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Extracellular matrix as a cellular information microenvironment

Naumova L.A., Starodumova V.A.

Surgut State University
1, Lenina Str., Surgut, Khanty-Mansiysk Autonomous Area – Yugra, 628412, Russian Federation

ABSTRACT

The article discusses modern ideas about the role of extracellular matrix (ECM) and cellular elements of connective tissue (CT) in tissue homeostasis in normal and pathological conditions. The works of recent years reflect a shift of interests concerning the study of many pathological processes, particularly carcinogenesis, to the state of the ECM and CT cells, which are considered as active components of the tissue that determine the processes of cellular proliferation, differentiation, migration, and apoptosis. The most important properties of the ECM attracting the attention of researchers include mechanotransduction, leading to the activation of cytoskeletal mechanisms and various cell signaling pathways; modeling of the effects of various cytokines, growth factors, and hormones; maintenance of the stem cell niches; influence on the emergence and course of the tumor process, in particular, formation of a cancerized field and premetastatic niches; and the epithelial-mesenchymal transition (EMT). Currently, CT cells are also an important object of study, in particular, fibroblasts, which are the main producers of ECM components. The attention of researchers is directed primarily to cancer-associated fibroblasts, the phenotype of which forms in the tissue long before the tumor appears. New knowledge about the role of ECM and CT cells in tissue homeostasis determines new approaches to treatment of many diseases, such as systemic sclerosis, tumors, etc.

Key words: extracellular matrix, epithelial-stromal relations and carcinogenesis, fibroblast, field cancerization, premetastatic niches.

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Экстрацеллюлярный матрикс как информационная клеточная микросреда

Наумова Л.А., Стародумова В.А.

Сургутский государственный университет (СурГУ)
Россия, 628412, Ханты-Мансийский автономный округ – Югра, г. Сургут, ул. Ленина, 1

РЕЗЮМЕ

Рассматриваются современные представления о роли экстрацеллюлярного матрикса (ЭЦМ) и клеточных элементов соединительной ткани (СТ) в тканевом гомеостазе в норме и патологии. Работы последних лет отражают смещение интересов при исследовании многих патологических процессов, в частности опухолевого роста, в область состояния ЭЦМ и клеток СТ, которые рассматриваются как активные компоненты ткани, определяющие процессы пролиферации, дифференцировки клеток, миграции и апоптоза. К важнейшим свойствам ЭЦМ, привлекающим внимание исследователей, относится механотрансдукция, ведущая к активации цитоскелетных механизмов и различных сигнальных клеточных путей; моделирование эффектов

✉ *Naumova Lyudmila A.,* e-mail: naumovala@yandex.ru.

цитокинов, факторов роста и гормонов; поддержание ниш стволовых клеток; влияние на возникновение и течение опухолевого процесса, в частности формирование опухолевого поля и преметастатических ниш, а также эпителио-мезенхимальный переход. Важным объектом исследования в настоящее время являются и клетки СТ, в частности фибробласты – основные продуценты компонентов ЭЦМ. Внимание исследователей привлекают, прежде всего, опухоль-ассоциированные фибробласты, фенотип которых формируется в ткани задолго до появления опухоли. Расширение представлений о роли ЭЦМ и клеточных элементов СТ в тканевом гомеостазе определяет новые подходы к лечению многих заболеваний – органных склерозов, опухолей и других.

Ключевые слова: экстрацеллюлярный матрикс, эпителио-стромальные отношения и канцерогенез, фибробласт, опухолевое поле, преметастатические ниши.

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INTRODUCTION

In recent years, ideas about extracellular matrix (ECM), which was previously considered mainly as a physical scaffold for cells and tissues, have changed significantly. Numerous studies confirm that the ECM is a physiologically active component of living cells and tissues that is responsible for the most important processes in them. The ECM (fibrous structures and the ground substance) along with various cellular elements (fibroblasts, macrophages, lymphocytes, mast cells, endothelium of microvessels) belongs to the components of connective tissue (CT) that functions under normal and pathological conditions on the basis of the feedback and cooperative interaction of its cells with one another, ECM, blood cells, and organ parenchyma (or epithelium of the mucous membranes) [1–3].

Due to communication between cellular elements and their microenvironment, which evolves during the development of tissues, a unique molecular composition of the ECM is formed, which has a powerful effect on the biochemical and biophysical processes in cells through adhesive contacts between cells and ECM proteins and determines the epithelial-stromal interactions [4].

The most important properties of the ECM that are attracting the attention of researchers at present include the mechanotransduction, which leads to the activation of cytoskeletal mechanisms and various cell signaling pathways; modeling of the effects of various signaling molecules, which makes it possible to consider the CT system as the most important information environment of the body [2]; maintenance of stem cell niches; influence on the emergence and course of the tumor process, in particular, formation of a cancerized field and premetastatic niches; and the epithelial-mesenchymal transition (EMT). New knowledge about the role of the ECM and cell elements of CT in maintaining tissue homeostasis is essential for understanding the treatment strategy in many diseases, including the tumor process.

EXTRACELLULAR MATRIX AS A PHYSIOLOGICALLY ACTIVE COMPONENT OF THE LIVING TISSUE

ECM is a complex which has a three-dimensional composition represented by fibrous structures, or different types of collagens (28 types are currently known), and various proteins of the ground substance (glycoproteins and proteoglycans), which forms a cellular microenvironment determining intercellular and cell-matrix adhesive contacts and is responsible for cell polarity, phenotype, proliferation, migration, and intercellular communication processes [4–6].

ECM components are created and arranged by resident cells in accordance with the characteristics of the tissue. Thus, type I and V collagen predominate in the interstitial stroma and type IV collagen predominates in the basement membranes. The main producers of ECM components are fibroblasts, the most important CT cells [4, 7].

Being a network of proteins to which various signaling molecules can bind, the ECM controls the most

important processes in living cells and tissues through modeling the effects of growth factors (GF), cytokines (CK), and hormones. At the same time, the ECM is a highly dynamic structure; it is constantly remodeled by matrix metalloproteinases (MMPs) with participation of their tissue inhibitors and various GFs [4, 7, 8].

Most post-translational modifications in the ECM are not encoded by DNA but are the result of physiological and pathological processes in the tissues. Factors of post-translational changes in the ECM include changes of MMP activity (during inflammation, fibrosis), nitrosylation and glycosylation of the ECM proteins, as well as cross-linking and isomerization, which significantly change the viscosity, elasticity, and rigidity of the ECM. Post-translational remodeling of the ECM can be both a consequence of the pathological process, and a link in its pathogenesis, for example, in development of fibrosis and cancer. Therefore, the products of degradation of type II collagen, which are formed during rheumatic diseases and enter the systemic circulation, acquire the properties of signaling molecules [4], and endostatin that is produced from degradation of type XVIII collagen becomes the most powerful anti-angiogenic factor [7].

The main functional modifiers of the ECM include proteoglycans, or proteins containing glycosaminoglycans (GAGs), which are covalently linked to them. GAGs are long, negatively charged repeating disaccharide units that are represented by heparin sulfate, chondroitin/dermatan sulfate, hyaluronan, or keratan sulfate. Transmembrane proteoglycans (integrins, syndecans, discoidins), pericellular proteoglycans (perlecan, decorin, etc.), and extracellular proteoglycans (hyalectans and five classes of proteoglycans containing leucine, or small leucine-rich proteoglycans (SLRPs)) are distinguished [4].

Proteoglycans play a major role in transmission of signals from the ECM to the cell and are, in fact, cell receptors for the adhesive molecules of the ECM. They are responsible for the interaction of the cell with the microenvironment, or for intercellular and cell-matrix interactions, participate in tissue hydration and formation of collagen, modulate the effects of GF and CK, and influence cell proliferation, cell adhesion, reparative processes, and tumor growth [4, 7, 8].

Laminin and fibronectin are among the most important glycoproteins of the ECM. Laminin is a three-dimensional glycoprotein consisting of α, β, and γ-chains. It can form up to 60 unique laminins, but only 16 combinations are currently known. Due to integrin binding, laminins are able to create a dynamic connection between the cell and the ECM. Fibronectin as a multi-domain protein provides mechanosensitive interaction between the cell and the ECM and formation of the fibrillar network [4].

Thus, protein molecules of the ECM are considered as paracrine signaling molecules, which, along with GFs, CK, and hormones, have a profound effect on tissue homeostasis.

MECHANICAL PROPERTIES OF THE EXTRACELLULAR MATRIX

A cell cannot exist or undergo proliferation and differentiation without its mechanical environment. The cells assess the stiffness of their microenvironment using lamellopodia and universal transmembrane proteins of the ECM: integrins and syndecans. Integrins, which are the main mechanosensors of the cell, bind to fibronectin, laminin, and collagens of the ECM through various combinations of their heterodimers. On the other hand, they bind to the intracellular actin cytoskeleton through vinculin, talin, and other proteins, mechanically integrating extracellular and intracellular compartments. Recognition of the mechanical properties of the microenvironment by a cell and its reaction to the biophysical properties of the ECM are called mechanotransduction [4, 9].

Conformational changes in the integrin cytoplasmic domains lead to activation of several cell signaling pathways associated with the activity of kinases and phosphatases, in particular, mitogen-activated proteinkinases (MAPs) and guanosine triphosphate phosphatases (Rho GTPase). The association of syndecans with fibronectin leads to synergy of the effects of integrin and syndecan with respect to the activation of signaling cascades through focal adhesion kinase (FAK) and subsequent stabilization of the focal adhesion complex. Tissue cells have diverse receptors for various components of the ECM [4, 6, 10]. Thus, CD34 is a receptor for hyaluronan, 67kDa-laminin is the receptor for laminin, and discoidin domain receptor (DDRs) is the receptor for collagen [4, 5, 11].

The mechanosensitivity of adhesion complexes formed by integrins and syndecans is currently an area of active research, yet much remains to be learned about the pathways providing ECM-mediated cellular responses [4, 7, 9].

To transmit external information, biochemical and biophysical repeaters work together. It is assumed that simultaneous interaction of thousands of intregrin receptors with binding sites in networks of anisotropic ECM allows cells to have a topological description

of the chemical and mechanical properties of the microenvironment, with subsequent conversion of this information into intracellular signals generating appropriate cellular responses, such as position, cell polarity, differentiation, growth, protein synthesis, and regulation of energy processes [9]. One striking example of a mechanosensitive genetic regulator is a pair of transcriptional coactivators: the yes-associated protein (YAP) and the transcriptional coactivator with the PDZ-binding motif (TAZ). The role that YAP/TAZ (which are proto-oncogenic transcriptional regulators) play in cellular processes reflects the importance of mechanotransduction both in normal conditions (regenerative processes) and in pathology, in particular, in the development of fibrosis and cancer [4, 7, 12].

It was found that during cultivation, the differentiation of mesenchymal stem cells (MSCs) into osteocytes, myocytes, or neurons depends on the elasticity of collagen matrices, i.e., mechanotransduction. When blocking myosin II, a key molecule of mechanotransduction signaling pathways, MSCs become insensitive to ECM elasticity and its effect on differentiation processes. The composition of the ECM, or the adhesion gradient (haptotaxis), is a coordinated process between adhesion and anti-adhesion which significantly affects the rate of cell migration. Thus, pronounced migration of fibroblasts is observed during cultivation on fibronectin, and it is absent during cultivation on a mixture of fibronectin/laminin or laminin [4].

In fact, the ECM exhibits a dynamic plan of cell development, which is best reflected by the phenomena of branching, budding and formation of tubular structures during embryogenesis [4].

Therefore, spatial orientation of cells and tissue homeostasis as a whole are determined by mechanotransduction or biophysical signals from the ECM, which are transformed into biochemical signals regulating gene transcription in the cell. Dysregulation of ECM remodeling, in particular, during the formation of fibrosis, significantly changes the fate of cells and leads to development of various diseases, such as disorders of the cardiovascular and musculoskeletal systems and tumor growth [4, 8, 9, 11].

EXTRACELLULAR MATRIX IN THE MAINTENANCE OF STEM CELL NICHES

The most important property of ECM is maintenance of stem cell niches [4, 13]. A niche is formed by an ensemble of stromal cells and components of the ECM produced by them. A niche is characterized primarily by spatial organization and provides intercellular and cell-matrix interactions necessary for implementation of appropriate differentiation of embryonic stem cells, which give rise to various cell lines, or somatic stem cells, which are necessary for tissue repair [14].

Maintaining stem cell niches is crucial for normal functioning of the epithelial tissues which belong to the border tissues of the body and are characterized not only by high intensity of physiological regeneration, but also by high frequency of damage and intensive reparative regeneration [3, 13, 14]. At the same time, in tissues which cells were previously considered post-mitotic, a low level of cell renewal is maintained throughout life due to stem cells niches [14].

A niche is represented by stem cells, their progeny and specific niche ECM. The niche provides integration of various signals received by stem cells (autocrine, paracrine, systemic, and cell-matrix signals) through adhesion and mechanotransduction receptors, which allows to coordinate the responses of stem cells to changing tissue needs. Stromal cells, in particular fibroblasts, play an essential role in maintaining niches of resident stem cells through activation of Wnt, Notch, and BMP signaling pathways [13].

The methodology for preservation of stem cell niches proves that adhesion to the basement membrane alone is insufficient for their maintenance; the proliferative capability of cells is related to their spatial location and the influence of mechanical force, in particular rotational force which ensures the mitotic spindle orientation during the final phase of the cell cycle [15]. The function of many stem cells decreases during life, which may underlie aging of the body [14], as well as formation of various types of pathology. Thus, it was found that the development of habitual miscarriages is largely associated with aging of endometrial stem cell niches [16].

Progress in understanding the principles of stem cell niche existence (identification of niche factors and signaling pathways) can be of great importance for regenerative medicine and tissue engineering [13, 14]. Although some intercellular and cell-matrix interactions in the niches of resident stem cells are well-studied (such as stem cell niches of the skin, intestines, hair follicles, mammary gland, and nerve trunks), many components of stem cell niches are still understudied. [14]. So, the question of cell dedifferentiation or regress of differentiated cells to a less differentiated state within their cell line with loss of stem cell niches in this tissue for implementation of the reparative processes (in particular, in the gastric mucosa) remains relevant [13].

EXTRACELLULAR MATRIX AND EPITHELIAL-MESENCHYMAL TRANSITION

The most striking example of the influence of the physicochemical properties of ECM on the phenotype and behavior of cells is the epithelial-mesenchymal transition (EMT), or the trans-differentiation phenomenon, when epithelial cells acquire a mobile fibroblastoid/ mesenchymal phenotype. The EMT program, along with the endothelial-mesenchymal transition (EndMT), revealed commonality of mechanisms working both in embryogenesis and wound healing, and in carcinogenesis, in particular during the formation of cancer stem cells and at the stage of invasive tumor growth [8, 17–19].

Activation and expression of transcription factors causing EMT occur through various signaling pathways, such as the Wnt-pathway, Sonic Hedgehog (Shh), Notch, and others, which can be triggered by different GFs (transforming growth factor beta (TGFβ), bone morphogenetic protein (BMP), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor 1 (IGF1), hepatocyte growth factor (HGF), platelet growth factor (PDGF)), signals from the ECM, and effects of hypoxia. Cells can integrate certain signals differently or respond to extracellular molecules with different sensitivity, depending on their state and microenvironment [18].

EMT is associated with loss of the apical-basal polarity in epithelial cells, tight intercellular contacts involving adhesive molecules (claudins, occludin, E-cadherin), which ensure the formation of an epithelial layer that is located on the basement membrane normally represented by type IV collagen and laminin, as well as by loss of expression of epithelial markers – cytokeratins. Epithelial cells acquire a fibroblastoid or mesenchymal phenotype due to the loss of intercellular contacts and a change in cell polarity. The ECM composition changes in parallel; fibronectin, N-cadherin, type I and III collagens begin to prevail, and the cells that have already changed their phenotype begin to express mesenchymal markers, such as vimentin, fibroblast-specific protein-1 (FSP-1), and α-smooth muscle actin (α-SMA). The acquired mobile mesenchymal phenotype allows the cells to invade through the basal layer with subsequent migration along the secreted fibronectin matrix [17, 18].

It is possible that EMT and reverse mesenchymal-epithelial transition (MET) exist as binary states of the cell. The flexible nature of the epithelial/mesenchymal state makes this process difficult to observe [18].

During EMT, the amount of type I and III collagen drastically increases in the ECM. Placing epithelial cells on matrices containing these types of collagen can induce EMT through various signaling pathways. The pathways of signal transmission also induce the expression of genes encoding MMP-2 and MMP-9 which cleave type IV collagen in the basal plate, facilitating tumor invasion [8, 18, 19].

As the composition of the ECM changes, the number of integrins on the cell surface increases, which contributes to the progression of EMT. Binding of latent TGFβ to integrins aVb6 and aVb8 induces proteolytic release of latent associated peptide (LAP) and activation of TGFβ. In response to TGFβ signaling, synthesis of type I collagen and fibronectin increases, which makes an additional contribution to EMT. Therefore, local accumulation of TGFβ is associated with the risk of initiating tumor growth through the development of EMT, while a decrease in the expression of TGFβ weakens EMT, invasion and tumor transformation itself.

Type I collagen, in turn, causes phosphorylation of IkB (inhibitor of kB) via integrin-linked kinase (ILK), leading to an increase in the amount of NF-κB localized in the nuclear region (kappa B nuclear transcription factor), which stimulates expression of Snail1 and lymphoid enhancer-binding factor-1 (LEF1) and induces EMT. An increase in the amount of type I collagen also activates the JNK pathway, the pharmacological inhibition of which cancels collagen-mediated migration and metastasis of breast cancer cells. The interaction of integrin b1 subunits with type I collagen in ECM correlates with direct suppression of E-cadherin and induction of N-cadherin [18, 19]. ECM remodeling not only changes the types of matrix proteins that interact with the cell membrane, but also affects the environment of soluble cytokines that promote EMT [18].

EMT precedes the emergence of stem cell properties in tumor cells [17, 20], which represent a small part of tumor cells capable of self-renewing, generating heterogeneity of the tumor cell population, metastasizing [21–23], and generating secondary tumors [24].

THE IMPORTANCE OF DYSREGULATION OF EXTRACELLULAR MATRIX MOLECULES IN THE DEVELOPMENT AND PROGRESSION OF CANCER

Modern ideas about carcinogenesis are shifting towards the crucial role of the ECM in this process, forming the cellular microenvironment and actively

regulating cell proliferation, adhesion, migration, and apoptosis [4, 18, 25–27]. The effect of ECM on cell differentiation has been proven, in particular, by return of the breast tumor cells to a normal phenotype during their culture on a basement membrane created on the basis of 3D substrates coated with antibodies blocking β1-integrin [4].

The main components of ECM interacting with tumor cells include fibronectin, laminin, collagen, proteoglycans, and glycosaminoglycans, in particular hyaluronic acid (HA). HA plays a crucial role in determining the compression properties of most tissues, participates in constant remodeling of ECM during embryogenesis and repair through interaction with cells of the immune system, is involved in tumor angiogenesis, and is an important modulator of the behavior of various cells in the tumor microenvironment [27]. HA is both a signal inducer for EMT and a substrate for cell migration; therefore, a change in the concentration of HA in some tissues (breast, prostate gland) is considered as a predictor of malignancy [4, 8, 27].

The formation of the HA-CD44 complex causes activation of the RhoGTPase signaling, leading to structural changes in actin assembly, cytoskeleton reorganization, transcription activation, growth of tumor cells, their migration and invasion, and disruption of the endothelial barrier. Many exact mechanisms of the effect of HA on immune cells are not known yet, but this interaction can both stimulate tumor angiogenesis and inhibit tumor growth through the induction of active immunity. One of the effects of the HA-CD44 signaling is involvement of β-catenin, the main protein of the Wnt-signaling pathway that controls cell polarity, proliferation, and a number of angiogenesis factors, such as VEGF-A97 and Il-898. The development of anti-angiogenic tumor therapy is based on the study of these mechanisms; moreover, such therapy is more effective in combination with α-interferon and chemical inhibitors of HA synthesis [27].

The most important role in tumor-stroma interaction is attributed to cancer-associated fibroblasts (CAFs), which are a heterogeneous cell population formed from various cells, including resident stromal fibroblasts, endothelial and smooth muscle cells, adipocytes, and vascular pericytes [28–30]. It is possible that the source of CAFs can also be epithelial cells that underwent EMT [30]. An important role in the formation of CAFs is assigned to bone marrow stromal cells recruited into the tumor microenvironment under the effect of chemokines secreted by tumor cells (CCL12, CCL16, etc.) and giving rise to MSCs [28, 29, 31].

Cross-talks between tumor epithelial cells and CAFs are formed by means of exosomes, extracellular vesicles from 30 to 100 nm in diameter, which are released by cells into the intercellular space and transmit information due to diverse biomolecules contained in them, such as proteins, DNA fragments, lipids, various GFs, miRNAs, and proteolytic enzymes [8, 17, 30, 32]. Exosome exchange determines the change in the spectrum of genes expressed by fibroblasts, the VCAM-1 integrin α4 signaling pathway is activated, the level of fibronectin in the ECM is increased, and the expression of VCAM-1 receptors in endotheliocytes is enhanced [17]. CAFs begin to secrete various CKs (TGFβ, CCL-10, CCL-5, etc.) that model the behavior of both immune cells (macrophages, lymphocytes) and tumors [17, 30, 33]. For example, CD9 positive fibroblast exosomes in scirrhous gastric cancer stimulate the migration and invasion of tumor cells associated with increased expression of MMP-2 [32].

Thus, CAFs begin to play an active role in tumor progression and metastasis, causing loss of E-cadherin expression and development of EMT [34]. During angiogenesis, formation of the immune status of the tumor microenvironment, and development of drug resistance, they become the main cells in the tumor stroma and can be important therapeutic targets [29–31, 33].

Moreover, in recent years it has been shown that changes in the stroma and the emergence of a CAFs-like fibroblast phenotype can precede the formation of a tumor, or the appearance of malignant epithelial cells. CAFs-secreted factors increase proliferative activity and mutagenesis in epithelial cells, activate angiogenesis, disrupt intercellular adhesion contacts, and suppress apoptosis, initiating a malignant phenotype in morphologically and genotypically normal epithelial cells [30].

It was found that CAFs-like fibroblasts are characterized by a decrease in the expression of CD36 (glycoprotein expressed on the surface of most cells) associated with high production of collagen and fibronectin [30]. Such CAFs-like fibroblasts were obtained, in particular, from healthy women with increased mammographic density; compared with fibroblasts obtained from women with low breast density, they were characterized by a pronounced ability to form desmoplasia [35]. With precancerous changes in the stroma, a decrease in the expression of CD36 and impairment of their functional state are observed in different stromal cells (adipocytes, fibroblasts, en-

dotheliocytes, immune cells). Thus, suppression of CD36 in endotheliocytes is accompanied by an increase in the angiogenesis; in preadipocytes, suppression of CD36 is associated with impairment of their differentiation into adipocytes; and in immune cells, it is accompanied by an increase in the population of M2 macrophages [30].

Repression of CD36 is associated with epigenetic changes, such as DNA methylation, modification of histones and nucleosome structure, or changes in miRNA expression, leading to a change in gene expression typical of CAFs [30, 36]. The phenotype of CAFs is associated with dysregulation of various signaling pathways, such as TGFβ, BMP, Wnt, Sonic hedgehog (Shh), PDGF, and some others [30].

Immune modulation and the development of inflammation caused by CAFs are considered as one of the mechanisms contributing to the growth and metastasis of the tumor. The immunosuppressive effect of CAFs in the tumor microenvironment is mediated by the activation of glycoprotein Chitinase 3Like1 (Chi3l1) in them, which determines the ability of fibroblasts to control the behavior of the tumor and its pro-inflammatory and immune environment and promotes tumor growth and a shift in the balance of the immune system to type 2 immunity [33].

CAFs can be the main cellular component of the tumor stroma and the main source of connective tissue components in ECM and various classes of proteolytic enzymes. Thus, in breast and pancreatic cancer, CAFs can make up to 80% of the tumor mass, causing pronounced desmoplasia [8, 30].

With an increase in the mass of tumor cells, their tissue microenvironment begins to experience the so-called solid stress, which causes compression of the blood and lymph vessels, deformation of healthy tissues and an increase in its resistance, which, in turn, causes solid stress in the tumor cells, leading to a change in gene expression and an increase in the rate of tumor cell proliferation and migration. The microvascular compression in the tumor area also results in a decrease in the effectiveness of chemotherapy and immunotherapy [4, 8].

Severe desmoplasia in pancreatic cancer is associated with the activation of stellate myofibroblast-like cells accompanied by an increase in the mechanical density of the microenvironment, that reflects the formation of positive feedback. Lysyl oxidase (LOX) secreted by tumor cells is responsible for cross-linking of collagen and elastin and an increase in the ECM rigidity, which leads to activation of integrins and a rise in Rho-generated tension in the cell and its facilitated movement along thickened and straightened collagen.

Inactivation of the ability of the stellate cells to remodel the ECM prevents mechanical release of TGFβ from its latent form. Persistent tumor rigidity induces high expression of TGFβ and EMT. Solid stress enhances hypoxia in the tumor cells, their proliferative activity, and resistance to chemotherapy. Inactivation of mechanosensory and remodeling ability of the stellate cells may be one of the areas in the treatment strategy. As such, all-trans retinoic acid suppresses the mechanosensitivity of stellate cells by decreasing the contractility of actomyosin (MLC-2), which disables the positive feedback between the increased ECM rigidity and stellate cell activation, resulting in reduced fibrosis and invasiveness of tumor cells [4].

The modulating effect on the tumor microenvironment is exerted by various ECM components and stromal cells (macrophages, dendritic cells, various populations of lymphocytes). So, an increase in the number of cancer-associated macrophages in the tumor microenvironment leads to an increase of the synthesis of type I, VI, and XIV collagen by fibroblasts. By modeling the activity of MMPs, it is accompanied by deposition, cross-linking, and straightening of collagen fibers, which facilitates the invasion of tumor cells [37]. Mast cells also affect remodeling of ECM, invasion and metastasis of tumor cells, and angiogenesis via neutral proteases (chymases, tryptases) secreted by them, which change the activity of MMPs, secretion of histamine, heparin, various growth factors (VEGF, FGF, TGFβ), tumor necrosis factor (TNF-α), and individual interleukins (IL-1, IL-6, IL-8) [38].

Therefore, tumor cells change the microenvironment in which they grow, interacting with cells of CT and ECM, and vice versa, which can cause both favorable conditions for tumor growth, invasion, and metastasis, and unfavorable conditions, in particular through activation of the immunity. Findings of recent studies drastically contrast with historically established ideas about the initiation of tumor growth in epithelial cells due to their mutations. In some types of cancer, the stroma goes beyond the role of a mediator in the tumor process. The changes in the signal and transcriptional program of stromal cells can precede (or act independently of) changes in epithelial cells and actually act as a driver of the tumor process [25, 27, 30, 35], that dramatically shifts the focus of the treatment strategy towards returning the original phenotype to the stromal elements. Such therapy can be aimed at removing CAFs by interfering with their

survival or normalizing their phenotype through pro-tumorigenic signal management [20, 30].

EXTRACELLULAR MATRIX AND FIELD CANCERIZATION

The concept of field cancerization formulated by R. Willis back in 1953 and actively developed at the present time is closely related with the system of CT, its cellular composition, the nature of the ECM, the spectrum of signaling molecules, and the activity of MMPs [39–43]. The main provisions of the concept are well confirmed in practice and include non-instantaneity of transformation; mosaic histological picture; tumor growth both due to transformation of cells entering the field zone and tumor proliferation; relapses caused not so much by non-radical treatment, but by maintaining this field or forming a new one with the emergence of a tumor according to the same laws [39, 44].

Zones of dysplasia and formation of blood vessels are considered as the first signs of field cancerization, while the size of the field can occupy both a part of the organ and extend to the entire organ. According to some authors, the field is associated with genetic and epigenetic lesions of the epithelium bordering the tumor growth zone [45–47], while others believe that the formation of the field is associated with the expression patterns of proteases and their inhibitors, inflammatory mediators, and immune signaling molecules. Big importance is attributed to the present macrophage line, accumulation of Treg lymphocytes [40–43], and the metabolic and hormonal status in the peritumoral zone [48].

The theory of field cancerization is constantly revised and updated and generally based on complex interaction between stromal and tumor cells by means of various signaling molecules changing the microenvironment of the tumor (GFs, CK, chemokines). Recently, great importance has been attributed to impairment of the regulatory role of miRNAs involved in posttranscriptional regulation of genes [34, 43]. So, in gastric cancer, an increase in the activity of some miRNAs (hsa-miR-10a, hsa-miR-483; hsa-miR-664a), which regulate cancer suppressor genes APC, RUNX1, PTEN, TP53, etc., is observed, which, in turn, is a consequence of chronic inflammation developing in the peritumoral zone [47].

A manifestation of field cancerization is evolution of somatic cells, as a result of which they acquire individual phenotypic characteristics (focal hyperplasia, metaplasia, dysplasia), which do not yet fully correspond to the tumor phenotype. The basis of this evolution is pro-oncogenic mutations which determine the formation of a mutant clone growing with formation of fields of cells predisposed to progression of changes to tumor cells; the cellular microenvironment plays a crucial role [43, 46]. In this context, precancerous diseases characterized by an increased risk of developing a tumor are an example of field cancerization [43, 46]. This is best illustrated by such precancerous diseases as Barrett's esophagus, prostatic intraepithelial neoplasia, and ductal carcinoma in situ in the breast, reflecting the growth of mutant cell lines evolving along the way to cancer [43].

An indirect indicator of field evolution or a high risk of tumor transformation is genetic or clonal diversity of cells within the field. The existence of field cancerization raises a number of practically important questions. Is medical modification of field cancerization possible, for example, the use of non-steroidal anti-inflammatory drugs that reduce the frequency of mutations in Barrett's esophagus or aspirin and 5-aminosalicylates in inflammatory bowel diseases, to prevent colorectal cancer? In addition, the effectiveness of treatment within the tumor field in precancerous diseases has been proven with respect to squamous cell carcinoma of the skin [46]. Is it necessary to remove the tumor field, which causes the risk of metachronous tumors, simultaneously with the tumor? Besides, the question of methods for visualizing the field (dysplastic lesions) during surgery also arises [43].

EXTRACELLULAR MATRIX IN THE FORMATION OF PREMETASTATIC NICHES

In recent years, ideas about the interaction between the tumor and the ECM during its progression and metastasis have significantly evolved. It has been shown that primary tumors have the ability to induce at a distance such microenvironment that will support the growth of tumor cells that have entered it. This new microenvironment formed away from the primary tumor site is called a premetastatic niche (PMN) [4, 49, 50]. Tumor cells entering the bloodstream from the primary focus die in large numbers, and only a part of them are able to survive and progress, being in the PMN [8, 49].

Tumor-derived secreted factors (TDSFs) and tumor exosomes, as well as stromal, immune cells and bone marrow-derived cells, through complex intercellular communication, determine the development of processes in niches, such as matrix remodeling, angiogenesis, immunosuppression, organotropism, and the

nature of biomarkers expressed by niche cells [4, 49].

It is assumed that remodeling of the ECM (accumulation of fibronectin, cross-linking of collagen using LOX) in the PMN occurs before tumor cells from the primary focus migrate into them, and then tumor cells that enter the vessels accumulate in the niches, that are characterized by higher tissue rigidity (this process is known as durotaxis) [4, 49]. An important role in ECM modification also belongs to prolyl 4-hydroxylase (P4HA), the expression of which increases under the effect of hypoxia and / or TGFβ and which causes deposition of high-stability collagen.

Fibronectin and hyaluronan in the ECM determine directional migration and increase the metabolic activity in the tumor cells. Fibronectin also promotes recruitment of bone marrow monocytes that turn into macrophages expressing VEGFR1. Neutrophils appearing in PMN due to leukotriene signaling (in particular, in lung PMN) also participate in the formation of an immunosuppressive environment by inhibiting CD8 + T-lymphocytes [49].

Many mechanisms of PMN formation are still not clear, in particular, the mechanism of metastatic tropism to certain organs, although recent studies have shown that tumor exosomes have specific integrin expression patterns that determine organotropism. Exosome proteomics revealed that oc6β4 and α6β1 exosomal integrins are closely associated with lung metastasis, and αvβ5 exosomal integrins are closely associated with liver metastasis. Targeted removal of these integrins reduces the absorption of exosomes by resident tissue cells and decreases metastasis to the lungs and liver [4].

It is assumed that cancer treatment can become more effective when it is targeted to various mechanisms of metastasis, in particular to the exosome-mediated mechanism. Exosomes can probably be used as a means of drug delivery to tumor cells, but many functions and mechanisms of exosome exchange have to be studied [4, 49, 50].

CONCLUSION

Thus, the works of recent years reflect a shift in the study of many pathological processes, in particular, tumor growth, to the area of the ECM state and CT cellular elements. The extracellular matrix and CT cells, primarily fibroblasts, are considered as active tissue components that determine cell proliferation, differentiation, migration, and apoptosis, as well as progression and metastasis of tumors, and initiation of tumor growth.

In recent years, the tissue (as well as its constituent elements) has been considered as a integrated structure, which is more than a simple sum of its constituents. The unique properties of the tissue arise from the collective behavior of its constituent components, and the architectural (structural), temporal, and dynamic aspects of tissue existence are integrated through feedback loops, which is clearly reflected in the formation of a cancerized field [30]. This understanding of the tissue, in particular cell-matrix interactions, defines a completely new treatment strategy in many diseases, including cancer.

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Authors information

Naumova Lyudmila A., Dr. Sci. (Med.), Professor, Department of Pathological Physiology and General Pathology, Surgut State University, Khanty-Mansiysk Autonomous Area – Yugra, Russian Federation. ORCID 0000-0003-1145-3710.

Starodumova Valentina A., Post-Graduate Student, Department of Pathological Physiology and General Pathology, Surgut State University, Khanty-Mansiysk Autonomous Area – Yugra, Russian Federation. ORCID 0000-0001-8362-0608.

(✉) **Naumova Lyudmila A.,** e-mail: naumovala@yandex.ru.

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