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REVIEW

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MODERN VIEWS ON THE ROLE OF MAIN COMPONENTS OF STROMA AND TUMOR MICROENVIRONMENT IN INVASION, MIGRATION AND METASTASIS

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The review presents modern ideas about tumor microenvironment, which most researchers recognize as the main "player" in tumor cell invasion, cell migration and metastasis. The current data on the main components of the stroma and the microenvironment, which play the role of the driving force in tumor progression, are analyzed. In particular, the review highlights the issues of origin, biological traits, phenotypic plasticity, functional heterogeneity of activated fibroblasts — myofibroblasts and tumor-associated fibroblasts, which in recent years have received much attention. Such components of the extracellular matrix proteome as collagen and matrix metalloproteinases are discussed in detail. They are mostly produced by activated fibroblasts and, on the one hand, initiate the development of desmoplasia due to type I collagen and, on the other hand, promote degradation of extracellular matrix proteins due to metalloproteinases, which generally leads to tissue remodeling that promotes tumor progression. Possibilities of using the most important indicators of extracellular matrix remodeling as potential markers and targets of clinical strategy are discussed.
***Key Words*: tumor microenvironment, extracellular matrix, activated fibroblasts, myofibroblasts, tumor-associated fibroblasts, collagen, matrix metalloproteinases, invasion, migration, metastasis, tumor progression.**

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Despite significant advances in the diagnosis, prognosis and treatment of patients with malignant neoplasms having been achieved through the widespread introduction of molecular and molecular genetic methods in experimental and clinical oncology, which has contributed to the development of various panels of diagnostic and prognostic markers [1], many challenges remain to be addressed [2].

Most studies in cancer biology focus on the morphological characteristics of the parenchymal component of the tumor, which is the basis for the classification of tumors, while until recently only a secondary role was given to the stromal component. To date, this issue is already being reconsidered by many experts. *In vitro* and *in vivo* experiments and clinical findings have demonstrated an importance of the tumor stroma, its microenvironment, as well as their relationship with the parenchymal component of the primary lesion in tumor progression, in particular in the formation of metastatic phenotype [3]. Nowadays, solid tumors are considered as complex organ-like structures, which include not only tumor cells but also their microenvironment with different types of cells in the altered extracellular matrix (ECM), as well as elements of the vascular system [4]. It is known that often the microenvironment in solid tumors occupies most of the total mass, and all components of tumors are significantly different from those in normal organs.

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Abbreviations used: α-SMA – smooth muscle α-actin; BC – breast cancer; FN – fibronectin; MF – myofibroblast; TAF – tumor-associated fibroblasts; ECM – extracellular matrix; MMPs – matrix metalloproteinases; TGF-β – transforming growth factor beta.

The factors of the stromal microenvironment include the following complex: 1) non-tumor cells (endothelial pericytes, smooth muscle cells, fibroblasts, myofibroblasts (MFs)); 2) extracellular molecules, namely non-classical elements of the stroma (adhesion molecules, growth factors, hormones, proteins, enzymes, metabolites); 3) ECM (connective tissue elements, including collagen, elastic, argyrophilic fibers, as well as nerves) [5, 6].

Many studies have shown that intratumoral signaling, transport mechanisms, metabolism, oxygenation and immunogenicity are influenced by ECM that also controls and regulates cell-cell, and cell-matrix interactions. By exerting such control, it affects not only the degree of malignancy of the growing tumor, but also its response to therapy [7]. Considering the microenvironment as a combination of the non-tumor matrix of the tumor with blood vessels, cells of inflammatory infiltrate and fibroblasts, ECM is considered to be of special importance [5] playing a leading role in the progression of malignant growth [8, 9]. It includes: 1) the basement membrane, which maintains balance in the location of epithelial/endothelial cells, and 2) the interstitial matrix that supports the ordering of the lower stromal compartment. 28 types of collagen are the main components of ECM [10].

It has been established that the interstitial matrix is the base of the stroma and plays a major role in cell migration, adhesion, angiogenesis, tissue development and repair. The currently identified types of collagen form a unique composition of the interstitial matrix and can be divided into several separate subgroups, such as fibrillar and reticulate collagen. It should be noted that among the components of this matrix fibrillar collagen is the most abundant, in particular I,

II, III, V types, as well as beaded threads with the synthesis of collagen type VI, which is produced by stromal fibroblasts and is the most studied one [11, 12].

THE KEY ROLE OF ACTIVATED FIBROBLASTS OF CONNECTIVE TUMOR TISSUE IN ECM REMODELING

Recently, comparisons of tumors with the wounds that never heal have become widely accepted [13], as wound and tumor stroma have many common features, including activation of fibroblasts, increased production of ECM proteins, and intensive remodeling processes [14]. It is known that fibroblasts are the main type of cells in both normal and tumor stroma of all tumors. Recently, researchers have focused on two important features of fibroblasts in tumors: their acquisition of the smooth muscle cell phenotype, i.e. differentiation into MFs, and the acquisition of embryonic features of differentiation and functioning (so-called tumor-associated fibroblasts (TAF)). There has long been a debate about the differences between MF and TAF. It was found that both cell types are activated fibroblasts, morphologically similar to each other, but with certain differences, and with significantly different functional characteristics [15–18].

In depicting the portrait of MF, it should be noted that these cells have differences in the following criteria: altered membrane and highly active endoplasmic reticulum, expression of smooth muscle α-actin (α-SMA), increased levels of vimentin, the formation of complex organized stress fibers and complexes called fibronexus, which combines contractile microfilaments and fibronectin (FN), matrix expression protein [18]. Expression of α-SMA is most often used as a marker of MF. In addition, FN, periostin, and proline 4-hydroxylase have also been identified as potential markers of these cells [19]. There is a report on a difference between MF and TAF in their transcription profiles [20].

MFs were first identified in the granulation tissue by Gabbiani [21] who gave them this name, and these cells were characterized as modified fibroblasts, in which the bundles of microfilaments with dense bodies located between them and connective tissue cracks could be determined.

To date, many researchers have proven that in case of tissue damage as well as malignant transformation of the epithelium, a complex process of activation of fibroblasts into MF occurs, due to which the latter acquire a migratory phenotype, and inhabit the tumor tissue. Such transformations are a response to changes in the composition and organization of ECM and to cytokines that are locally released from inflammatory and tumor cells [22]. The second stimulus of such a phenotypic transition is a change in the mechanical microenvironment [23]. It was found that the so-called “crosslinked” ECM, which usually protects fibroblasts of intact tissues, loses its structure due to constant reconstruction of ECM during damage and oncogenesis, which contributes to the formation of transmembrane complex ECM protein — FN in activated fibroblasts, i.e. MF. Architectonically altered cells are considered as an intermediate stage of fibroblast differentiation into MF, so-called protomyofibroblasts. Such cells acquire smooth muscle function through the formation of neoexpression of α-SMA and already have the status of mature MF. Cytokines, including platelet-derived growth factor, interleukin-4, insulin-like growth factor-2, and transforming growth factor (TGF)-β, have been reported to be involved in fibroblast transdifferentiation in MF. TGF-β plays a primary role in this process [24]. In addition, it is argued that fibroblast differentiation into MF occurs in the invasive tumor front with the participation of the ECM component of the glycoprotein tenascin C, and this can be used as a new target and marker for the identification of MF [25].

The response of connective tissue to the development of epithelial tumor manifests itself in fibrosis with increased tissue stiffness, which is associated with dense production and deposition of extratumor matrix molecules, including collagen type I, and occurs with involvement of MF, the number of which increases significantly [26]. The conditions thus created in the tumor environment together with the mechanical stress toward connective tissue promote migration, invasion of malignantly transformed cells and subsequent metastasis, and therefore play a key role in tumor progression [27].

In addition, it has been shown that adhesion molecules, such as intercellular adhesion molecules, vascular cell adhesion molecules and nerve cell adhesion molecules, are expressed upon MF activation. Due to this, lymphocytes, mast cells, neutrophils can come into contact with MF and in this interaction to participate in immunological reactions and inflammation and affect the peculiarities of the tumor process. Depending on the type of tissue to be remodeled, the source of MF progenitors may be different. The main progenitors of MF are intact fibroblasts, others include mesenchymal cells such as pericytes and vascular smooth muscle cells, bone marrow fibrocytes, and mesenchymal stem cells. The role of epithelial-endothelial-mesenchymal transition due to which differentiated or malignantly transformed epithelial cells, as well as endothelial cells, undergo phenotypic transformation and are alternative sources of MF, is considered [28, 29].

This indicates that MF represent a heterogeneous population of cells, and this should affect the course of cancer, especially since these cells themselves express numerous growth factors and inflammatory chemokines, which are involved in remodeling of the tumor stroma, regulation of motility of malignantly transformed cells and induction of chemotherapy-resistant cell subpopulations. In addition, it is reported that the increased number of MF is associated with poor overall survival of patients and is a reason to predict unsatisfactory 3- and 5-year recurrence-free survival. These data are based on a meta-analysis of published clinical trials of 2,606 patients with solid

tumors in which MF were identified using α-SMA staining [30]. Some studies have confirmed that an increase in stromal MF is a poor prognostic factor in patients with solid tumors [31, 32], and it has been shown that an increased MF count is directly related to the aggressive biological behavior of tumors and increased susceptibility to recurrence [33, 34]. However, not all publications confirm these conclusions [35, 36]. Given the ambiguity of the literature on the use of quantitative characteristics of MF in primary cancer patients as markers of the tumor process, in the future it is advisable to use molecular factors involved in the acquisition of phenotype of mature MF, to find ways to counteract the aggressive behavior of these cells and develop anticancer therapy.

Thus, MF represents a unique subpopulation of fibroblasts, which is phenotypically intermediate between smooth muscle cells and fibroblasts [37], and plays an important role in tumor progression. At the same time, the inconsistency of the data on the prognostic value of these cells as clinical criteria requires further research on large cohorts of cancer patients.

TAFs represent another type of activated fibroblasts. These cells are also activated during malignant growth and play a key role in the development of local tumor progression and metastasis. TAFs differ from normal fibroblasts of tumor stroma by increased collagen formation, expression of ECM proteins, mostly tumor factors, vimentin, desmin, fibroblast activation protein [38–40]. TAFs also secrete MMPs, which significantly affect neovascularization due to the release of VEGF from the degraded matrix [41, 42]. In addition, neoplastic cells have been shown to be able to recruit fibroblasts through a variety of growth factors and cytokines [43, 44]. Many researchers note that TAFs, on the one hand, are extremely influential cells in such important aspects of tumor development as tumor growth, progression, metastasis and response to therapy because they interact with cellular and matrix components of the microenvironment, such as endothelial, immune cells, collagen, FN, elastin. On the other hand, TAFs are recipients of chemical and physical signals generated by tumor microenvironment, and due to such interactions, TAF phenotype is constantly evolving along with the tumor progression [45].

According to the literature, there are several ways of TAF formation, in particular, activation of resident fibroblasts or other progenitor cells. Such cells may include mesenchymal stem cells, bone marrow derivatives, epithelial cells, pericytes, smooth muscle cells, adipocytes, fibrocytes, carcinoma cells, as well as some specialized cells such as stellate cells of pancreas and liver, myoepithelial cells of mammary gland, and pericryptal cells of the gastrointestinal tract. This spectrum of TAF progenitors partly explains the heterogeneity of these cells, which has recently been pointed out by many researchers. In addition, the heterogeneity may be due to the fact that the activation of TAF occurs in various ways, including those of malignantly transformed cells — TGF-β1, platelet-derived growth factor α, platelet-derived growth factor β, as well as hypoxia, oxidative stress, and matrix stiffness. All of them can interact and form different phenotypes of TAF. Now some studies have shown that the heterogeneity of TAF may be due to the existence of TAF subtypes differing by protein expression, paracrine signaling, tumorigenicity, invasion profile, the ability to modify the ECM [38, 41, 46–48].

Recently, a number of publications focused on the existence of two subtypes of TAF, one of which has protumoral properties, while the other — antitumoral ones. This is associated with a complex interaction between TAF, the biological characteristics of tumor and immunocompetent cells, which largely determines the response of the tumor to therapy or the further tumor progression and metastasis. In particular, the expression of oncogenes c-MYC, c-Fos and p62 protein in activated fibroblasts and macrophages of patients with breast cancer (BC) has been shown to correlate with a more favorable prognosis [49]. In general, the currently established ways of TAF generation, their functional heterogeneity and phenotypic plasticity, on the one hand, can promote migration and invasion of tumor cells and metastasis, on the other hand, lead to a positive response to therapy [50, 51].

TAF has been reported to be involved in tumor invasion and metastasis by induction of epithelial-mesenchymal transition and secretion of TGFβ and hepatocyte growth factor. Tumor cells that undergo epithelial-mesenchymal transition are characterized by enhanced migratory and invasive properties, while losing adhesive ones [6, 50]. A specific marker of TAF has been shown to be a fibroblast activation protein, which is a cell surface serine protease type II and exerts both dipeptidyl peptidase and endopeptidase activities with the ability to cleave gelatin and type I collagen [52]. The important role of TAF in the processes of malignant transformation of epithelium, tumor growth and metastasis has been proven by experimental studies *in vitro* and *in vivo* in their cocultivation with cells of different histogenesis or their administration to animals together with TAF [41, 53–55].

Recently, many studies have shown that TAFs make a major impact to the process of fibrotic changes in the stromal component of tumor, i.e. its desmoplasia and ECM remodeling, which are considered the most favorable factors of tumor progression [43, 56, 57]. The development of desmoplasia occurs in several stages: cross-linking of collagen, elongation of fibers, and their restructuring, which is associated with reduced survival of cancer patients [58, 59]. In this case, TAFs secrete an increased amount of metalloproteinases (MMPs) and lysyl oxidase proteins, which catalyze these stages. MMPs expressed by these cells play a key role in neovascularization due to the release of VEGF from the degraded matrix [41, 42]. An important step in desmoplasia is the increased expression of collagen derived from stroma fibroblasts [60]. Collagen accumulation is accompanied by increased

crosslinking and density of connective tissue, as well as increased intercellular fluid pressure. This effect reduces the income of drugs during chemotherapy and immunotherapy. It also promotes an invasion of tumor cells. Desmoplasia and high levels of TAF have been reported to correlate with poor prognosis and low survival in patients with many cancer types, including BC [61].

Thus, the available information in the literature on the highlighted aspect of research has shown that the main feature of cancer is changes in the microarchitectonics of tumor tissue, in particular the tumor microenvironment, where a key role is played by activated fibroblasts, namely MF and TAF, which results in increased invasive and metastatic properties of tumor cells, poor prognosis and cancer resistance to chemotherapy.

INFLUENCE OF ECM COMPONENTS ON MIGRATORY, INVASIVE AND METASTATIC PROPERTIES

According to the numerous literature data, tumor progression is impossible without ECM degradation, which occurs due to the action of the MMPs accelerating the invasion and migration of malignantly transformed cells from the primary lesion. MMPs are proteolytic enzymes of the secretory or membrane type that act on the protein components of the ECM, such as collagen, gelatin, elastin, laminin, FN and integrins. In addition, they can affect the function of endothelial cells, namely their migration, proliferation, Ca^{2+} signaling, and contraction. MMPs are synthesized as zymogens and are activated into functional forms by autoproteolysis or with the involvement of other proteases [62].

These endoproteases belong to the family of a constantly growing group of zinc and calcium-dependent endopeptidases, which accounts 20 enzymes, and in contrast to other proteolytic enzymes, including cathepsins, serine proteases are able to completely decompose all ECM structures of the tumor due to specific hydrolysis of basic proteins. Their role is to destroy the collagen of the basement membrane and degrade the ECM, and according to clinical observations, their activity always correlates with the invasion of tumor cells and metastasis [63, 64].

Today there are two systems of MMP classification. The first, the best known, is based on substrate specificity and divides MMPs into 6 main groups: collagenase, gelatinase, stromelysin, matrilysin, membrane type MMP, metalloelastase, emalysin, and others [62]. A more recent new classification of MMPs is based on the genomic studies of their domain organization, which showed that there are 24 different genes encoding members of the MMP family [65–75].

MMP are produced by various cells (epithelial cells, fibroblasts, macrophages, neutrophils, smooth muscle cells of vascular wall) [76]. Under the influence of inflammatory cytokines, the synthesis of MMPs increases. The relationship between the production of these enzymes and the presence of TAF in tumors has been established. The lysyl oxidase family and MMPs are considered to be the two main types of remodeling enzymes synthesized by TAF. These cells control and tune the TAF-ECM interaction.

MMPs play a role in tissue reconstruction during various physiological processes, such as angiogenesis, morphogenesis, wound healing, as well as various pathological processes, in particular malignant growth. MMP activity can be controlled by tissue endogenous MMP inhibitors and transcriptionally regulated by miRNAs. MMP/tissue endogenous MMP ratios are often used to determine the degree of ECM protein degradation and tissue reconstruction and have been proposed as biomarkers for cancer diagnostics [76–79]. Since tissue remodeling is a dynamic process, the spatial distribution of different MMPs in the tissue may vary. Due to the differences in the proteolytic activity of MMPs relative to different substrates, their activity may change during the course of the disease. This makes it important to determine different MMPs and tissue endogenous MMP at all stages of the disease [76].

The most studied MMPs involved in oncogenesis are MMP-2 and MMP-9, which are classified as gelatinases and play an important role in the destruction of ECM, thus promoting migration, invasion and metastasis of tumor cells [80–83]. If the function of these MMPs is well defined in tumors of various genesis, including BC, the role of MMP-8, which is expressed in polymorphonuclear neutrophils and is also known as neutrophilic collagenase or collagenase-2 is poorly studied yet. Moreover, recently the impact of MMPs is considered not only from the standpoint of promoting the generalization of the tumor process due to their destructive effect, but also in terms of positive effects on the survival of cancer patients. However, the mechanisms of such opposite effects are not fully understood, and experimental studies in this area are scarce.

In vitro experiments on various BC cultures, including primary normal cells and ductal carcinoma-associated myoepithelial cells *in situ* (DCIS), as well as normal (N-1089) and DCIS modified myoepithelial (β6-1089) cell lines, have studied the effect of MMP-8 on the adhesion and migration of cells into ECM. It has been established that normal myoepithelial cells of the mammary ducts play the role of tumor suppressor and express MMP-8, while during the development of cancer (namely ductal carcinoma *in situ*), they acquire the function of a promoter and lose this enzyme. As a result of experimental simulation of high expression of MMP-8 and its knockdown using 2D and 3D analysis of tumor cell invasion, it was shown that MMP-8 can exert different effects on cell adhesion and TGF-beta signaling and gelatinolytic activity of MMP-9. Increased expression of MMP-8 in β6-1089 cells increased their adhesion to ECM proteins and decreased cell migration. At the same time, the MMP-8 knockdown in N-1089 cell line reduced adhe-

sion and enhanced the migration of tumor cells into the environment. High MMP-8 expression reduced TGF-β signaling and gelatinolytic activity, whereas MMP-8 knockdown enhanced these processes. Thus, MMP-8 has been shown to be an important component of myoepithelial tumor suppressor function, which in addition to the destructive effect is able to restore the interaction of myoepithelial cells with the environment, counteract TGF-β signaling and proteolysis of MMP-9, which inhibits tumor invasion [84]. This study design allows determining the risk of progression of ductal BC *in situ* by assessing the expression of MMP-8.

There are few genetic studies of MMP-8. In particular, it was found that melanomas are characterized by somatic MMP-8 mutations, which reduce the activity of MMP-8 and lead to increased colony formation and cell migration *in vitro*, and the formation of metastases *in vivo*. Analysis of microchips of epigenetic regulation of MMPs in BC and glioma cells revealed epigenetic inactivation of MMP-8 in contrast to other MMPs, which explains a decrease of MMP-8 activity in various malignant neoplasms not associated with genetic changes [85].

The study of antitumor molecular mechanisms of MMP-8 action in *in vitro* and *in vivo* experiments showed that collagenase-2 triggers oncosuppressive molecular cascades after cleavage of various non-extracellular substrates. In particular, in lung adenocarcinoma cells treated with hepatocyte growth factors inhibition of proliferation, reduced invasion, and increased active MMPs were observed [86]. At the same time, MMP-8 mRNA expression was increased along with other MMPs in chemotherapy-resistant aggressive lung cancer cell lines [87]. The lack of MMP expression in prostate cancer cells has been shown to enhance β1-integrin ligand binding and increased invasion of prostate cancer cells *in vitro*, as well as increased lung extravasation in BC *in vivo* [88].

Three independent experimental studies have shown that MMP-8 reduces intercellular fluid pressure in tumors, increases fluid flow in various mouse tumors, including lung cancer, soft tissue sarcoma, and increases the effectiveness of chemotherapy with liposomal forms of drugs [89–91]. On the other hand, some clinical studies suggest that certain treatments may increase MMP levels, in particular MMP-8, and promote resistance to chemotherapy, in particular, resistance to sunitinib in patients with renal cancer [92]. There are interesting results of *in vivo* studies conducted on the model of BC in mice, which proved the possibility of visualizing different levels of MMP, including MMP-8 by tomography. It seems reasonable to suggest this phenomenon as a diagnostic tool for assessing tumor progression [93].

In terms of the above bidirectional action of MMP, namely the ability to destroy and alter the function of various bioactive molecules, which leads to stimulation of tumor growth, and the opposite effect — inhibition of invasion and proliferation of tumor cells by cleavage of non-structural substrate (non-matrix bioactive molecules), an analytical study of 171 publications was conducted to determine the levels of MMP-8 in tumors of different genesis and the possibility of using the data in clinical practice as prognostic factors or treatment targets. As it has been shown, in BC, skin cancer, cancer of oral cavity, elevated levels of MMP-8 inhibit the invasion and proliferation of tumor cells, thereby preventing metastasis by cleavage of non-structural substrates of the microenvironment. In contrast, high levels of MMP-8 in patients with hepatic and gastric cancer worsen the prognosis. Thus, many researchers have shown that MMP-8 levels are differently associated with invasive and metastatic properties of tumors depending on their histogenesis and therefore have a prognostic potential [94].

Recently, the attempts have been made to elucidate the mechanisms underlying protective role of MMP-8 in tumor progression and metastasis. Using BC cells with different metastatic activity (highly metastatic MDA-MB-435 cells and the cells with lower metastatic potential — MDA-MB-468 and MDA-MB-231), it has been shown that MMP-8 is expressed in all these cell lines, but the expression of MMP-8 in MDA-MB-231 cells causes a decrease in the level of miR-21, which regulates a large number of target genes involved in carcinogenesis. This in turn leads to a decrease of tumor growth and the formation of lung metastases demonstrated in *in vivo* experiments. The mechanism of the protective role of MMP-8 in tumor progression and metastasis of MDA-MB-231 BC cells has been associated with decorin cleavage and subsequent reduction of TGF-β signaling that control miR-21 levels. In addition, it is noted that inhibition of miR-21 induced by MMP-8 increases the level of tumor suppressors and promotes programmed cell death, which may also contribute to the suppression of tumor progression and metastasis of BC cells that express this MMP [95]. Therefore, the data obtained in the experiments can be taken into account when developing a personalized approach to the treatment of patients with BC.

It is known that one of the most important components of ECM of the tumor, which is a target for the destructive effect of MMP, is collagen. On the other hand, collagen plays a primary role in the processes of fibrosis, which are now known as desmoplasia and are considered the driving force of invasion and migration of tumor cells outside the primary lesion. It has been established that collagen biosynthesis can be regulated by malignantly transformed cells via mutations in genes, transcription factors, signaling pathways and receptors. Collagen has also been shown to affect the properties of tumor cells through inhibitors, tyrosine kinase receptors, and some signaling pathways. Experimental studies suggest that hypoxia, prevalent under conditions of increased collagen content, enhances tumor progression, and ECM molecules such as FN, hyaluronic acid, laminin and MMP, when interacting with collagen, affect the invasive properties of tumor cells that ultimately relates

to prognosis, recurrence, metastasis and resistance to chemo-radiation treatment [96].

It is believed that one of the factors in the tumor microenvironment, which regulates proliferation, migration, invasion and survival of cells is collagen type I [97]. Recently, the relationship between the expression of collagen type I alpha 1 in tumor cells and the clinical and pathological characteristics and survival of patients has been shown using the clinical material of patients with BC. Elevated collagen type I alpha 1 levels have been shown to be associated with poor survival, especially in women with ER^+ tumors. At the same time, in the case of high levels of this form of collagen, a better response to cisplatin-based chemotherapy was observed. According to the authors, collagen type I alpha 1 may serve as a new prognostic biomarker and a potential therapeutic target in patients with BC, especially ER^+ tumors [98].

Many studies have shown that the leading role in oncogenesis belongs to fibrillar collagen types I and II, which belong to stromal collagen and are produced by TAF [99–101]. Collagen and hyaluronan have also been found to be the most common components of the extracellular matrix, and their increased expression in tumors is associated with tumor progression and metastasis [102]. It has been shown that type I collagen, which is the main component of fibrous ECM, in patients with invasive ductal BC undergoes a complex chain of changes that accompany tumor progression and is associated with changes of collagen composition and reorganization with collagen fiber alignment. Correlation analysis between such alignment and a large set of proteins showed a different direction of the relationships between them. Among these proteins, candidate proteins have been identified to study the structural and cellular effects on the alignment of collagen, in particular tenascin-C and thrombospondin-2 [103]. It is also noted that the large expression of type I collagen contributes to the rigidity of tumor tissue, increases the mechanical stress that promotes the proliferation of malignantly transformed cells, their metastasis due to the activation of the TGF-β signaling pathway. Attention is drawn to the fact that the increased mechanical stress causes compression of blood vessels and leads to hypoperfusion with impaired flow of drugs to the tumor tissue [104].

Other studies have shown that the high density of collagen in the ECM, which is strongly aligned and leads to a strictly directed, so-called contact migration of tumor cells from the focus of malignant growth, can be reconstructed due to traction force controlled by myosin contractility or MM proteolytic activity resulting in the increased or decreased contact migration of cells [105]. The data based on the results of morphometric, immunohistochemical study and application of RGB modeling have shown that under oncogenesis of invasive BC of non-specific type, in contrast to fibroadenoma, there are changes in physicochemical properties of collagen fibers of tumor stroma [106]. According to the researchers, this may be due to the synthesis of TAF type II collagen, which is not characteristic for loose connective tissue of the breast, however its secretion by connective tissue cells has been found along with that of type I collagen. It has been suggested that in such cells the genes characteristic of cartilage tissue are expressed, i.e. the cells acquire osteoblastic phenotype features. Such findings seem to be related to the known fact of frequent metastasis of BC to bone tissue.

Also interesting were the features of collagen fibrous structures, identified by the morphological examination of the tumor stroma of patients with gastric cancer and stained with picrofuxin, in comparison with such connective tissue of patients not affected by cancer. The data obtained showed that patients with gastric cancer have more immature components of collagen, which indicate its qualitative changes. Weak, moderate and significant changes in collagen fibers were noted. It turned out that with weak and moderate changes, they have the appearance of normal collagen fibers, except for such features as alignment and density. In the case of strong changes, the fibrous structures were of significant density, thickness and less intense staining. In addition, a large amount of MF and increased expression of mesyloxidase, an enzyme that mediates the "crosslinking" of collagen molecules, which may contribute to increased collagen deposition and denser "crosslinking", has been revealed. Using the high-tech technique of collagen fiber visualization 5 parameters of its architecture, in particular alignment, density, width, length, and straightness were found to be increased in the tumor microenvironment. When comparing these indices with patient's survival, it was determined that of all 5 characteristics, the width is the most significant parameter in the prognosis, as confirmed by two independent cohort studies in groups of patients with gastric cancer involving 225 and 151 patients [107]. In addition, the researchers found that overall 1-year survival and increased collagen width were inversely related. It is supposed that the prognostic value of collagen width is much better than conventional clinical and pathological indicators.

With the prospect of implementation in clinical practice, studies of the concentration of serum collagen type I (aminoterminal propeptide) and markers of degradation (carboxyterminal telopeptide) in patients with BC of different molecular subtypes are being conducted. It was found that before and after surgery, the concentration of carboxyterminal telopeptide increased linearly from ductal cancer *in situ* to stages I–III and the disease characterized by metastatic spread of tumor cells. High preoperative levels of carboxyterminal telopeptide were associated with better survival of patients with luminal BC subtype, and in patients with triple negative BC high levels of the index were recognized as an objective predictor of recurrence-free survival, as evidenced by both single-factor and multifactorial analysis [108].

Finally, drawing parallels between the biological effects on the tissue components of the microenvironment in the developed tumor — MMP-8 as a protease that have destructive power, and collagen as the substrate to which this action is directed — the bilateral nature of their manifestation can be traced. For the most part, the progression of malignant growth is determined by increasing the proliferation, invasion and migration of tumor cells, as well as inhibition of these processes. This ambiguous development in the microenvironment of the tumor can be explained by the existence of two domains in each MMP, one of which has a suppressive effect, and the second promotes tumor progression [94].

PROSPECTS FOR FURTHER RESEARCH

Analysis of the literature allowed us to understand how complex are all the components of the microenvironment by signaling, metabolic-immunogenic relations, and as a consequence heterogeneous and variable in the dynamics of the tumor process, and why it is impossible to make a stable idea of the true role of each of them as prognostic factors without comprehensive assessment using modern high-tech methods of visualization and machine learning, which are part of the methodology of artificial intelligence. It is not surprising, therefore, that more and more publications have recently appeared with the data on the development and testing of cancer-on-chip platforms designed for further integration with artificial intelligence. Undoubtedly, this methodology will play a crucial role in the near future in all areas of medicine. In particular, research in 3D cell culture, tissue engineering, and microfluidics has led to the development of on-chip cancer platforms that enhance *in vitro* tumor environment modeling for drug development with future integration with artificial intelligence to improve prognostic models for anticancer screening. At the same time, on-chip cancer platforms are being created that simulate the microenvironment *in vivo* and, when integrated with artificial intelligence, help to expand understanding of cancer pathophysiology, optimize diagnosis, personalize treatment, and improve prognostic models for drug screening [109]. It is noted that the integration of artificial intelligence and nanotechnology, in terms of the well-known fact of tumor heterogeneity, can overcome the difficulties of diagnostic, prognostic and therapeutic accuracy due to algorithms for analysis and classification of certain criteria for objective assessment of metastatic potential of tumor cells from the primary lesion [110].

In terms of the significant influence of biology of stroma and collagen on oncogenesis and metastasis, the qualitative characteristics of collagen structures, in particular aggregated thick (dense) collagen and dispersed thin one in patients with triple negative BC in comparison with invasive intraductal BC and benign neoplasms are being studied. Finally, new approaches to image processing and quantification of profiling of collagen structures using numerical imaging parameters in integration with artificial intelligence have contributed to the development of a prognostic model and the assertion that only scanning of histological specimens and not the results of discrete numbers obtained manually with a certain subjectivity in the assessment, is the future of pathology with the prospect of transition from basal staining with hematoxylin and eosin to immunohistochemical and iminofluorescent staining [111, 112]. The importance of using modern research methods such as multiplex imaging, digital pathology, flow cytometry, and microscopy in combination with artifical intelligence, provides a powerful basis for fundamental and translational cancer research [113, 114].

As a result of the application of artificial intelligence approaches, in particular spectroscopy and machine learning, in determining changes in such biochemical components of ECM as collagen, lipids, nucleic acids in different molecular subtypes of BC identified during the studies, there were obtained informative data sets which accurately reflect the molecular subtypes of tumors. This allowed us to make assumptions about the possibility of creating a methodology for accurate diagnosis and monitoring of cancer in real time [115].

The data showed that on the basis of such characteristics of collagen fibers as shape, size and structure of the image in the tissues of atherosclerotic arteries they can be divided into 5 groups using the methods of multiphoton microscopy and machine learning [116], which many researchers regard as the most effective approach for analyzing the spatial structures of collagen.

The artificial intelligence model was used to determine the possibility of predicting the overall survival of patients with diffuse large B-cell lymphoma by the proximity of the location of collagen VI in relation to tumor cells. It has been shown that the significant proximity of collagen to malignantly transformed cells is associated with better survival of patients with this pathology [117]. Other researchers have shown that the use of spatial interference microscopy to screen for colorectal cancer, followed by the use of artificial intelligence, makes it possible to create a powerful combination of data for screening, as well as paving the way for internal objective markers that do not depend on training and bias. In addition, it was found that due to the selective sensitivity to collagen fibers, this method allows to obtain information of prognostic value with an accuracy of detection of benign and malignant tumors of 97% [118].

Due to the use of a wide range of parameters to identify changes in puncture biopsies of the mammary gland based on histological evaluation of various structural components and pathological processes using machine learning based on neural networks, a high level of differential diagnosis of atypical ductal breast hyperplasia was demonstrated. It is hoped that such an approach can be used to separate the patients who require the surgery from those assigned only to active observation [119].

Therefore, today, in the era of evidence-based medicine, extensive mastery and improvement of artificial intelligence methodology based on convincing comprehensive information about the connective tissue component and microenvironment using modern methods of image visualization create a real perspective of personalized prognosis and choice of treatment tactics for cancer patients.

CONCLUSION

The analysis of the modern literature on the state and role of the components of tumor microenvironment showed a key role of a process of remodeling of ECM microarchitectonics. The main players that significantly affect the remodeling of tumor ECM are activated fibroblasts, namely MF and TAF, because they, on the one hand, intensify the expression of a number of MMPs, on the other — produce collagen, including type I collagen, a large content of which leads to desmoplasia. Finally, such a complex of interactions changes the physicochemical properties, qualitative and quantitative characteristics in the non-tumor matrix and initiates accelerated proliferation, invasion and migration of tumor cells with subsequent metastasis.

In addition, *in vitro* and *in vivo* experiments, and the studies conducted on clinical material, have shown that MMP-8 and type I collagen under certain conditions exhibit not only protumoral but also antitumor effects, and some of the underlying mechanisms have been established. Through clinical observations, a number of biomarkers have been identified that are considered potential targets for the development of new markers for the prognosis and treatment of cancer patients.

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