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COLLAGEN MATRIX REMODELLING AS A FACTOR OF TUMOUR PROGRESSION AGGRESSIVENESS: THE ROLE OF PROTEASES AND CROSS-LINKING ENZYMES

Collagen matrix remodelling is one of the key processes determining the progression of malignant tumours and the development of an aggressive tumour phenotype. This review summarises current data on the role of collagen degradation and cross-linking enzymes in the progression of malignant tumours. It is shown that an imbalance between the processes of degradation and stabilisation of collagen fibres contributes to increased tissue stiffness and the activation of signalling pathways associated with invasion and metastasis. The review focuses on matrix metalloproteinases, cathepsins, meprins, lysyl oxidases and enzymes of the procollagen-lysine family, 2-Oxoglutarate 5-Dioxygenases (PLODs) as enzymes involved in extracellular matrix remodelling, regulation of the epithelial-mesenchymal transition, and the progression of malignant tumours. The results of recent studies demonstrating the link between abnormalities in the expression of collagen-modifying enzymes and the aggressiveness of the tumour process in patients with various types of cancer have been analysed.

Keywords: *cancer, collagen, matrix metalloproteinases, cathepsins, meprins, LOX, PLOD.*

Recent data indicate that the metastatic potential of malignant neoplasms is largely determined by the characteristics of the tumour's cytoarchitecture and the composition of its microenvironment, in particular the structural and biochemical properties of the extracellular matrix (ECM). The ECM is a complex, multicomponent system comprising structural fibrous proteins, including various types of collagens, elastin, proteoglycans, laminins, fibronectin, hyaluronan and other glycoproteins. In addition to its function as a mechanical scaffold for tissues, the ECM acts as a reservoir for biologically active molecules and participates in the regulation of intercellular signalling [1, 2].

Collagen, which is the main structural component of the ECM, is regarded as one of the key factors modulating the growth and progression of malignant tumours. The spatial organization of collagen fibres, their packing density, thickness, length and orientation relative to the tumour invasion front determine the mechanical properties of the stromal component of tumours and the ability of tumour cells to migrate and invade [3]. The formation of the collagen scaffolds of tumours is modulated by both tumour and stromal cells through the secretion of a range of structural proteins, enzymes and ECM remodelling factors [4].

During tumour growth, ECM remodelling occurs, accompanied by the degradation of the existing matrix and the formation of a tumour-specific matrix characterised by increased collagen deposition, enhanced collagen cross-linking, fibers linearization, and increased tissue stiffness [5]. The most pronounced changes are observed in areas of tumour invasion, where there is a reorganisation of fibrillar collagens, particularly types I and III, and basement membrane collagen type IV, accompanied by accumulation of fibronectin and proteoglycans, as well as activation of stromal cells. Such structural rearrangements of the ECM contribute to the loss of cell polarity and intercellular adhesion, the enhancement of growth factor signalling, and the creation of conditions favourable for invasive tumour growth and metastasis [6].

ECM remodelling has a direct impact on the biological properties of tumour cells, including the regulation of gene expression, proliferation, differentiation, migration, and the development of resistance to therapy. Proteases and collagen cross-linking enzymes

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play a key role in regulating these processes, controlling the degradation, reorganisation and stabilisation of ECM components. The coordinated activity of these molecules determines the degree of matrix stiffness, the orientation of collagen fibers and the intensity of intercellular signalling in the tumour microenvironment [7] (*Fig.*).

Matrix metalloproteinases. Collagenases and gelatinases belong to the family of matrix metalloproteinases — zinc-dependent endopeptidases that are involved in the remodelling of the extracellular matrix and the maintenance of the structural and functional organisation of tissues. This group of enzymes is characterised by multi-level regulation, including transcriptional control, secretion as inactive zymogens, proteolytic activation, and inhibition by tissue inhibitors of metalloproteinases (TIMPs), which normally restricts their activity in a spatiotemporal manner. The biological significance of collagenases and gelatinases is not limited to the degradation of the extracellular matrix, as they also modulate the bioavailability of growth factors, cytokines, chemokines and matrix-associated

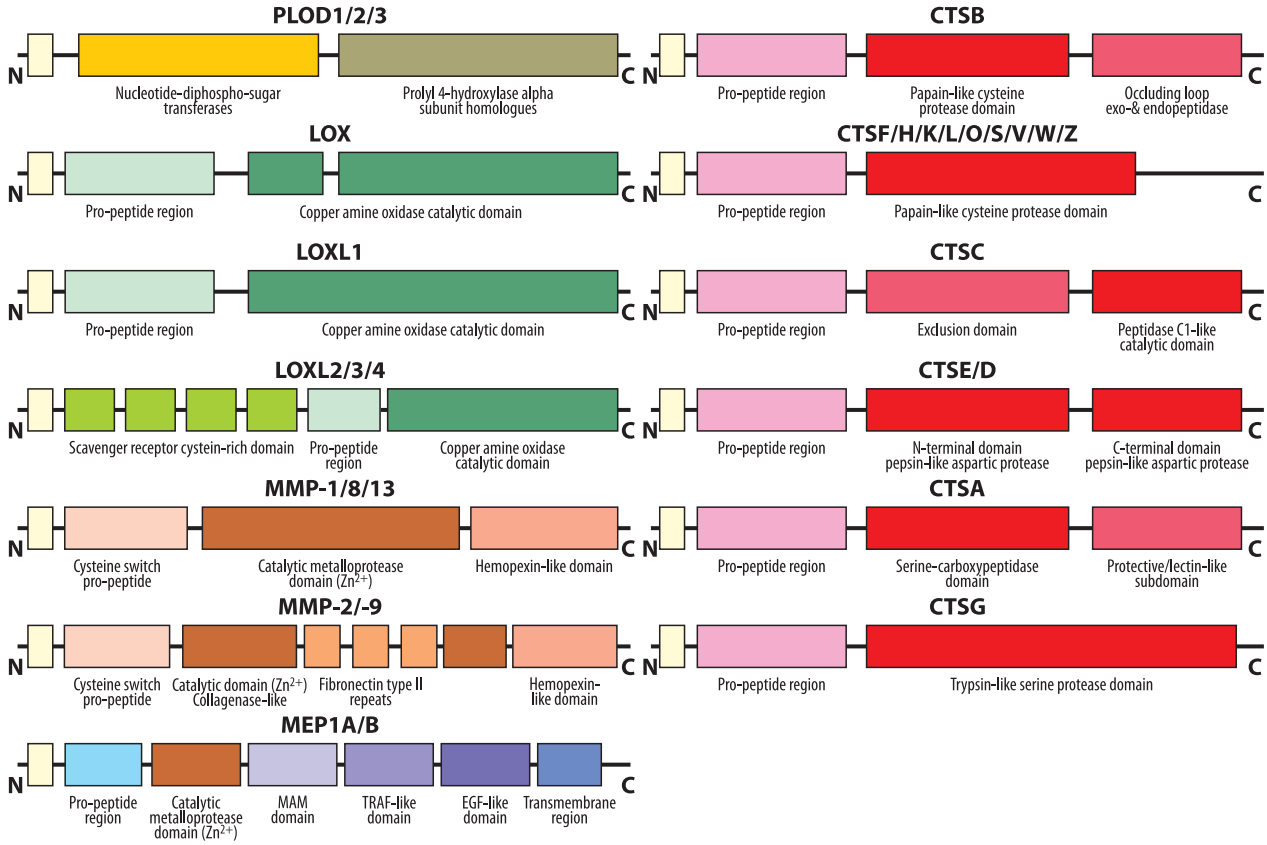

Fig. Domain organization of key remodeling proteins of the collagen scaffold of the extracellular matrix (ECM)

The presented proteins form a functionally interconnected network of enzymes that provide both modification, stabilization, degradation, and remodeling of collagen and non-collagen components of the ECM. Despite the significant diversity of families, all the shown molecules are united by the presence of N-terminal signal peptide, and clearly organized functional domains that determine their substrate specificity, activation mechanisms, and spatial regulation of activity in the tumor microenvironment. The group of post-translational collagen modification enzymes includes PLOD1/2/3 and LOX/LOXL1–4. PLOD proteins are characterized by the presence of domains associated with glycosylation and hydroxylation of lysine residues in procollagen, while the LOX and LOXL families contain a copper-dependent amino oxidase catalytic domain that provides covalent crosslinking of collagen and elastin fibers, increasing matrix stiffness. LOXL2–4 additionally have scavenger receptor cysteine-rich (SRCR) domains, which expand the possibilities of interaction with ECM components and regulatory proteins. Proteolytic degradation of the matrix is represented by several large families of cysteine, serine and aspartate proteases. Cathepsins (CTSB, CTSF/H/K/L/O/S/V/W/Z, CTSC, CTSE/D, CTSA, CTSG) have a common feature — synthesis as proenzymes with a propeptide region that blocks the active site from proteolytic activation. Papain-like cysteine cathepsins (CTSB and others) contain a characteristic catalytic domain with an occluding loop or modified inserts that determine endo- and exopeptidase activity. Aspartate cathepsins (CTSE/D) have a two-domain organization similar to pepsin, with N- and C-terminal domains that form the active site. Serine cathepsins (CTSA, CTSG) contain trypsin- or carboxypeptidase-like domains, which expands the spectrum of their substrates in the ECM. Matrix metalloproteinases (MMP-1/8/13, MMP-2/9) are characterized by a modular structure that includes a signal peptide, a pro-domain with a "cysteine switch", a catalytic Zn^{2+}-dependent domain and additional regulatory elements. Collagenase MMPs (MMP-1/8/13) contain a hemopexin-like domain, which determines specificity for fibrillar collagen. Gelatinases (MMP-2/9) additionally contain fibronectin type II repeats, which increase the affinity for denatured collagen and gelatin. A special group is made up of membrane-bound metalloproteinases (MEP1A/B, meprin family), which have a multidomain structure with MAM-, TRAF- and EGF-like domains, as well as a transmembrane segment, which ensures their localization on the cell surface and spatially controlled proteolytic activity. Thus, all the proteins presented demonstrate a common organizational principle — the presence of a catalytic core surrounded by regulatory and targeting domains, which determine their activation, substrate specificity and functional role in the dynamic remodeling of the extracellular matrix, which is critically important in the processes of tumor progression and invasion.

signalling molecules, thereby influencing cell–cell and cell–matrix interactions and the functional state of the microenvironment [8].

Despite the basic modular organisation typical of many matrix metalloproteinases, which includes a signal peptide, a prodomain with a 'cysteine–switch' mechanism, a catalytic domain with a zinc-binding motif, a linker region and a C-terminal hemopexin-like domain, the spatial arrangement of these modules and their contribution to substrate recognition in collagenases and gelatinases differ significantly [9]. For collagenases, the coordinated interaction between the catalytic and hemopexin-like domains is of decisive importance, since the effective cleavage of the intact collagen triple helix requires not only the hydrolysis of the peptide bond, but also the prior recognition, binding and local destabilisation of the fibrillar substrate [10]. In this regard, it is of fundamental importance for collagenases not only a presence of both domains, but also maintaining the functionally necessary flexibility of the linker region, without which collagenolytic activity is sharply reduced or lost [11]. Unlike collagenases, gelatinases possess three fibronectin type II-like modules inserted into the catalytic domain near the active site. These modules act as exosites — additional substrate recognition sites — and ensure high-affinity binding of extracellular matrix macromolecules [12, 13]. Consequently, the structural organisation of gelatinases is adapted primarily to the proteolysis of partially destabilised, denatured or non-fibrillar components of the extracellular matrix, rather than to the initial cleavage of intact fibrillar collagen triple helices. It has been experimentally demonstrated that the interaction of the substrate with this specific domain is critical for the hydrolysis of gelatin and other large protein substrates, whereas its contribution to the cleavage of short peptides is significantly smaller [14]. A distinctive feature of the structure of MMP-9 is the presence of an O-glycosylated linker, which should primarily be regarded as a regulatory structural element that modulates enzyme availability, interactions with inhibitors, and capacity for receptor–mediated binding and endocytosis, whereas its contribution to the actual catalytic cleavage of substrates is limited [15].

Collagenases (MMP-1, MMP-8 and MMP-13) are characterised by high substrate specificity, as their primary target is the stable native fibrillar collagen of the extracellular matrix. Accordingly, their main substrates are type I, II and III collagens. It is worth noting that collagenases carry out the initial and critical stage of collagenolysis — the cleavage of the intact triple helix at a single characteristic site, resulting in fragments of 3/4 and 1/4 the length of the collagen molecule [16]. However, the range of collagenase substrates is not limited to fibrillar collagens alone. Although MMP-1 is usually regarded as an interstitial collagenase with pronounced activity against type I, II and III collagens, it is also capable of cleaving gelatin, aggrecan, entactin, tenascin, fibronectin and vitronectin [17]. MMP-8 exhibits the typical collagenase activity against type I, II and III collagens, but its substrate spectrum is somewhat broader and includes non-fibrillar type IX, XII and XIV collagens, as well as fibronectin, laminin, entactin, tenascin C and aggrecan. In addition, MMP-8 can cleave certain soluble proteins and mediators, including angiotensin I, bradykinin and IL-8 [18]. MMP-13 is considered the collagenase with the highest proteolytic activity, particularly towards type II collagen; however, this MMP also effectively cleaves type I and III collagens, as well as type IV, IX, X and XIV collagens, gelatin, fibronectin, aggrecan, osteonectin, tenascin and plasminogen [17, 19].

Gelatinases (MMP-2 and MMP-9), unlike collagenases, exhibit broader substrate specificity and are functionally specialised primarily in the proteolysis of destabilised matrix components, basement membrane proteins and other extracellular molecules, rather than in the initial cleavage of intact fibrillar collagen, thereby ensuring further disorganisation of the ECM, disruption of tissue barriers and facilitation of cell invasion [20, 21]. The typical substrates of MMP-2 include gelatin and several basement membrane and extracellular matrix components, particularly types IV and V collagen, fibronectin, laminin and elastin, whereas expanded lists of substrates for this enzyme include type I, III, VII and X collagens, as well as aggrecan, vitronectin and tenascin [17, 22]. The substrate spectrum of MMP-9 primarily encompasses gelatin, type IV collagen and other basement membrane proteins, as well as type V and XI collagens, elastin, laminin, entactin, aggrecan and decorin [9]. At the same time, MMP-9 can also act on a number of non-matrix targets, in particular CXCL8/IL-8 and TNF-α, which indicates its involvement not only in the proteolysis of the extracellular matrix but also in the modulation of inflammatory processes [23].

The activation of collagenases and gelatinases is a prerequisite for their proteolytic function, as within the proenzyme, the sulfhydryl group of a conserved cysteine residue in the prodomain coordinates the catalytic Zn^{2+} and thereby keeps the active site in a blocked state. Only after this contact is disrupted does the active site become accessible for substrate binding, and a zinc-bound water molecule is recruited into the mechanism of hydrolytic cleavage of the peptide bond [16].

Following the initial recognition of fibrillar collagen, collagenases form an enzyme-substrate complex, within which local destabilisation of the triple helix occurs and one of the polypeptide chains is moved into a position favourable for hydrolysis. In the next stage, the glutamate residue of the catalytic domain activates a water molecule coordinated by a Zn^{2+} in the active site, which performs a nucleophilic attack on the carbonyl carbon atom of the substrate's peptide bond. The formation of a tetrahedral intermediate is

completed by the cleavage of the peptide bond, resulting in the cleavage of collagen at a characteristic site to form 3/4 and 1/4 fragments, which are subsequently much more susceptible to proteolysis [24].

Unlike collagenases, for which the key mechanistic step is the preliminary local destabilisation of the intact collagen triple helix, gelatinases act predominantly on macromolecular substrates that are already structurally accessible and therefore do not require a preliminary 'unwinding' stage of the native collagen triple helix. Although the chemistry of peptide bond cleavage by gelatinases, as in other matrix metalloproteinases, retains a common zinc-dependent mechanism, the fundamental difference in gelatinases lies in the manner of substrate recognition, binding and spatial preparation for catalysis. Gelatinases function predominantly in sites of pericellular matrix remodelling, carrying out sequential hydrolysis of numerous accessible peptide bonds, rather than through a single specific cleavage, which is characteristic of collagenases. It is for this reason that gelatinases should be regarded as 'second-wave' proteolytic enzymes, which, following the initial action of collagenases, deepen matrix degradation, fragment the components of the basement membrane and complete the disorganisation of the extracellular matrix [20].

Understanding of the role of collagenases and gelatinases in carcinogenesis has now moved beyond the initial, somewhat simplified notions of their involvement in the clearance of old or damaged fibrils within the ECM, analogous to the processes of traumatic tissue regeneration. And although their primary physiological functions determine their role in the onset and progression of malignant neoplasms, under tumour conditions, matrix metalloproteinases begin to perform a number of specific functions.

One of the key new functions of MMPs in carcinogenesis is their ability to act as regulators of signalling cascades through the proteolytic release and activation of growth factors, cytokines and receptors. In particular, the gelatinases MMP-2 and MMP-9 are capable of releasing bound forms of TGF-β, VEGF and FGF from the matrix, which promotes the transition of local tissue remodelling into a state of chronic proliferative stimulation [25, 26]. In this context, the proteolytic activity of MMPs forms positive feedback loops between tumour cells and the stroma, supporting proliferation, survival and resistance to apoptosis. Thus, MMPs become key mediators of intercellular communication, capable of modulating signalling cascades directly at the microenvironmental level.

Equally important is the role of the collagenases MMP-1 and MMP-13 in shaping the invasive phenotype of tumour cells. The degradation of interstitial type I collagen is accompanied by the formation of biologically active matrix fragments (matrikines), which act as chemotactic factors and stimulators of cell migration [27, 28]. This process not only creates physical pathways for invasion but also facilitates the signalling reprogramming of tumour cells by activating integrin-dependent and MAPK signalling pathways. Thus, matrix proteolysis becomes not merely a consequence of invasion but its active driver [29].

Particular attention is drawn to the role of MMPs in initiating the epithelial-mesenchymal transition. MMP-1, MMP-2 and MMP-9 are capable of modifying intercellular contacts by degrading adhesive molecules and activating TGF-β-dependent signalling, which contributes to the loss of the epithelial phenotype and increased cell plasticity [30]. In this regard, MMPs act as regulators of the cell phenotype, facilitating the transition of tumour cells to an invasive and metastatic state.

Gelatinases, particularly MMP-9, play a central role in tumour angiogenesis. They facilitate the remodelling of the vascular basement membrane, the mobilisation of VEGF and the recruitment of endothelial cells, thereby creating the conditions for the formation of a pathological vascular network. It has been shown that increased expression of MMP-9 correlates with the intensity of neoangiogenesis and a poor prognosis in many tumour types [31].

An important new paradigm is the immunoregulatory function of MMPs. In the tumour microenvironment, these enzymes are capable of proteolytically modifying cytokines, chemokines and immune cell receptors, contributing to the formation of an immunosuppressive environment [32]. Particularly illustrative is the role of MMP-8, which exhibits context-dependent effects: alongside its pro-oncogenic properties, its potential anti-tumour functions have also been described, highlighting the complexity of the MMP regulatory network in the tumour process [33].

Taken together, these mechanisms indicate that in carcinogenesis, matrix metalloproteinases perform functions that are fundamentally different from their physiological role. They transform from enzymes involved in controlled tissue remodelling into key regulators of the tumour microenvironment, coordinating proliferation, invasion, angiogenesis, immune modulation and the formation of the metastatic niche.

Cathepsins. Cathepsins represent another group of proteases actively involved in ECM remodelling, including collagen turnover. These are predominantly lysosomal proteases that, under normal conditions, facilitate intracellular proteolysis, antigen processing and the maintenance of cellular homeostasis. However, under pathological conditions, cathepsin activity is often disrupted and plays an important role in the remodelling of the local tissue microenvironment [34, 35].

In humans, cathepsins are represented by several catalytic classes: the cysteine-type includes cathepsins B, C, F, H, K, L, O, S, V, W and X/Z; the aspartate-type includes cathepsins D and E; and the serine-type includes cathepsins A and G [36]. However, in the

context of ECM remodelling and tumour progression, it is the cysteine cathepsins that are of greatest significance, primarily B, L, S and K, which are capable of acting not only as enzymes of mass proteolysis but also as selective regulators of extracellular proteins, signalling molecules and cell-matrix interactions [34, 37].

Most cysteine cathepsins are synthesised as inactive precursors, undergo intracellular processing and exhibit maximum activity in a weakly acidic environment, which determines their physiological localisation in endosomes and lysosomes. At the same time, during inflammation, the development of neoplasms, or local microenvironmental acidosis, they can be secreted externally and retain functional activity in the pericellular space [35, 38]. Unlike MMPs, the activity of cathepsins depends to a large extent on local pH, redox status and the presence of endogenous inhibitors, primarily cystatins; therefore, the execution of their proteolytic function is determined by the enzyme's location and the specific conditions of the microenvironment [34, 35].

Under pathological conditions, increased expression of cathepsins, changes in their subcellular localisation and enhanced secretion are frequently observed; this is associated with the degradation of ECM components, the activation or inactivation of other proteolytic cascades, the modification of cytokine signalling, as well as involvement in the processes of invasion, angiogenesis and immune dysregulation [34, 37].

From a structural perspective, in the context of ECM remodelling and the tumour microenvironment, it is advisable to focus primarily on cysteine cathepsins, as they constitute the main papain-like group of human cathepsins and have the best-characterised domain organisation [34, 39].

Cysteine cathepsins are synthesised as proenzymes, comprising a signal peptide, a prodomain and a catalytic domain. The signal peptide directs the protein to the endoplasmic reticulum, whereas the prodomain simultaneously facilitates the correct folding of the enzyme and maintains it in an inactive state. The mature cysteine cathepsin molecule has a two-domain structure typical of papain-like proteases: an N-terminal domain rich in α-helices and a C-terminal domain formed predominantly by β-structures. A V-shaped cleft lies between them. The catalytic centre is located within this cleft; in most cysteine cathepsins, it is represented by three amino acid residues (Cys–His–Asn), whereas the spatial environment of the active site and the configuration of additional substrate-binding sites largely determine the individual substrate specificity of each enzyme [39].

Despite their general structural similarity, cysteine cathepsins are divided into cathepsin L-like and cathepsin B-like subfamilies, which differ primarily in the characteristics of the prodomain and certain structural elements of the mature form. Catepsin L-like proteases (cathepsins L, V, K, S, W, F and H) are characterised by a prodomain of approximately 100 amino acid residues containing the conserved ERFNIN and GNFD motifs, whereas in cathepsin B-like enzymes these motifs are partially or completely absent, reflecting differences in their evolution and autoprocessing mechanisms [39, 40]. Some cathepsins possess additional structural elements that determine their functional specialisation. The best-known example is the occluding loop in cathepsin B, which partially overlaps the enzyme's active site and determines its ability to function not only as an endopeptidase but also as a dipeptidyl carboxypeptidase [40].

Cysteine cathepsins are activated by the proteolytic removal of the prodomain, which, in the proform, blocks substrate access to the active site and prevents premature enzymatic activity. In the acidic environment of the endo- or lysosomes, the prodomain partially loses its stable interaction with the catalytic domain, which contributes to the emergence of low residual activity of the proenzyme and triggers autoactivation. Subsequently, the process takes on a cascade-like nature: a partially activated molecule or a mature cathepsin cleaves the prodomain of other proforms, ensuring rapid accumulation of the mature enzyme. For cathepsins with endopeptidase activity, in particular B, H, L, S and K, this mechanism may be realised through auto- or trans-proteolytic maturation, whereas exopeptidases require activation by other endopeptidases [40]. In the extracellular space during pathological processes, particularly in the tumour microenvironment, the activation of cathepsins may be enhanced by glycosaminoglycans and other negatively charged ECM components, which facilitate prodomain cleavage and accelerate the maturation of proenzymes even under less acidic conditions [41].

The substrate specificity of cysteine cathepsins is relatively broad; however, in the context of ECM remodelling, their most relevant targets include collagens, elastin, laminin, fibronectin, proteoglycans and other ECM components. Cathepsin K exhibits the most pronounced collagenolytic activity, as it is capable of effectively cleaving fibrillar collagens, primarily type I collagen, which is of particular importance for the remodelling of the bone and tumour matrix. Cathepsins B and L are primarily involved in the degradation of basement membrane components and the destabilised matrix, in particular type IV collagen, laminin and fibronectin, which contributes to the disruption of tissue barriers and pericellular proteolysis [42]. Cathepsin S, unlike many other cysteine cathepsins, retains its activity at near-neutral pH, and can therefore act in the extracellular environment, participating in ECM remodelling, proteolytic receptor activation, and the modulation of pro-inflammatory signalling cascades [43].

The mechanism of proteolytic action of cysteine cathepsins is based on the activation of the thiol group of the cysteine residue within the active site of the

enzyme. In the Cys–His pair, histidine facilitates the deprotonation of the cysteine sulfhydryl group, resulting in the formation of the reactive Cys-S$^-$ form, which attacks the carbonyl group of the substrate's peptide bond. This results in the formation of a thioacyl enzyme-substrate intermediate, which is subsequently hydrolysed with the participation of a water molecule, culminating in the cleavage of the substrate, the release of proteolysis products, and the restoration of the initial configuration of the enzyme's active site [44].

As mentioned above, changes in the expression, localisation and activity of cathepsins are associated with several pathological conditions, including the progression of malignant neoplasms. It has been established that cysteine cathepsins secreted into the ECM promote its degradation and remodelling, whereas intracellular cathepsins are key components of signalling pathways that stimulate the growth of tumour cells [34, 40].

Cathepsin B is the most common and widely expressed cysteine protease. Normal regulation and distribution of cathepsin B are essential for cellular function, and any aberrant expression of cathepsin B resulting from altered splicing or changes in expression levels can significantly alter cellular homeostasis and lead to a malignant phenotype [45]. Cathepsin B is encoded by the *CTSB* gene, located in the 8p22-p23.1 region of chromosome 8 [46, 47]. This cathepsin is a key factor in ECM degradation. The enzyme's substrates include collagen types I, II, IV, IX, X and XI, laminin, osteonectin and fibronectin. It has been established that cathepsin B can initiate a cascade of proteolytic reactions by hydrolysing the pro-urokinase type plasminogen activator (pro-uPA), converting plasminogen into plasmin, which leads to the activation of MMPs, and, consequently, the active cleavage of ECM components, particularly collagen, and the promotion of invasion [48]. Furthermore, cathepsin B can stimulate tumour cell proliferation via the ERK/MAPK signalling pathway, induce epithelial-mesenchymal transition (EMT), and acts as a regulator of angiogenesis by modulating VEGF and MMP-9 [45, 49]. It is known that cathepsin B can inactivate the TLR3-mediated apoptosis, leading to the survival of tumour cells [45]. High levels of cathepsin B expression are observed in numerous types of malignant neoplasms, including colorectal, breast, lung, pancreatic and gastric cancers. For example, in astrocytoma, cathepsin B expression gradually increases 3–6-fold during progression to high-grade glioblastoma. In general, high levels of cathepsin B expression in tumour tissue are associated with tumour progression and a poorer clinical prognosis [46, 47, 50].

Cathepsin L is an endopeptidase and is encoded by the *CTSL* gene. The cathepsin L locus is located adjacent to the chromosomal region 9q22.2, where the *CTSV* is localised. Both enzymes demonstrate a high degree of structural similarity; however, cathepsin L is expressed ubiquitously, whereas cathepsin V is organ-specific. Increased expression of cathepsin L has been observed in a wide range of oncological conditions, including glioma, melanoma, and cancers of the pancreas, breast and prostate [46].

Cathepsin L is proteolytically active in the acidic lysosomal compartment, and in the acidified tumour microenvironment, extracellular cathepsin L effectively degrades ECM components such as type I and IV collagen, fibronectin and laminin [46]. Cathepsin L is involved in the processes of disrupting cell junctions, which is associated with its specific sheddase function (ectodomain shedding of the transmembrane and membrane-anchored proteins). Together with cathepsin S, cathepsin L cleaves a number of cell adhesion molecules and tight junction molecules from the cell surface, including ALCAM, MCAM and JAM–B, which promotes increased invasive activity and motility of tumour cells. At the same time, cysteine protease L mediates EMT via the NF-kB pathway, which stimulates the expression of the transcription factors Snail and Slug and facilitates the translocation of this cathepsin to the nucleus, where the enzyme cleaves its substrate — the homeodomain protein Cux-1. This leads to the inhibition of E-cadherin expression and the induction of Snail transcription itself, which further stimulates EMT. It is also worth noting that cathepsin L plays an important role in neoangiogenesis by modulating the CDP/Cux/VEGF-D pathway. At the same time, the cleavage of type XVIII collagen by cathepsin L can generate endostatin, an anti-angiogenic factor, suggesting a possible dual role for the enzyme [49].

High levels of cathepsin L expression are associated with the aggressive course of malignant tumours of various histological origins. In particular, it has been found that high levels of cathepsin L in the blood plasma of patients with pancreatic cancer are associated with a poor clinical prognosis. In patients with breast cancer, high levels of CTSL expression in tumour tissue were associated with an increased frequency of disease recurrence and an elevated risk of metastasis [50, 51]. Recent studies have demonstrated that cathepsin L is involved in regulating the sensitivity of tumour cells to chemotherapy [52].

Cathepsin H, encoded by the *CTSH* gene, owes its pronounced aminopeptidase activity to an octapeptide mini-chain linked by a disulfide bond to the main enzyme structure within the active site pocket in the direction of substrate binding. This spatial arrangement determines the enzyme's predominant exopeptidase activity, although under certain conditions it may exhibit weak endopeptidase activity [46, 48].

Cathepsin H plays an important role in tumour progression. In particular, cathepsin H-mediated processing of talin, a major focal adhesion protein, promotes tumour progression by modulating integrin activation and altering the strength of cell adhesion

[47]. High levels of cathepsin H not only stimulate the invasive activity of tumour cells through ECM degradation but also enhance their migratory capacity. It is also worth noting that cathepsin H is involved in the regulation of angiogenesis [53].

It has been established that high levels of cathepsin H expression in the tissue of colorectal cancer, breast cancer, prostate cancer, melanoma and glioma are associated with a more aggressive course of these diseases [47, 53]. Thus, the ability of cathepsins to remodel the ECM, activate proteolytic cascades, disrupt the barrier function of cell adhesion molecules and initiate EMT creates the conditions for the spread of tumour cells.

Although the degradation of ECM components involving MMPs, cathepsins and a number of other proteases is a prerequisite for tumour cell invasion, mechanisms of matrix stabilisation and compaction, mediated by cross-linking enzymes, play an equally important role in tumour progression. The formation of covalent intermolecular bonds between collagen fibres leads to changes in the mechanical properties of the ECM, an increase in its stiffness, and the creation of a microenvironment conducive to the migration, invasion, and metastasis of tumour cells. Enzymes involved in the remodelling and stabilisation of collagen fibres play a key role in these processes, in particular members of the lysyl oxidase (LOX/LOXL), meprin and Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase (PLODs) [7, 20, 34, 54–56].

Meprins are zinc-dependent metalloproteinases belonging to the astacine endopeptidases [11]. These enzymes are involved in ECM remodelling processes and play a role in organogenesis, angiogenesis, wound healing, cell adhesion, fibrosis, inflammatory and neurodegenerative processes, as well as the development of malignant neoplasms. Meprins function as extracellular endopeptidases characterised by unique biochemical properties and substrate specificity, distinct from other ECM metalloproteinases [54, 57, 58].

In humans, meprins are encoded by two separate genes: *MEP1A*, located on the short arm of chromosome 6 (6p11–p12), and *MEP1B*, located on the long arm of chromosome 18 (18q12.2–q12.3) [59]. Meprins are complex, highly glycosylated multidomain proteins. They are characterised by the presence of conserved zinc-binding motifs (HExxHxxGxxHxxxRxDR) in their structure and a sequence located near the active site cleft, the so-called methionine turn — SxMHY. Their molecule comprises an N-terminal signal peptide that directs the polypeptide chain to the endoplasmic reticulum, an N-terminal propeptide, an astacin-like catalytic protease domain, a MAM domain (meprin A5 protein tyrosine phosphatase μ) and a TRAF domain (tumour necrosis factor receptor-associated factor), which mediate protein-protein interactions, as well as an EGF-like domain, a transmembrane segment and a short C-terminal cytosolic tail [54, 58].

Meprins α and β have a similar modular structure; however, the key difference between them lies in the presence of an insert fragment in the meprin α molecule, which contains a cleavage site for the protease furin. During protein maturation in the Golgi apparatus, furin cleaves this domain, resulting in the loss of the EGF-like and transmembrane segments of meprin α and the secretion of the mature enzyme outside the cell. Consequently, meprin α forms noncovalently bound oligomers that can reach a size of ~6 MDa — representing one of the largest known secreted protease complexes [54, 57, 58, 60]. In contrast, meprin β remains anchored to the plasma membrane as a type I dimeric integral protein, but can be released from the cell surface by the α-secretases ADAM10 and ADAM17 [54, 57, 60]. It is worth noting that meprin β can stimulate the activation of ADAM10 by cleaving its proform. Consequently, there is a positive feedback loop between meprin β and ADAM10: ADAM10 cleaves meprin β from the cell membrane, whilst meprin β increases the activity of ADAM10, which together leads to an enhancement of the effects of both molecules [54].

Meprins form homodimers linked by disulfide bridges within the MAM domains. Both enzymes are synthesised as inactive zymogens, which require proteolytic cleavage of the N-terminal propeptides for activation [54]. Today, the activation of both meprins by trypsin and kallikrein-related peptidase (KLK5) has been described; furthermore, meprin α can be activated by plasmin, whereas meprin β can be activated by KLK4 and KLK8 [60, 61, 62]. In particular, it has been reported that in a colorectal cancer model, pro-meprin α on the surface of Caco-2 cells is activated by plasmin, which, in turn, is generated from plasminogen by urokinase-type plasminogen activator (uPA) secreted by stromal fibroblasts [61]. A specific membrane-bound activator has also been identified for membrane-bound meprin β — the serine protease matriptase-2 (MT-2), which ensures effective enzyme maturation directly on the cell surface [63].

Meprins demonstrate a selectivity for substrates based on the amino acid sequence around the cleavage site that is unique among extracellular proteases. In particular, both enzymes prefer the presence of negatively charged amino acids (aspartate or glutamate) at the P1' position (immediately following the cleaved peptide bond) [54, 58, 60]. In meprin β, this feature is more pronounced: it has been shown that this enzyme is capable of cleaving even polyacidic peptide sequences, making it unique among all extracellular matrix metalloproteases.

It has been established that under conditions of tumour growth, the regulation of meprin expression is disrupted. In particular, it has been shown that the chromatin-remodelling oncogenic factor Reptin acts as a positive regulator of *MEP1A* transcription, and its inhibition leads to a reduction in meprin α levels in

hepatocellular carcinoma cells [64]. Meprin α and meprin β catalyse the proteolytic cleavage of a very wide range of protein substrates, which accounts for their involvement in multiple biological processes — from morphogenesis and ECM maturation to inflammation and the immune response. In total, 151 extracellular substrates have been identified with high confidence for meprins, including growth factors, matrix proteins, inhibitors and other proteases [54, 57].

One of the best-characterised physiological functions of meprins is to facilitate the maturation of fibrillar procollagens types I and III by cleaving their N- and C-terminal prodomains. This stage is critical for the formation of collagen fibrils; therefore, reduced expression of meprins is associated with connective tissue defects, whereas their excessive activity is associated with the development of fibrosis [54, 57]. Furthermore, meprins cleave a wide range of basement membrane and ECM components, including collagen IV, fibronectin, nidogen-1, tenascin-C, fibulin, laminin and the glycoprotein SPARC/osteonectin [54, 57, 64], which disrupts the organisation of the basement membrane and may contribute to the infiltration of immune cells and the formation of a pro-inflammatory microenvironment [57].

This pathological aspect of meprin activity is also illustrated by models of acute kidney injury, where atypical localisation of active meprins on the basolateral surface of renal epithelial cells leads to degradation of the collagen IV–laminin network of the tubular basement membrane and the cleavage of intercellular adhesion proteins (E-cadherin, tenascin-C), accompanied by tissue damage and leukocyte infiltration [54]. Furthermore, it has been shown that proteolytic processing of the adhesion molecule CD99 by meprin β modifies transendothelial migration of both immune cells and Lewis lung carcinoma cells *in vitro*, whereas in *MEP1B* knockout mice or following pharmacological inhibition of meprin β, the full-length form of CD99 accumulates in the lungs, further confirming the contribution of this protease to the regulation of cell movement during inflammation and tumour growth [65].

Growth and differentiation factors also belong to the substrates of meprins. The best-characterised of these are pro-EGF and pro-TGF-α: meprin α can proteolytically process these precursors, generating mature epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), which are released into the extracellular space and initiate signalling via the EGF receptor (EGFR). In Caco-2 colorectal cancer cells, the activation of meprin α by plasmin is accompanied by EGFR transactivation and the induction of the ERK1/2 cascade, which stimulates cell proliferation and migration [66]. In addition to EGF/TGF-α, vascular endothelial growth factor A (VEGF-A) and connective tissue growth factor (CTGF) — key mediators of angiogenesis and fibrogenesis, respectively — have been identified as meprin substrates [64]. Thus, in experiments on *Danio rerio* embryos, knockout of the *MEP1A* gene was accompanied by marked inhibition of vascular network formation [67], similar to the phenotype observed in VEGF-A deficiency [68], indicating the indirect involvement of meprin α in angiogenesis, likely through the proteolytic processing of VEGF and other pro-angiogenic factors.

Furthermore, the spectrum of meprin targets includes other cellular proteins and proteases. In particular, meprin α cleaves the cell junction protein occludin, which may affect the barrier function of the epithelium [64]. The ability of meprins to activate certain matrix metalloproteinases has also been described: for example, partial proteolysis by meprin α may lead to the activation of pro-MMP-1, the precursor of type I collagenase [54].

The wide range of substrates that meprins are capable of proteolytically modifying underlies their involvement in several key aspects of carcinogenesis — invasion and metastasis, tumour cell proliferation, angiogenesis, as well as the regulation of the tumour microenvironment. Although meprins have long remained outside the main focus of oncological research, recent studies have demonstrated their association with the progression of malignant tumours of various histological origins [64, 69].

Dysregulated meprin expression has been observed in various types of cancer. In particular, increased expression of *MEP1A* has been observed in hepatocellular carcinoma (HCC) cells. It is worth noting that high expression levels of this metalloproteinase were associated with a poor clinical outcome in patients with this cancer. Functional studies indicate that meprin α enhances the malignant phenotype of HCC cells: siRNA-mediated knockdown of *MEP1A* in HuH7 and Hep3B cell lines led to a reduction in their migration (Transwell assay, wound-healing assay) and invasion (Boyden chamber assay), whereas overexpression of *MEP1A* or the addition of recombinant enzyme restored or enhanced these effects [64]. It has been established that meprin α stimulates proliferation and induces EMT in HCC cells: in cells with high *MEP1A* expression, increased levels of ZEB1, vimentin, MMP-2 and MMP-9 were observed against a background of reduced E-cadherin expression [70]. This highlights the importance of meprins in tumour progression and makes them promising targets for anticancer therapy.

One of the key pathways through which meprins contribute to tumour progression is ECM remodelling. Meprins α and β are capable of directly degrading structural components of the ECM. Proteolysis of basement membrane and ECM components leads to the disruption of physical barriers for tumour cells, intravasation/extravasation and metastatic dissemination. Furthermore, meprins indirectly enhance proteolysis by activating other proteases: in particular,

meprin β can proteolytically activate the metalloproteinase ADAM10, and meprin α/β complexes are capable of promoting pro-MMP-9 activation via MMP-3-mediated processing [69]. Increased MMP-9 activity may further enhance matrix degradation and support invasive and metastatic behaviour.

Another important effect of meprins is their impact on intercellular contacts. It has been shown that meprin β directly cleaves the extracellular domain of E-cadherin — a key adhesive protein in epithelial cells. Proteolysis of E-cadherin by meprin β leads to the formation of a truncated fragment and the loss of integrity of the adhesive complexes, thereby reducing the strength of cell junctions and the ability of cells to aggregate. It is known that the loss or inactivation of E-cadherin is a typical step in EMT and correlates with the progression of adenomas to invasive carcinomas [71]. Thus, the ability of meprin β to induce E-cadherin cleavage may significantly enhance the invasiveness of tumour cells, promoting metastasis.

Meprins also modulate the tumour microenvironment through the proteolytic processing, release or activation of signalling molecules — cytokines and growth factors. In particular, it has been established that these proteases are capable of initiating the release or activation of the pro-inflammatory cytokines IL-1β and IL-18, as well as TGF-α. By cleaving the inactive precursors of these molecules, meprins α and β enhance the generation of active signalling mediators, leading to a pro-inflammatory response in the tumour site [54].

Another aspect of the pro-tumour action of meprins is the stimulation of angiogenesis. Proteolytic degradation of the matrix by meprins promotes the release of pro-angiogenic factors and creates permissive conditions for endothelial cell migration. *In vitro* experimental data confirm the pro-tumour role of meprin α in angiogenesis: the addition of recombinant human meprin α significantly enhances the formation of capillary-like vascular structures in a rat aortic organ culture model. Cell models have also shown that the presence of active meprin α increases the motility of epithelial cells in response to growth factors (e.g., hepatocyte growth factor (HGF)), particularly under conditions of plasmin-mediated activation of this proteolytic cascade [72]. Thus, meprins potentiate both the invasive and migratory properties of malignant cells and their ability to stimulate blood vessel formation.

However, the effect of these enzymes on the course of the tumour process may depend on the tissue type. For example, in colorectal cancer, high levels of meprin β expression in tumour tissue are paradoxically associated with better overall patient survival, whereas the opposite trend is observed in prostate cancer [73]. The same enzyme can evidently have different effects — anti-tumour or, conversely, oncogenic — depending on the cellular environment, tumour type and subcellular localisation of its activity [69, 73]. This underscores the need for a more in-depth study of meprin functions in various neoplasms. Nevertheless, the accumulated data generally suggest that, in many tumour contexts, meprins support tumour progression and aggressiveness through their complex effects on the ECM, cell interactions and signalling within the tumour microenvironment.

Experimental data indicate that meprin α is involved in the formation of the malignant phenotype of prostate cancer cells. In particular, according to the results of genetic screening conducted on a *Drosophila* accessory gland model, the human orthologue *MEP1A* was identified among candidate genes whose activation stimulates the growth and migration of secretory cells. The authors demonstrated that *MEP1A* is expressed in human prostate cancer cells and is associated with their increased replicative activity and invasiveness [74]. These data are consistent with the findings of Wang et al., who, in summarising the role of *MEP1A* in malignant neoplasms of various histological origins, emphasise that this enzyme promotes both the proliferation and invasive behaviour of tumour cells [75]. In the study by Koistinen et al., which describes the role of proteolytic enzymes in prostate cancer, meprin α is classified among the proteases the expression of which is elevated in castration-resistant prostate cancer tissue compared with primary hormone-sensitive tumours, further indicating the potential role of meprin α in the progression of the disease to more aggressive forms [76].

Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenases (PLODs). Among the enzymes involved in the cross-linking processes of collagen fibres are the lysyl hydroxylases of the PLOD family, which comprises three main members: PLOD1, PLOD2 and PLOD3. The lysine residues of the triple helix undergo hydroxylation with the participation of PLOD1 and PLOD3, whereas the lysine residues of collagen telopeptides are hydroxylated by PLOD2. It is worth noting that the hydroxylation of collagen fibres by enzymes of the PLOD family leads to their stabilisation and maturation, ensuring the integrity of tissue structures through the formation of a rigid three-dimensional ECM. PLODs catalyse the hydroxylation of lysine intracellularly prior to collagen secretion, and subsequently lysyl oxidase (LOX) binds to hydroxylated lysine residues in extracellular collagen fibers and induces the formation of cross-links [55].

With an overall protein sequence identity of approximately 47%, members of the PLOD family are highly homologous. Proteins of the PLOD family belong to the secretory enzymes of the endoplasmic reticulum lumen, which have a fairly conservative modular organisation typical of Fe(II)/2-oxoglutarate-dependent dioxygenases [77]. At the N-terminus of the polypeptide lies a signal peptide 26 amino acid residues in length, which ensures co-translational transport into the endoplasmic reticulum and subsequent localisation

of the enzyme in its lumen. Following cleavage of the signal peptide, a luminal enzyme is formed, capable of glycosylation and the formation of dimers or multimeric complexes, which is important for stability and catalysis. The main part of the protein is occupied by a large C-terminal catalytic domain, which contains a characteristic iron-binding motif (His-X-Asp...His) and a 2-oxoglutarate-binding site — key elements necessary for the hydroxylation of the ε-amino groups of lysine in procollagen. The N-terminal region of PLOD proteins primarily performs structural and regulatory functions: it is involved in the correct folding of the enzyme, interaction with procollagen substrates, and retention of the enzyme in the ER lumen. In some isoforms, particularly PLOD3, additional domains associated with glycosyltransferase activity are present, which enables further modification of hydroxylysine and highlights the functional multi-domain nature of the family. Thus, PLOD proteins combine a signal-secretory N-terminus with a highly conserved C-terminal dioxygenase catalytic domain, reflecting their specialisation in the post-translational modification of procollagen during the early stages of extracellular matrix biosynthesis [78].

It has been demonstrated that different types of tissues and organs exhibit a predominant functional activity of various members of the PLOD family. PLOD1, amongst other things, is essential for the formation of healthy bone tissue. It influences wound healing and vascular stability [79–81]. PLOD2 is essential for the continuous cross-linking of collagen in the extracellular matrix of peripheral organs [82]. The activity of PLOD3 is important primarily for the biosynthesis of type IV and VI collagen, making it a key regulator of the formation of intact basement membranes in epithelial tissue [83]. Additionally, PLOD3 possesses glycosylation activity, which induces the attachment of monosaccharides or disaccharides to collagen hydroxylysines [84].

Elevated expression of PLOD1/2/3 has been described in many types of malignant tumours and is consistently associated with invasiveness, metastasis and a poorer patient prognosis. Mechanistically, these enzymes enhance the hydroxylation of lysine residues in collagen, which promotes the formation of stable intermolecular cross-links in collagen fibres, increases ECM stiffness, and activates mechanotransduction signalling pathways (notably NF-κB, integrin-regulated FAK–Src and PI3K/AKT pathways) [85–87]. PLOD2 is considered most closely associated with the metastatic phenotype: its expression is induced by hypoxia via HIF-1α and stimulates the formation of rigid collagen bundles for tumour cell migration, as well as the epithelial-mesenchymal transition [88]. PLOD1 and PLOD3 also contribute to tumour progression, although their role is less specifically linked to metastatic ECM remodelling than that of PLOD2. Elevated expression of PLOD1 has been described in gliomas, gastric, lung and breast cancers and is associated with increased proliferation, invasiveness and reduced overall survival [89–92]. In the TME, PLOD1 expression correlates positively with the level of infiltration by monocytes, macrophages and tumour-associated fibroblasts, and negatively with $CD8^+$ T-cells, B-cells and CD4+ T-cells. Furthermore, PLOD1 expression is associated with immune checkpoint molecules and immunomodulatory genes. At the same time, PLOD1 knockdown reduces the proliferation, migration and anti-apoptotic capabilities of tumour cells [93].

PLOD3 is a unique member of the family because it combines lysyl hydroxylase and glycosyltransferase activities, which together mediate the complete cycle of collagen post-translational modification; its overexpression is associated with angiogenesis, basement membrane remodelling, and the formation of premetastatic niches, as well as with increased invasiveness and an unfavourable prognosis in the tumour process [94–96]. Overall, the accumulated data indicate that PLOD enzymes are important regulators of fibrotic tissue remodelling of the tumour microenvironment and promising prognostic markers and therapeutic targets.

Lysyl oxidases. The lysyl oxidase family comprises five members — lysyl oxidase (LOX) itself and LOX-like proteins (LOXL1–4). This group of proteins consists of extracellular amino oxidases, whose primary function is the post-translational modification of collagen and elastin in the extracellular matrix (ECM) [56]. All family members exhibit similar catalytic activity due to the presence of a highly conserved C-terminal region containing a copper-binding domain, residues for the formation of the lysine tyrosylquinone (LTQ) cofactor, and a cytokine-like receptor (CRL) domain. At the same time, the N-terminal regions of LOX and LOXL1 differ significantly from the corresponding regions of LOXL2–4, which is the basis for their division into two subfamilies [97].

The first subfamily is considered phylogenetically younger and is primarily associated with the cross-linking of phylogenetically younger substrates, such as fibrillar collagen (types I and III) and elastin; therefore, LOX and LOXL-1 are considered matrix-oriented enzymes, and they do indeed interact with other extracellular matrix proteins, such as BMP-1, fibronectin, fibulin-4 and 5, as well as tropoelastin [97, 98]. In contrast, LOXL-2, LOXL-3, and LOXL-4, with their phylogenetically ancient and conserved SRCR domains, may primarily act as cross-linkers of the basement membrane (collagen IV) and more strongly regulate the stiffness of the extracellular matrix. In addition, SRCR domains likely mediate protein-protein interactions, expanding the substrate or signalling capabilities of LOXL-2, LOXL-3, and LOXL-4 [97].

It has been established that LOX catalyses the oxidation of peptidyl lysine side chains of fibrillar collagens, leading to the formation of corresponding lysine

aldehydes. These aldehydes can spontaneously form condensation products with each other or with a lysine residue, thereby cross-linking two fibrillar collagen proteins. The condensation products undergo further non-enzymatic rearrangements, leading to the formation of stable end products, such as pyrrolidine or deoxypyrrolidine, which provide covalent cross-linking of collagen fibers. The presence of such cross-links determines the resistance of collagen fibers to degradation by MMPs [99].

LOX expression has been detected in various cell types, including basal and suprabasal keratinocytes, fibroblasts, adipocytes, osteoblasts, smooth muscle cells, and endothelial cells. It has been established that LOX plays an active role not only in the physiological remodelling of the ECM but also in the initiation and progression of malignant neoplasms, enhancing the proliferative and invasive activity of tumour cells, as well as the processes of angiogenesis and metastasis. At the same time, the scientific literature also contains data on the potential tumour-suppressor properties of LOX [100].

High levels of LOX expression are detected in tumours of various histological origins, including oral cavity cancer, gastric cancer, breast cancer, and thyroid cancer. It has been demonstrated that LOX overexpression in tumour tissue is associated with high proliferative activity of malignantly transformed cells in oral squamous cell carcinoma, colorectal cancer, and astrocytoma [100]. Furthermore, it has been established that a decrease in LOX expression leads to increased E-cadherin expression and decreased vimentin levels, indicating the pro-metastatic activity of this protein. In particular, LOX is an important component of the CD44–Twist signalling axis. Under hypoxic conditions, LOX stimulates *TWIST1* expression, thereby promoting tumour metastasis. At the same time, LOX can translocate to the cell nucleus, where it binds to the *SNAI2* (*SLUG*) gene promoter — one of the key regulators of E-cadherin suppression — and stimulates its expression. This leads to increased expression of tissue inhibitor of metalloproteinases-4 (TIMP-4), which also enhances EMT [101]. It has been found that high levels of LOX expression are associated with the development of metastases in squamous cell carcinoma of the oral cavity, colorectal cancer, and lung adenocarcinoma [100].

LOXL proteins, which are also actively involved in the progression of malignant tumours, deserve special attention. In particular, LOXL2 contributes to the development of colorectal cancer, gastric cancer, esophageal squamous cell carcinoma, cholangiocarcinoma, hepatocellular carcinoma, lung squamous cell carcinoma, non-small cell lung cancer, and renal cell carcinoma. LOXL3 is involved in the progression of melanoma and also promotes the invasiveness and metastasis of breast cancer [102]. Meanwhile, LOXL4 stimulates the proliferative and metastatic activity of gastric cancer, as well as the invasion and metastasis of hepatocellular carcinoma [101].

It has been demonstrated that overexpression of *LOXL2* or *LOXL3* induces the EMT. It was first shown that these enzymes oxidize lysine residues 98 and 137 of the Snail transcription factor, which ultimately leads to its ubiquitin-mediated degradation. This is accompanied by the suppression of E-cadherin expression — one of the key triggers of the EMT [101].

In addition, it has been established that LOX in breast cancer cells activates the secreted protease HTRA1, which inhibits TGF-β1 signalling. In turn, this leads to increased expression of *MATN2* (matrilin-2), which inhibits the internalization of EGF receptors and promotes their accumulation on the cell surface. Elevated concentrations of EGF receptors increase the sensitivity of tumour cells to EGF family ligands, stimulating tumour growth and metastasis [102].

It is important to note that LOX secreted by primary tumour cells can enter the bloodstream both as soluble enzymes and as components of extracellular vesicles. Upon reaching distant organs, they are capable of modifying the ECM in these tissues, forming so-called premetastatic niches that become more favourable for colonization by circulating tumour cells [103].

To summarize the above, it is worth noting that the ECM is a dynamic, multicomponent structure that provides mechanical support to tissues, regulates intercellular interactions, and participates in the control of proliferation, differentiation, migration, and survival of normal and malignantly transformed cells [104]. Under conditions of malignancy, the ECM undergoes profound remodelling, accompanied by changes in its composition, organization, and mechanical properties. It has been established that disruption of ECM homeostasis is one of the key characteristics of the tumour microenvironment, determining the aggressiveness of malignant neoplasms, their invasive potential, and their ability to metastasize. Among the central participants in ECM remodelling are proteolytic enzymes, primarily MMPs, cathepsins, and other proteases capable of degrading the structural components of the matrix. Excessive activity of these enzymes leads to the destruction of the basement membrane, disorganization of collagen fibers, and the release of numerous biologically active molecules stored in the ECM. This, in turn, creates favourable conditions for tumour cell migration, stimulates angiogenesis, and promotes the formation of an immunosuppressive microenvironment. In addition, proteases are involved in the regulation of signalling pathways associated with EMT, the maintenance of tumour stem cells, and the development of therapeutic resistance. Along with ECM degradation processes, mechanisms of collagen matrix stabilization and compaction, mediated by cross-linking enzymes, play a crucial role in tumour progression [7].

It should be noted that ECM remodelling is the result of complex interactions between tumour cells and components of the stromal microenvironment, particularly fibroblasts, immune cells, and endothelial cells. Activation of cancer-associated fibroblasts is accompanied by increased collagen synthesis, secretion of proteases and LOX, as well as production of cytokines and growth factors that support tumour progression. At the same time, changes in the mechanical properties of the ECM influence cellular signal transduction via integrins and mechanosensitive signalling pathways, which contributes to the acquisition of an aggressive phenotype by tumour cells [7, 25].

Thus, current data strongly suggest that collagen degradation and cross-linking enzymes are important regulators of ECM remodelling and key components of the tumour microenvironment. Their dysregulation contributes to the formation of a mechanically and biochemically favourable environment for tumour growth, invasion, and metastasis. Further study of the molecular mechanisms of ECM remodelling is of great importance for the search for new diagnostic markers and the development of targeted therapeutic approaches in modern oncology.

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РЕМОДЕЛЮВАННЯ КОЛАГЕНОВОГО МАТРИКСУ ЯК ФАКТОР АГРЕСИВНОСТІ ПЕРЕБІГУ ПУХЛИННОГО ПРОЦЕСУ: РОЛЬ ПРОТЕАЗ І ФЕРМЕНТІВ КРОСЛІНКІНГУ

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Резюме. *Ремоделювання колагенового матриксу є одним із ключових процесів, що визначають прогресування злоякісних новоутворень та формування агресивного фенотипу пухлин. У представленому огляді узагальнено сучасні дані щодо ролі ферментів деградації та крослінкінгу колагену у прогресуванні злоякісних новоутворень. Показано, що порушення балансу між процесами деградації та стабілізації колагенових волокон сприяє підвищенню жорсткості тканини, активації сигнальних шляхів, пов'язаних з інвазією та метастазуванням. Детально охарактеризовано значення матриксних металопротеїназ, катепсинів, мепринів, лізилоксидаз та ферментів родини проколаген-лізин, 2-оксоглутарат-5-діоксигенази (PLODs) у ремоделюванні позаклітинного матриксу, регуляції епітеліально-мезенхімального переходу та прогресуванні злоякісних новоутворень. Проаналізовано результати сучасних досліджень, які демонструють зв'язок порушень експресії колаген-модифікуючих ферментів із агресивністю перебігу пухлинного процесу у хворих на різні типи раку.*

Ключові слова: рак, колаген, матриксні металопротеїнази, катепсини, меприни, LOX, PLOD.

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