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CHARACTERISTICS OF TNF-ALPHA, TYPE I, III COLLAGEN, AND MATRIX METALLOPROTEINASES. THEIR CLINICAL APPLICATIONS

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Abstract

The world of science, particularly in oncology, continually strives to detect cancer or its recurrence at the earliest possible stage of the disease. Consequently, imaging techniques are constantly being refined, while molecular biology is concurrently advancing to understand the mechanisms and pathomechanisms at the cellular level. Immunodiagnostics, which quantitatively determine circulating tumour markers, are also subject to development. Researchers are also attempting to identify markers that enable a fast and accurate diagnosis of diseases with inflammatory, autoimmune, or genetic backgrounds. In this study, we addressed the construction and formation of TNFα, collagen I and II, as well as extracellular matrix metalloproteinases. These molecules also have a significant impact on various processes in the body, including inflammatory and cancer processes. Understanding them in detail allows for a better comprehension of the mechanisms and pathomechanisms of certain diseases. This publication explores their utility in diagnosis and potential treatment approaches for some illnesses. A literature review indicates a consistent increase in interest in these molecules, particularly in certain gastrointestinal, gastric, connective tissue disorders, and central nervous system cancers.

Key words: sisease markers, type I collagen , type III collagen, TNFα, matrix metalloproteinases

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Introduction

The world of science, primarily oncology, continually strives to detect cancer or its recurrence in the earliest possible stages of the disease. Consequently, imaging techniques are constantly being refined, while molecular biology simultaneously advances to understand their mechanisms and pathomechanisms at the cellular level. Immunodiagnostics, which quantitatively determine circulating tumour markers, is also undergoing development. Researchers also seek to identify markers that allow for the rapid and accurate diagnosis of diseases with inflammatory, autoimmune, or genetic bases.

Tumour necrosis factor alpha (TNFα), type I and III collagen, as well as matrix metalloproteinases, participate not only in the normal functioning and structure of the body but also in processes leading to various pathological conditions. Therefore, it is crucial to understand their precise characteristics and roles in the pathomechanism of these disorders. This paper introduces the structure, formation process, and clinical use of these molecules.

Characteristics of TNFα

TNFα (tumour necrosis factor alpha) belongs to the basic proinflammatory and immunoregulatory cytokines. It exists in two forms – a membrane-bound form with a mass of 26 kDa and a soluble form with a mass of 17 kDa [1]. The soluble form is generated by the metalloproteinase TACE (TNFα-converting enzyme), which, by acting on the membrane-bound form, releases a segment corresponding to the external portion of the cell membrane [2].

The action of these molecules is made possible by two receptors: p55 TNF receptor 1 and p75 TNF receptor 2. These molecules mediate the production of other proinflammatory cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6), and also stimulate leukocyte migration through the expression of adhesion molecules in endothelial cells and leukocytes [3]. Tumour necrosis factor alpha is primarily produced by macrophages and monocytes in response to various stimuli, including inflammation, infection, infectious agents (such as viruses or bacterial lipopolysaccharide), and proinflammatory cytokines. It is also produced by other normal cells [3,4].

An important feature of TNFα is its pleiotropic action. This is due to its ability to activate multiple intracellular signalling

pathways, thereby activating various transcription factors that influence the expression of many genes. This action is likely present in all nucleated cells as they possess TNF receptors. The effect of TNFα may therefore vary among different cell types [4,5].

As mentioned earlier, TNFα influences the ongoing inflammatory process in organs. Manifestations of this influence include an increase in the level of acute-phase proteins, induction of fever, and loss of appetite [4].

Disrupted secretion of TNFα contributes to the development of many diseases, such as rheumatoid arthritis [6], diabetes [7], myocarditis [8], and multiple sclerosis [9]. In the case of cancer, both overproduction and deficiencies in TNFα can disrupt the immune response and accelerate the process of metastasis formation. Persistent secretion of this factor can also lead to disturbances in metabolic processes and, consequently, to its depletion [10].

TNFα is also presented as a factor causing increased collagen accumulation and proliferation of intestinal myofibroblasts, with the involvement of insulin-like growth factor 1 (IGF-1). TNFα stimulates the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) and reduces the activity of matrix metalloproteinase, while IGF-1 stimulates the activation of the collagen I gene. These phenomena may contribute to the fibrosis process during intestinal inflammation [5].

Collagen Type I and III

Collagens are the main polypeptide components of the extracellular matrix. These proteins, rich in proline and glycine, form a network of amino acids in the connective tissue. In addition to their structural function, they also participate in signal transduction by binding to integrins (transmembrane receptors present in the cell membrane). Fourteen different types of collagen have been discovered, all of which are constructed from a triple helix composed of subunits - α chains, which can be heterotrimeric or homotrimeric. Collagen Type I consists of two α1 chains and one α2 chain, while Collagen Type III consists of three α1 chains [11].

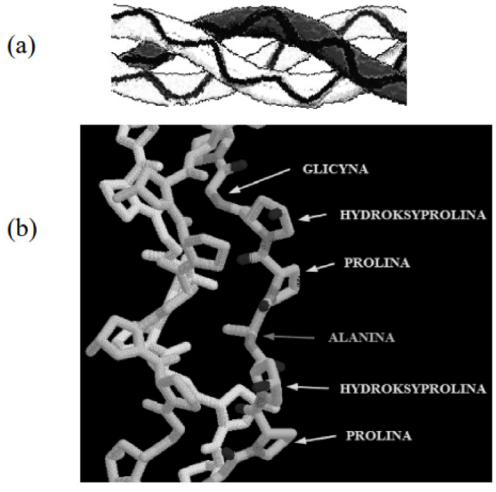

Fig. 1 Superhelical Model of Collagen: (a) spatial structure; (b) amino acid sequence - GXY, to demonstrate that adjacent polypeptide chains undergo crowding in a position typically occupied by glycine, alanine with a larger molecule is placed [12].

Collagen Type III is also a homotrimer, although the α1 chains are connected by disulfide bridges. Collagen Type I constitutes 90% of all human collagen and is responsible for the majority of the organic substance in bones. The formation of these molecules involves genes such as COLIA1 and COLIA2, pre-mRNA processing, and post-translational modifications through hydroxylation and glycosylation [13]. During the formation of collagen, every third amino acid is incorporated into the interior of the triple helix. In the case of Type I collagen, glycine is the mentioned amino acid due to its compact molecule. It is present in at least 38 consecutive GXY triplets. Other positions are occupied, among others, by proline and hydroxyproline, and their protrusions facilitate cross-linking with other helices [13,14].

The triple helix of collagen, under the influence of twisting, allows the formation of structures that undergo polymerization into different higher-order structures. Due to their structure, collagen triple helices are resistant to the action of all proteases, except for specific collagenases, including metalloproteinases 1 and 13, which will be discussed later in the article.

As mentioned above, collagens are components of the bone matrix, but they exhibit numerous structural and functional abnormalities in various pathological conditions, including stomach cancer. Some of these disorders are associated with mutations in collagen I and III genes, e.g., the COLIA1 and COLIA2 mutations are responsible for 90% of cases of spontaneous bone fragility, also known as incomplete ossification [13].

The multi-step synthesis of collagen, which also includes processes independent of genetic information, allows for the production of a vast number of different proteins, even in the same individual, although they "originate" from the same genes. The basis of this heterogeneity is not known and is likely the cause of collagen diversity in different tissues. Therefore, it can be hypothesized that understanding this cause will help in the comprehension of many pathophysiological processes related to connective tissue [12].

Collagen Type I is found in bones, ligaments, skin, tendons, dentin, and other elastic structures but is absent in the vitreous body and cartilage [15]. On the other hand, Collagen Type III is localized in internal organs and blood vessels but is absent in bones and dentin [16].

The genes encoding collagen I and III molecules share a similar intron-exon structure, suggesting their common origin from a shared gene [12].

Characteristics of Matrix Metalloproteinases in the Extracellular Matrix

The extracellular matrix (ECM) is a collection of diverse molecules that form a scaffold supporting cells and tissues. Its components include glycosaminoglycans (GAGs), proteoglycans, proteins forming the fibres of connective tissue, and glycoproteins. Besides its structural function, the ECM also serves other purposes, such as playing a crucial role in tissue formation, cell localization and migration, intracellular signalling, and the determination of cell shape. Under normal conditions, cells are ancho-

red in the extracellular matrix. However, this situation changes during the progression of cancer when cancer cells partially lose their adhesive properties, leading to the loosening of intercellular interactions and adherence to the ECM [17,18].

The movement of cancer cells within the ECM is made possible by the secretion of metalloproteinases that break down collagen and other proteins. It can be inferred, therefore, that remodelling of the extracellular matrix is crucial for the growth, invasiveness, and formation of distant metastases in cancer [17,19,20]. The degradation of the extracellular matrix is regulated by proteolytic enzymes, among which matrix metalloproteinases (MMPs), cysteine proteases, aspartic proteases, and serine proteases with the plasminogen activation system can be mentioned [21].

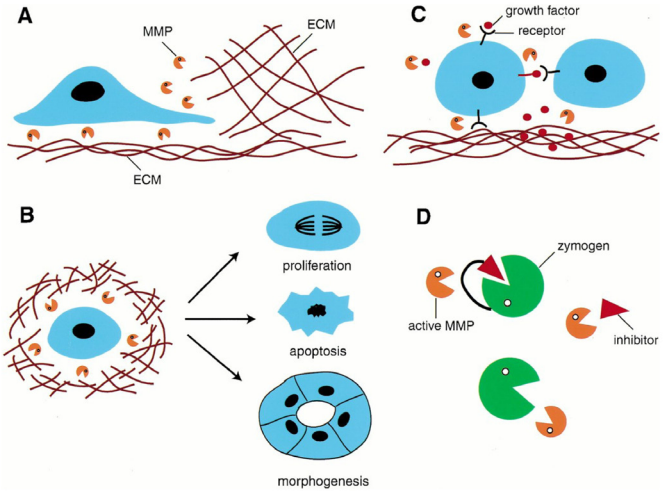

Fig. 2 Stages of Metalloproteinase Action. A - Influence on the cell through changes in cell adhesion and degradation of the extracellular matrix; B - Interaction of metalloproteinases with the microenvironment of the extracellular matrix, leading to proliferation, apoptosis, and morphogenesis; C - Impact of metalloproteinases on growth factors or receptors for growth factors and their release into the extracellular matrix; D - Change in the activity of metalloproteinases through the elimination of enzymes and their inhibitors [22].

In the category of extracellular matrix metalloproteinases, ion-dependent zinc and calcium endopeptidases can also be included. They play regulatory roles through their involvement in the enzymatic cascade components of the extracellular matrix. Cytokines, adhesion molecules, growth factors, and hormones influence the production of these enzymes. Matrix metalloproteinases (MMPs) are synthesized and secreted as inactive proenzymes, mainly by leukocytes, macrophages, fibroblasts, and vascular endothelial cells. Differences in the structure of ECM metalloproteinases affect their substrate specificity, regulate binding to matrix proteins, and are significant in interactions with tissue inhibitors of matrix metalloproteinases (TIMPs), which naturally inhibit their activity. Currently, 24 ECM metalloproteinases have been identified, which may exist in membrane-bound or free forms.

The synthesis of metalloproteinases begins with the production of preproenzymes, known as zymogens, which are secreted in this form. The activation of proenzymes is a two-step process. First, the prodomain is cleaved between two α-helices, thereby weakening the interaction between the prodomain and the catalytic domain. This process enables autolytic cleavage of the prodomain. In the next stage, autolytic cleavage of the propeptide occurs. Membrane-type metalloproteinases (MT-MMPs) activate proenzymes by forming triple complexes with TIMP-2 and the propeptide of the zymogen (proMMP). MT-MMPs act as a receptor for the TIMP-2 molecule. Only the combination of MT-MMP with TIMP-2 functions as a receptor for proMMP, which attaches to it using the C-terminal domain. Currently, only two enzymes from the matrysin family are known to undergo activation involving MT-MMP, namely MMP-2 and MMP-13.

The regulation of MMP activity is also ensured by another mechanism, specifically through the inhibition of TIMP protein activity. So far, four genes responsible for proteins called TIMPs have been identified: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. TIMP-1 and TIMP-2 form non-covalent complexes with MMP proteins in a 1:1 molar ratio. TIMP-2 exhibits twice the affinity for MMP-2 and MMP-9 compared to TIMP-1. However, both particles have an inhibitory effect on MMP activity. The exact mechanism of inhibition is not fully understood, but it is likely that TIMP binds to MMP at several sites. In the case of MMP-2, it appears that there are two places through which TIMP-2 forms a complex with it, precisely the active centre and the "stabilizing" site in the C-terminal region of the molecule. This leads to a change in the molecule's conformation and the formation of fully active enzymes.

A review of the literature suggests attempts to use this knowledge of the role of MMP and TIMP particles in tumour invasion processes to develop drugs acting as anti-invasive agents. Some authors have proposed the use of protein inhibitors that mimic the N-terminal fragment of MMP (propeptide), which keeps the enzyme in an inactive form. Peptides with sequences such as RCGVP-NH2 (Arg, Cys, Gly, Val, and Pro) and RCGVPDP--NH2 (Arg, Cys, Gly, Val, Pro, Asp, and Pro), inspired by the sequence in the propeptide of all MMPs, have been obtained. As a result, it has been shown that these peptides inhibit the activity of stromelysin (one of the metalloproteinases) with an IC50 (the concentration of the inhibitor causing a 50% reduction in enzyme activity) ratio of 11 and 5 μM, respectively. It has also been demonstrated that the addition of exogenous TIMP-1 protein to in vitro cancer cell cultures significantly limits their invasiveness. A similar effect was observed in in vitro cultures of glioblastoma cancer cells.

In an in vitro study, it was found that the ability of glioma cells to pass through a filter coated with ECM components is inhibited by the administration of a peptide with the sequence TMRKPRCGNPDVAN (Thr, Met, Arg, Lys, Pro, Arg, Cys, Gly, Asn, Pro, Asp, Val, Ala, and Asn).

Conclusions

For many years, attention has been focused on the increased expression of genes encoding various cytokines, including TNFα, in cancer processes, inflammatory conditions, and autoimmune diseases such as rheumatoid arthritis, lupus, diabetes, thyroid diseases, etc.

Type I and III collagen dominate in cancer-altered tissues, but their structural abnormalities also underlie many connective tissue diseases.

Metalloproteinases, which degrade extracellular matrix proteins of the basement membrane and blood vessel walls, play a significant role in the development of cancerous changes. This enables the ability to invade and create metastases.

In summary, further research on the characteristics of these mentioned particles is worthwhile to discover more advanced diagnostic methods that allow the detection of diseases at the earliest stage possible or to improve the methods of their treatment.

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