape and tumor angiogenesis (Tang *et al*., 2020; Zhang *et al*., 2021) (Figure 3).

Intracellularly, the activities and structures involved in promoting the fusion and lysis of molecules by the action of digestive enzymes mainly encompass Rab GTPases, attachment proteins that control intracellular vesicle transport, and SNAREs (Figure 2). Rabs, such as Rab7, serve to activate and recruit the attachment proteins of the HOPS (homotypic fusion and vacuolar protein sorting) complex. This complex allows for the proximity of the membranes of the

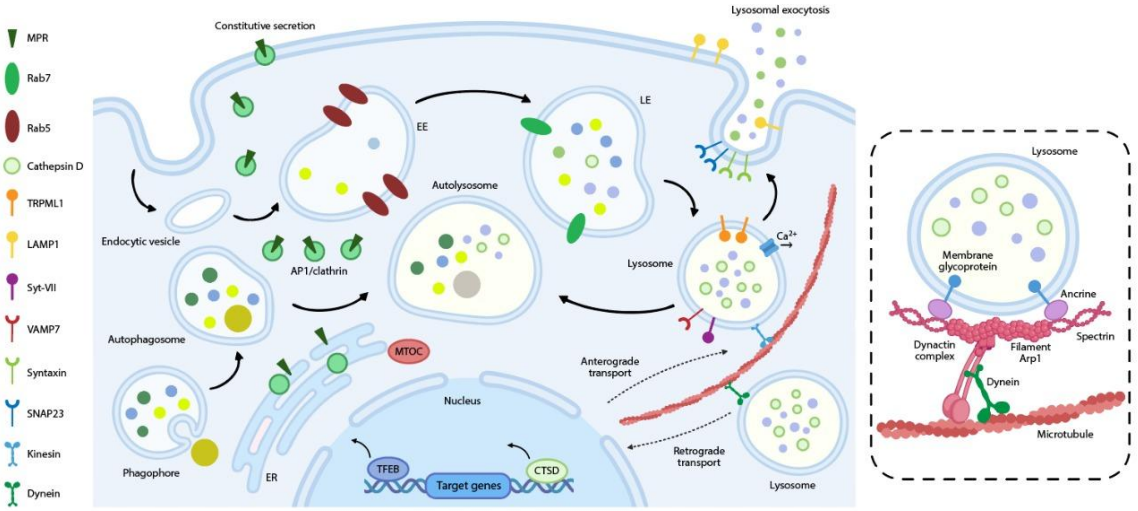
organelles to be fused (autophagosome and lysosome), functioning as a link between them and thus collaborating in the support of the fusion mediated by SNAREs (Koike & Ganley, 2013; Jahn, 2024; Stenmark, 2009). SNAREs are membrane proteins located in opposing membrane complexes that may or may not interact with each other. In the autophagy process, the SNAREs STX17 (Syntaxin 17) and SNAP29 (synaptosomal-associated protein 29) in autophagosomes and the SNAREs VAMP7 and VAMP8 in lysosomes have already been cited to mediate the fusion of these organelles (Kato & Tagaya, 2022; Wang & Diao, 2022; Itakura *et al*., 2012; Shen & Mizushima, 2014).

After the contents of the autophagosomes are released into the lumen of the lysosome, lysosomal enzymes degrade and recycle the digested components back into the cells. In this process, more than 50 types of acid hydrolases in the lysosomes have been described as phosphatases, proteases, peptidases, nucleases, glycosidases, sulfatases, and lipases— all capable of degrading any cellular macromolecule. Among this enzyme complex, the proteases cathepsins B, L, and D are the most active molecules in autophagic degradation (Kallunki & Jaattela, 2013; Kaminskyy & Zhivotovsky, 2012; Saftig, 2006; Trybus & Król, 2023).

In the extracellular activities of catD, some cell types possess structures called secretory lysosomes (Andrews, 2000; Brix *et al*., 2008). These endocytic compartments resemble late endosomes and lysosomes in their characteristic biochemical composition. According to Tancini *et al.* (2020), the organelles move from the perinuclear region along microtubules towards the plasma membrane. Data shows that lysosomal targeting is, although not well understood, mediated by two M6P receptors (46 kDa cation-dependent and 300 kDa cation-independent M6PR) (Kornfeld, 1990; Větvicka *et al*., 2010; von Figura & Hasilik, 1986). The secretory lysosome then fuses with the plasma membrane through a Ca^{2+}-dependent process. Thus, lysosomes secreting biomolecules such as catD, accumulated or late endosomes and lysosomes can be transported retrogradely, allowing vesicles to fuse with the plasma membrane, usually after signaling (Tancini *et al*., 2020). Furthermore, Větvicka *et al*. (2004) cite that one of the complex mechanisms that regulate this vesicle displacement process are molecular zip codes, such as RabGRPase and phosphoinositides, as well as other compounds that are stably associated with the membrane and are sufficient to confer specificity in targeting.

Figure 2

Cellular physiology highlighting the interactions of the lysosome in intracellular and extracellular digestion



Sources: From Amaral *et al*. (2023), Tancini *et al*. (2020), Alberts *et al*. (2014) apud Universidade Federal Fluminense (2025), Zhu *et al*. (2020) & Mehta *et al*. (2006).

In this process, the pathways represented demonstrate the transport mechanisms mediated by a complex of biomolecules involved in adhesion, vesicle signaling, digestion and transport within the cell. In this sense, considering that cells receive extracellular particles for degradation, events can occur through different forms of endocytosis, intracellular structures and different types of autophagy. Macroautophagy (digestion of large cytoplasmic entities and damaged organelles) occurs within a double-membrane structure, the autophagosome, which fuses with lysosomes to form an autolysosome. Lysosome biogenesis, initiated by the TFEB (Transcription Factor EB) gene, requires the participation of biosynthetic and endocytic pathways. Lysosomes receive their specific cargoes from soluble hydrolases, such as catD encoded by the CTSD (Continuous Traumatic Stress Disorder) gene, and membrane proteins of the “conventional” secretory pathway. Briefly, in the trans-Golgi, M6P receptors bind to proteins containing mannose 6-phosphate residues, which interact with AP1/clathrin complexes and bud off into vesicles in the Golgi complex. Proteins present in the lysosomal membrane are transferred to the lysosomes independently of the M6P receptor, therefore, the transport from the trans-Golgi network (TGN) to the lysosome is both direct and indirect, enabling the fusion of the vesicles with the plasma membrane – resulting in endocytosis. Subsequently, these vesicles fuse with endosomes and donate the lysosomal protein precursor complex present in the lumen. Vesicle

transport occurs through a complex of motor proteins attached to microtubules and centrioles (dashed image) and can follow two directions. However, most lysosomes are located close to the nucleus. Therefore, most lysosomes migrate from the perinuclear region, through motor proteins (kinesin) attached to the microtubule, fusing with the plasma membrane, where compounds, such as catD, present in the vesicle are released into the extracellular environment. However, lysosomes can also undergo retrograde transport from the periphery to the nucleus along the microtubules, mediated by dynein. In the interaction of the vesicle with the membrane, the lysosomal membrane protein VAMP7 interacts with syntaxin-4 and the 23 kDa synaptosome-associated protein (SNAP23) in the plasma membrane. During the process, the lysosomal Ca^{2+} channel TRPML1 supplies Ca^{2+} that aids in the fusion of the lysosomal membrane, which is detected by Syt-VII. In the image, the complementary acronyms: EE - Early endosomes; LE/MVB - Late endosomes/Multivesicular bodies; MPR - Mannose phosphate receptors; ER - Endoplasmic reticulum; MTOC, microtubule organizing center. Sources. Amaral *et al*. (2023); Tancini *et al*. (2020); Alberts *et al*. (2014) apud Universidade Federal Fluminense (2025); Zhu *et al*. (2020); Mehta *et al*. (2006).

Cathepsins (mature pro- and cathepsins) have been localized in peri- and extracellular environments under different physiological and pathological conditions (Brix, 2018). In extracellular proteolysis, cathepsin D has been shown to promote cancer cell invasion and tumor progression through degradation of the extracellular matrix (ECM) (Gocheva & Joyce, 2007; Liaudet-Coopman *et al*., 2006; Mohamed & Sloane, 2006; Joyce & Hanahan, 2004; Rochefort *et al*., 2000). On the other hand, Johnson *et al*. (1993) cite that cathepsin D levels were inversely correlated with aggressive behavior in vivo and in vitro in previously reported studies. Furthermore, the analyzed data suggest that cathepsin D secretion by tumor cells is not an important determinant of tumor cell invasiveness *per se*. For the authors, *op. cit*., and Garcia *et al*. (1996), these data also reinforce the view that the poor prognosis in clinical breast cancer associated with high tumor levels of cathepsin D is probably due to high levels of molecule (catD) in tumor stromal components, such as infiltrating inflammatory cells. In this context, the question arises whether the increased secretion of catD is responsible for the greater degradation of the ECM, the acidic environment in the tumor tissue, or if it is still due to other mechanisms? (Cavallo-Medved & Sloane, 2003; Brix, 2018). Thus, it is suggested that there is a synergistic action that enhances the events of variation of this pH.

The secreted procathepsin D (pCD) can be endocytosed via the mannose-6-phosphate receptor (M6PR) pathway or another, as yet unknown, receptor by cancer cells and fibroblasts. In addition, endocytosed pCD undergoes further maturation to intermediate forms weighing 48 kDa, 34 kDa, and 14 kDa (Benes *et al*., 2008; Haendeler *et al*., 2005; Okitani *et al*., 1981; Větvicka *et al*., 2010). Although the relationship through the M6PR pathway is not well understood, it is known that this process involves endocytosis, and that the M6PR receptor aids in the cellular uptake of M6P-bearing proteins, including the serine protease granzyme-B (Gzm-B), which plays an important role in the activation, migration, and contraction of T cells. Furthermore, among the possible relationships, it is mentioned that $CD8^{+}$ T cells provide protection against pathogens and cancer (Ahmed & Xiang, 2017; Prlic & Bevan, 2008). Notably, host immune responses to pathogens and host immunity to cancer can be enhanced by preventing regulatory T cell (T_{reg})-mediated suppression of T lymphocyte effectors (Ahmed & Sakaguchi *et al*., 2008; Salti *et al*., 2011; Xiang, 2017).

pH AND OXYGENATION IN CARCINOGENESIS

The human extracellular fluid, in homeostasis and in most of the body, has a slightly alkaline pH of 7.40 ± 0.02. This corresponds to a concentration of H^{+} ions of 40 ± 2 nEq/l and is regulated by the excretion of volatile and non-volatile acids produced in the body (Hosonuma & Yoshimura, 2023). Its regulation is based on the maintenance of the molecular geometry of proteins and other macromolecules that participate in biochemical reactions essential for life (Hall & Guyton, 2017). In this context, Jin *et al*. (2022) note that the formation of LLPS (*liquid-liquid phase separation*) has recently been associated with intracellular pH, and maintaining adequate intracellular pH homeostasis is essential for the survival of organisms. Additionally, oncogenesis is also related to LLPS via mutations in the substrate recognition domain of the tumor suppressor SPOP (*Spallated POZ protein*), which interfere with binding to oncogenic substrates and, consequently, with the formation of condensates with the ubiquitin ligase complex—this mechanism leads to failures in the ubiquitination of these substrates and proteasomal degradation. Furthermore, mutations in the P53 gene, which produces the p53 protein, can accelerate its transition from the solid phase to amyloid aggregates, as observed in more than 50% of human cancers. In other words, the mutation or overexpression of molecules – such as receptors – alters the signaling clusters, activating irregular cascades that can lead to the development of tumors.

Another factor described by Bivehed *et al*. (2023) is the stability of genomic DNA, which is essential for cell survival functions. However, exposure to destabilizing agents can cause primary DNA damage, such as single and double strand DNA breaks, which, if not repaired, can lead to different types of mutations that may result in critical stages of tumor development (Bivehed *et al*., 2023; Gates, 2009).

Different pathologies can cause variations in the balance of the acidification process, and in the case of tumors, the pH decreases to approximately 6.8 (Hosonuma & Yoshimura, 2023). Tumor cells exhibit altered metabolism, and an example of these changes is obtaining energy from glycolysis at high rates, to the detriment of oxidative phosphorylation, even under aerobic conditions (Wang *et al*., 2020; Warburg & Negelein, 1927).

Variations in pH value make extracellular acidosis a striking feature of inflammatory processes (Erra Díaz *et al*., 2018) and can typically be attributed to three factors: (1) tissue hypoxia caused by damage to small blood vessels and the metabolic activity of infiltrated leukocytes, resulting in a change in glycolytic metabolism that leads to the accumulation of lactic acid (Arnould *et al*., 2001; Menkin & Warner, 1937); (2) high proton production by neutrophils during increased respiratory activation (Borregaard *et al.*, 1984; Van Zwieten *et al.*, 1981); and (3) the accumulation of short-chain fatty acids produced, as for example and observed, by bacteria (Niederman & Kashket, 1997; Rotstein *et al*., 1989).

Although tumors have angiogenesis stimulated by chemical messengers as one of their general characteristics (Suresh & Diaz, 2021; Spill *et al*., 2015)), the vessels formed within the tumor present a disorganized network characterized by reduced blood flow – promoted by the rupture of some endothelial cell (EC) junctions and loss of pericytes, which lead to variable thickness and leaks, resulting in increased tissue hypoxia and intravasation of tumor cells (Lugano *et al*., 2020). Furthermore, the vascular network supplies oxygen (O_2) and nutrients to all cells in the body. Most transcriptional responses to low O_2 are mediated by hypoxia-inducible factors (HIF), conserved transcription factors that regulate the expression of several angiogenic, metabolic, and cell cycle genes. Consequently, the HIF pathway is currently regarded as a master regulator of angiogenesis (Krock *et al*., 2011), as well as interfering with cellular homeostasis and causing changes in the local environment.

TUMOR IMMUNE MICROENVIRONMENT (TIME) AND catD

The tumor microenvironment is a complex system represented by biochemical compounds and immune as well as non-immune cells of the host, which establish a dynamic crosstalk with cancer cells (Baxevanis *et al*., 2021; Wang *et al*., 2024). With the evolution of this system, organisms have developed the ability to generate numerous cells and molecules capable of specifically recognizing, containing, and eliminating a seemingly unlimited variety of foreign agents, functioning cooperatively (Conus & Simon, 2008, 2010). These cells constitute the critical components in the tumor microenvironment (TME), and studies of immunological relationships have focused on the processes of immune responses at TME sites. However, immunological processes are coordinated by cell lineages that act between tissues (Hiam-Galvez & Spitzer, 2021).

Immune cells are classified into two categories: adaptive, represented by T, B, and natural killer (NK) cells, which are activated by exposure to specific antigens mediated by immunological memory; and innate, characterized by a non-specific defense mechanism, represented by macrophages, neutrophils, and dendritic cells that come into action for immunological defense hours after an antigen enters the body (Wang *et al*., 2025; Anderson & Simon, 2020)).

In immune cells, cathepsins perform significant activities in endosomes and participate in innate and adaptive immune responses (Yadati *et al*., 2020). In innate responses, lysosomal cathepsins have been shown to cleave the ectodomains of Toll-like receptors (TLRs) 7 and 9 that are expressed on endo/lysosomal membranes, where TLRs recognize nucleic acids, as observed in assays with microbes and phagocytosed structures. The processed forms of TLRs 7 and 9 then recruit the adaptor protein MyD88, leading to the activation of TLR signaling pathways (Yadati *et al*., 2020; Creasy & Mccoy, 2011).

According to Yadati *et al*. (2020), Bird *et al*. (2009), and Guncar *et al*. (1999), during adaptive immune responses, T lymphocytes can recognize processed antigens on the surface of antigen-presenting cells (APCs) and bind to major histocompatibility complex (MHC) molecules. While MHC class I molecules present processed antigenic peptides derived from the cytosol, MHC class II molecules present peptides derived from the endo/lysosomal compartment. Furthermore, it has been shown that MHC class II molecules require an invariant chain (Ii), a glycoprotein necessary for their folding and assembly. Several cathepsins are involved in (I) the

proteolytic processing of antigens into short peptides and (II) the degradation of the invariant chain, thus facilitating adaptive immune responses. However, cathepsins B and D have not been shown to be essential in MHC II-mediated antigen presentation (Deussing *et al*., 1998).

Depending on the context, there is a dichotomy in the relationship between immune cells and the tumor microenvironment; these cells can either suppress tumor growth or promote it (Anderson & Simon, 2020). According to Sun *et al*. (2025), the stromal components of the host tissue in the TME are relevant in the process and consist of the basement membrane, fibroblasts, extracellular matrix, and immune and vascular cells, which play essential roles in maintaining healthy epithelial tissues and their malignant counterparts. Furthermore, in the progression of malignant tumors, the stromal elements change significantly, leading to enhanced tumor growth, invasion, and metastasis.

The tumor immune microenvironment (TIME) aids cancer cells in selecting dominant cells, allowing the tumor to progress more rapidly in a restricted environment (Wang *et al*., 2017). However, particularly during the early stages of cancer, the immune response generated by immune cells in the TME exhibits anti-tumor characteristics (Wei *et al*., 2020). In this context, the dynamic interaction between cancer cells and tumor stroma is crucial for tumor progression and metastasis, as well as for shaping the TIME (Bremnes *et al*., 2011; Sun *et al*., 2025).

In TIME, some response mechanisms are related to cytokines, soluble proteins of low molecular weight (15–20 kD) that transmit chemical messages to adjacent cells or distant organs. Most cytokines associate with specific receptors, which transmit intercellular signals to their target cells (Da Silva *et al*., 2019; Dinarello, 2007), while also playing diverse roles in regulating immunity, development, metabolism, aging, and cancer (Kang *et al*., 2020). Different types of cells can produce cytokines, including white blood cells such as macrophages, monocytes, lymphocytes, and in the nervous system, “neuroimmune” cells like microglia, along with glial and neuronal cells (Da Silva *et al*., 2019; Schirmer *et al*., 2010; Neumann, 2019)).

These molecules form a family of cytokines that include chemokines, interferons, interleukins, lymphokines, tumor necrosis factors (TNF), neurotrophins, colony-stimulating factors, and growth factors. Based on the type of immune response they induce—promoting or inhibiting inflammation—they are categorized into two main groups: (i) pro-inflammatory and (ii) anti-inflammatory (Da Silva *et al*., 2019; Schirmer *et al*., 2010; Neumann, 2019).

Of this family, interleukins (IL) are involved in signaling to adjacent cells or distant organs, where they primarily associate with specific receptors that transmit signals to their target

cells (Dinarello, 2007; Kang *et al*., 2020). The carcinogenic process and progression can be accelerated by the presence of chronic inflammation. IL-6 exhibits high levels in the TME and is also associated with the cause of inflammation by promoting the growth, survival, and metastasis of cancer cells. Notably, IL-6 increases the generation of inflammatory factors and angiogenesis-related factors, such as IL-1β, IL-8, CC motif chemokine (CCL)2, CCL3, CCL5, GM-CSF, and VEGF, affecting immune and non-immune cells in the TME through an autocrine and/or paracrine manner. Furthermore, IL-6 is also considered a prognostic factor in cancers (Johnson *et al*., 2018; Wu *et al*., 2025).

The participation of human cathepsins in this process includes their involvement in several normal physiological mechanisms, such as cellular homeostasis and immunological functions, as well as pathological conditions like cancers (Patel *et al*., 2018). In carcinogenic activities, where degradation of the extracellular matrix (ECM) is necessary for metastasis, cathepsins facilitate ECM remodeling and the disruption of cell-cell junctions to promote migration and invasion. They also promote angiogenesis and chemoresistance (Mohamed & Sloane, 2006)). Malignant cells exhibit increased proteolytic activities, which enable them to digest the ECM. This digestion is crucial for cancer cells to invade and migrate through the basal lamina, a hallmark of malignancy (Gocheva *et al*., 2006; Tan *et al*., 2013).

In this context, catD is one of the compounds present in the tumor microenvironment associated with invasion, metastasis, and progression. Thus, for the supply of proteolytic enzymes capable of degrading the basement membrane and the extracellular matrix to occur, in addition to uPA (urokinase-type plasminogen activator) and PAI-1 (uPA inhibitor type 1), catD, a proteolytic factor frequently implicated as a prognostic factor in cancer (Harbeck *et al*., 2000; Rodrguez *et al*., 2005), is included.

In the immune system, particularly the innate immune system, cathepsin D has been identified as a key molecule in neutrophil apoptosis by directly activating the initiator caspase-8 (Conus *et al*., 2008). This pathway, observed in neutrophils, is blocked during inflammatory conditions and is crucial for the resolution of innate immune responses. Delayed neutrophil apoptosis due to cathepsin D deficiency amplifies and prolongs neutrophilic inflammation *in vivo* (Conus & Simon, 2010). Additionally, besides catD, cathepsin B has also been shown to be involved in immune cell apoptosis. Blomgran *et al*. (2007) demonstrate that during microbial-induced apoptosis in human neutrophils, the reactive oxygen species (ROS)-dependent release of cathepsin B into the cytosol induces cleavage of the proapoptotic Bcl-2 family protein member

Bid, leading to mitochondrial damage, subsequent caspase activation, and apoptosis. These data suggest that cathepsins are important modulators of innate immune responses, and that azurophilic granules, in which cathepsins are expressed and stored, may represent a novel target for developing anti-inflammatory drugs (Conus, 2010).

Lee *et al*. (2024) cite that the loss of catD suppresses polarization in tumor-associated macrophages (TAMs), similar to M2 macrophages, through the upregulation of transforming growth factor beta **(**TGFBI) and downregulation of CCL20, known as LARC (*Liver and Activation-regulated Chemokine*). Furthermore, macrophages primed with Cat D KO (knockout), cancer cells remarkably suppress cancer metastasis. Thus, controlling the activity and expression of catD may serve as a potential combination therapy to treat cancer metastasis and immune escape.

Větvicka *et al*. (1997) reported that the activity of procathepsin D growth factor is mediated by a previously unknown receptor moiety and that the binding activity can be localized, by their analysis, to positions 27-44 of the procathepsin D activation peptide. Subsequently, it was shown that enzymatically inactive procathepsin D, secreted by cancer cells, plays a role in the development of human breast cancer, and the growth factor activity of the activating peptide is located in a nine-amino acid stretch (aa 36-44) of the activating peptide. Furthermore, assays with anti-activating peptide antibodies (27-44) have been shown to inhibit the growth of human breast tumors in mice (Větvicka *et al*., 1999).

Furthermore, in *in vivo* assays, they indicated that treatment with anti-procathepsin D antibodies could reverse the growth of human breast tumors. Recently, for Desroys *et al*. (2025), the use of anti-catD antibodies in different formats represents new avenues for cancer immunotherapy, particularly in triple-negative breast cancer (TNBC). In summary, these antibodies work by restoring antitumor immunity, especially through the activation of tumor-infiltrating natural killer cells. They exhibit dual antitumor effects on both cancer cells and fibroblasts associated with stromal cancer, data that have generated therapeutic interest for TNBC.

Wnt PATHWAY AND POSSIBLE ASSOCIATIONS WITH catD

The biological development of multicellular organisms, along with the genetic and structural factors of the cells, is regulated by the interaction of several signaling pathways that

communicate positional information and induce the specification of cell fate. Additionally, other families of secreted factors, such as the proteins involved in transforming growth factors beta (TGFßs), fibroblast growth factors (FGFs), Hedgehog and Notch proteins, and Wingless growth factors (Wnt), are crucially involved in these developmental processes (Hendriks & Reichmann, 2002)).

Wnt signaling comprises an evolutionarily preserved physiological mechanism that controls various processes, such as differentiation and other cellular functions in metazoans (Gao *et al*., 2021; Wen *et al*., 2020). It is mediated by a family of proteins that, in humans, include more than 19 characterized types with 350-380 amino acid residues; over 100 of these have been conserved throughout the evolutionary process (Gregorieff *et al*., 2005).

According to Hendriks and Reichmann (2002), all known vertebrate Wnt genes encode putative cysteine-rich glycoproteins ranging from 38 to 43 kDa. Additionally, the 19 identified Wnt proteins exhibit 27% to 83% amino acid sequence identity and a conserved pattern of either 23 or 24 cysteine residues. Furthermore, during the development of the metazoans analyzed, the proteins involved in the Wnt pathways have demonstrated diverse roles associated with cell fate, proliferation, migration, polarity, and death. However, in adults, the Wnt pathway operates in homeostasis. Conversely, inappropriate activation of the Wnt pathway may lead to cancer. This indicates that the coordinated functioning of cells in homeostasis relies on various communication mechanisms that can be mediated, for instance, between the Wnt protein and a ligand receptor known as Frizzled (FZD), as well as other coreceptors-proteins associated with the lipoprotein receptor (LRP5 and LRP6) (Haseeb *et al*., 2019). In the case of human cancers, Wnt/β-catenin signaling is highly activated, and has been targeted for the development of several Wnt signaling inhibitors for cancer therapies (Jung & Pai *et al*., 2017; Park, 2020)).

Wnt signaling, due to its chemical properties and mechanisms of action, is classified into canonical and non-canonical pathways (Figure 3 - B and C): (**1**) the canonical pathway involves *β-cadherin*-associated protein (β-catenin), T-cell factor (TCF), and lymphocyte enhancer-binding factor (LEF); and (**2**) the non-canonical pathway includes the planar cell polarity (PCP) pathway and the Wnt calcium pathway (Wnt-Ca^{2+}) (Niehrs, 2012; Staal *et al*., 2008). In this scenario, interest in the role of the Wnt/β-catenin pathway in regulating immune cell infiltration into the tumor microenvironment has been renewed, given its potential impact on responses to treatments also aimed at immunotherapy (Pai *et al*., 2017).

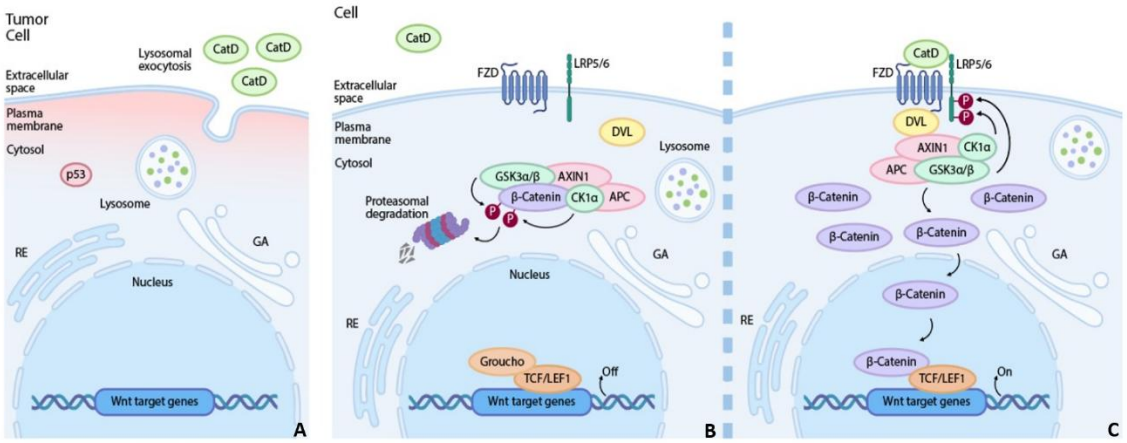
he Wnt pathway is activated when proteins bind to Frizzled receptors present in the cell membrane. Following this interaction, a signaling cascade is triggered that can influence gene expression and cell growth (Mendonça Sanches *et al*., 2018). In the case of the Wnt/β-catenin signaling pathway, the binding of Wnt proteins to the G protein-coupled receptor inactivates glycogen synthase kinase 3β (GSK3β), an enzyme that phosphorylates β-catenin for subsequent proteasomal degradation. This process increases the cytoplasmic content of β-catenin and facilitates its translocation to the nucleus, resulting in enhanced cell proliferation and survival (Mendonça sanches *et al*., 2018; Miller *et al*., 2006; Song *et al*., 2024).

Among the various pathologies associated with the Wnt/β-catenin pathway, silicosis, inflammation, and cancer are noted (Liu *et al*., 2022). In these processes, abnormalities in the activation of the signaling pathway may occur, leading to mutations in key components of the Wnt/β-catenin pathway target genes (Figure 3). With these epigenetic modifications and interference in other signaling pathways, dysregulations arise that may contribute to tumor initiation and progression (Maurice & Angers, 2025; Song *et al*., 2024)). Alternatively, through different cell signaling routes and mechanisms involving proteinases such as cathepsins B, D, and G, along with inflammatory pathways and Wnt/β-catenin signaling, catD can generate the peptide angiotensin II, resulting in increased pro-inflammatory activity (LI *et al*., 2020). Thus, it is suggested that the combination of synergistic actions promotes homeostatic imbalance, while catD interferes with the regulation of Wnt signaling and receptors, facilitating cell proliferation and metastasis.

According to Song *et al*. (2024), the involvement of Wnt signaling in carcinogenesis stems largely from its ability to promote the sustained proliferation of cancer stem cells (CSCs) in regions resistant to anticancer treatments. Additionally, it facilitates the invasion of CSCs into neighboring tissues and their dissemination into the bloodstream. Inhibition of the Wnt/β-catenin signaling pathway offers an efficient approach to regulating CSCs (Song *et al*., 2024). In this context, the relationship between catD and the Wnt system is dynamic, complex, and involves a network of various cellular signaling mechanisms. Identifying and understanding these processes may contribute to improving therapies related to intra- and extracellular signaling.

Figure 3

The images illustrate the overexpression of catD in tumor cells ***(a)*** *and possible relationships with the WNT pathway (****b*** *and* ***c****).*



Sources: From Maurice & Angers (2025), Liu *et al*. (2022) & Pai *et al*. (2017).

In the "OFF state" (**b**): In the absence of WNT ligand proteins, the β-catenin destruction complex - (Figure - off), a tertiary molecular complex consisting of AXIN1 - the main organizer of the degradation complex - has chemical properties (binding sites) of interaction with APC - adenomatous polyposis coli, cysteine kinase 1 α - CK1α and glycogen synthase kinase 3α or β (GSK3α/β), which phosphorylate β-catenin, where it subsequently undergoes proteasomal degradation. In addition to these properties, the binding of Groucho family proteins to TCF/LEF in nuclear DNA prevents the expression of WNT pathway target genes. In the canonical WNT/β-catenin pathway (on) - "ON state" (**c**): WNT pathway proteins, when binding to Frizzled receptors and the coreceptor LRP5/6, act to suppress the activity of glycogen synthase kinase-3β (GSK-3β). The E3 ligase enzyme (here suppressed), produced by the ZNRF3 gene, promotes the degradation of the WNT receptor, a pathway that normally acts as a tumor suppressor. This molecular interaction process prevents the phosphorylation reaction of molecules in the downstream cytoplasm, allowing the association of β-catenin with T-cell factor, bound to DNA, forming the lymphoid enhancer-binding factor (TCF/LEF) in the nucleus. These mechanisms can provide the intensification of cell proliferation – a characteristic of tumor cells (Maurice & Angers, 2025; Liu *et al*., 2022; Pai *et al*., 2017). In this context, catD overexpressed by cells in the tumor microenvironment (TME) can act, depending on the type of cancer, interfering with the complex's bonds or degrading, among other compounds, the proteins of the WNT and Frizzled (FZD) pathways, altering homeostasis and providing, in addition to these biochemical changes, the intensification of cell proliferation.

SCIENTIFIC EVIDENCE, INFERENCES AND HYPOTHESES

Scientific evidence (SE) contributes to scientific activities, especially to new discoveries; that is, the scientific data collected serves as evidence to derive new scientific knowledge (Park *et al*., 2021). Furthermore, SEs are considered worldwide to be important elements for the development of effective public policies, although their use is incipient (Ministério da Saúde, 2015). On the other hand, it has become the basis of security for diagnostic and therapeutic choices [...], and methodological advances in research make it much more reliable for clinical procedures (Siqueira, 2017). In this universe, hypotheses aim to identify and direct the investigation, as they compose the structures of the research processes (Bulajic *et al*., 2012).

In this context, the surveys of scientific evidence allowed the development of reflective approaches on published data, inferences and hypotheses about the mechanisms of action, possible interactions and academic questions for future scientific prospections (Table 1).

Table 1

Studies with data collected, compiled and tabulated showing evidence, inferences and hypotheses formulated from published scientific findings

References	Scientific evidence / Inferences	Hypotheses
Cavallo-Medved and Sloane (2003); Brix (2018).	Is increased catD secretion responsible for increased degradation of the ECM or the acidic environment in tumor tissue, or is it due to other mechanisms?	Altered cancer cell signaling results in excessive catD release to the ECM.
Gates (2009); Hall and Guyton (2017); Jin et al. (2022); Bivehed et al. (2023).	The regulation of pH in cells is based on maintaining the molecular geometry of proteins and other macromolecules, such as DNA, which act in biochemical reactions and are essential for life.	The low intra and extracellular pH, enhanced by the release of catD, mutates and/or degrades protein components, interfering with communication mediated by the SPOP domain.
Mutka et al. (2010); Masson et al. (2011).	Studies indicate the silencing of catD by shRNAs - short hairpin RNA - a type of RNAi (RNA interference) used to silence genes, in 3T3-F442A preadipocytes that leads to lipid depletion in the cells and reduced expression of adipocyte differentiation markers PPARα, HSL and aP2, indicating the essential role of catD in the preadipocyte differentiation process. Furthermore, they mention the role of catD in adipocyte differentiation - where it is abundantly expressed in fully differentiated adipocytes. Additionally, it was shown that murine catD deficiency led to the accumulation of cholesterol esters in the brain, suggesting that catD may participate in lipid metabolism.	catD may be involved in the onset of obesity (Masson *et al*., 2011)
Conus and Simon (2010); Lee et al. (2024).	Loss of catD suppresses polarization in tumor-associated macrophages (TAMs). Cathepsins modulate innate immune responses and azurophilic	

	granules, in which cathepsins are expressed and stored, may represent a novel target for anti-inflammatory drug development.	catD is an active molecule in the granulocyte-mediated innate immune response.
Haseeb et al. (2019).	The Wnts pathway functions in homeostasis and inappropriate activation may imply cancer, that is, they are coordinated in homeostasis and require several communication mechanisms that are mediated, for example, between the Wnt protein and ligand receptors called Frizzled (FZD), as well as with the other several coreceptors - lipoprotein receptor-related proteins (LRP5 and LRP6).	catD alters the communication mechanisms of the Wnts system and enhances carcinogenesis.
Moraes et al. (2022).	In molecular interaction assays by surface plasmon resonance (SPR) performed on a Biacore T200 system (GE Healthcare), cathepsin D was immobilized using a CM5 S-type sensor chip via amine coupling. The study demonstrated the formation of a functional molecular complex of human catD with phospholipase A_2 (PLA_2) from snake venom.	1- Formation of a functional complex between catD and PLA_2 from snake venom may promote synergistic events in tissue injury. 2- catD binds to human PLA_2.
Lee et al. (2024)	Controlling catD activity and expression may be a potential combination therapy to treat cancer metastasis and immune escape.	Control of catD expression represents a combinatorial potential for cancer treatment.
Desroys et al. (2025)	The use of anti-catD antibodies, in different formats, are new avenues for cancer immunotherapy, such as triple-negative breast cancer - TNBC, as they act to restore antitumor immunity.	Anti-catD antibodies act to restore attitudinal immunity.

Source: By the authors (2025).

FINAL CONSIDERATIONS

catD represents an important aspect of homeostasis, signaling, tissue remodeling, and immunological processes that must be kept adequately controlled to prevent damage and other instability factors to the surrounding cells or tissues, ensuring they do not promote the development of various pathologies, such as cancer.

In the tumor microenvironment, descriptions of pathways and metabolic relationships that emerge from homeostasis processes, signaling activation, and immune responses (both innate and adaptive) can, when unbalanced, result in chronic inflammatory diseases, autoimmune disorders, and carcinogenic processes. catD is a promising target for therapeutic studies.

The characterization and analysis of the mechanisms of control and release of cathepsin from lysosomes and their cytosolic targets, along with their relationships to alterations that lead to pathological processes like cancer, are relevant. Furthermore, this may contribute to clarifying signaling pathways, molecular aspects, and mechanisms that regulate and maintain cellular stability, inflammatory conditions, and other instabilities that result in various pathologies. In addition, the data may enhance understanding of pathways and strategies for controlling catD

activities and may aid in studies for therapeutic interventions that could mitigate or control the release and action of cathepsins in the tumor microenvironment.

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