TUMORAL MICROENVIRONMENT – PATHOGENIC ELEMENT OF TUMORAL DEVELOPMENT

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Summary

Cellular microenvironment is the reduced space around a cell or a biological multicellular structure in which there are cells, extracellular matrix, enzymes, signal proteins. Between the cells and cellular microenvironment elements a regular exchange of signals is possible.

By expanding to solid tumors the space around the tumor is considered as a tumor microenvironment formed by the same elements as the cell microenvironment.

Cellular microenvironment provides and promotes normal cell development.

Tumor microenvironment provides and promotes tumor development at all stages of its development including metastasation, site preparation of metastatic cell attachment situs (premetastatic niche).

It was observed that the tumor microenvironment, in particular cells, gain important protumoral properties.

Moreover, it was observed that the tumor microenvironment acts as a true biological barrier that protects tumor action of host defense mechanisms (immunological and non-immunological) or the action of antitumor drugs.

There are presented the main causes modifications of microenvironment of tumor cells to become protumoral, suppression of adoptive and innate immune response and therapeutic prospects by the action of the cellular elements that compose the tumor microenvironment.

Key words: *tumor microenvironment, immunity, therapeutic perspectives.*

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Microenvironment is a small space around a cell or multi-cellular biological structure, where there is a possible exchange of signals between elements of this area and the biological structure. In this space a series of signals of various origins are processed, coupled and can directly influence the normal evolution of the cell or multicellular biological structure.

There are a number of physical and chemical factors considered essential elements of a biological microenvironment. Among these, the most important are: viscosity, temperature, O2 concentration, pH, polarity, electrostatic potential factors [1].

In vitro microenvironment of a single cell is composed of: extracellular matrix, cells of the same type or of other types, surrounding a single cell, bioactive substances (cytokines, hormones, growth factors), with autocrine, paracrine or endocrine rol, mechanical forces resulting from the motion of the body fluids or entirely organism, plus structures as nanopores, nanofibers, nanocrystals, substances resulting from cellular metabolism [2].

Microenvironment of a cell or group of cells to be regarded as a dynamic element with a structure wherein the cells have a constant exchange of signals physical, chemical, biological. Cellular microenvironment structure is constantly changing and even cells / biological pluricellular structures, through its own evolution and development, contribute to the

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appearance of changes in the microenvironment and even its appearance [1,2,3].

Cellular microenvironment

Material support of cellular micro-environment is the extracellular matrix (ECM). The concept of the microenvironment of a cell or biological structure comprises cells that these structures interact with, without being extra-cellular matrix constituents.

The cell matrix is defined as a three-dimensional macromolecular network, non cellular, formed of collagen, proteoglycan, elastin, fibronectin, laminin, and a plurality of macromolecules [5].

To these is added a number of proteins acting as receptors but also as adhesion molecules such as integrins. Each protein which comprises extracellular matrix, has different functions: structural (collagen, elastin), adhesion, mechanical, biochemical. Integrins, especially b1 integrin acts as a link between ECM and the cell cytoskeleton. The signal received by the integrins may lead to the activation of MAP and Eco kinases within the cells, affects proliferation and polarity, differentiation and gene expression. There is also the reverse process in the sense that a number of changes in intracellular (as FAK protein and LLK) may induce conformational changes in integrin structure and activation of other extracellular ligands [5,6].

There is a permanent reshaping of ECM by matriceal metalloproteinases action, with collagen as the substrate of these enzymes. ECM may suffer changes under the influence of cytokines and oxidative stress but mechanical factors also. ECM biomechanical changes are affecting the morphology and differentiation of cells which they interact with [1].

Glycosylation is an essential process in the activity of extracellular matrix proteins. Malfunction of this process result in changes to the structure, production and function of intercellular adhesion proteins with disruption of the cellular microenvironment. There is a bidirectional relationship cell-ECM. The cell produces ECM remodeling and ECM signals influence cell activity [7].

Microenvironment consists of ECM, cells and various biological agents and is essential for the life of the cell. The absence of microenvironment, especially of cell adhesion, induces cell death by apoptosis. The composition of micro-environment may vary from cell to cell depending on the specific activity of each cell type.

Apart from structural function cellular microenvironment appears as a real informational entity integrating structural and functional signals without which the cell cannot survive.

On the other hand microenvironment can become "ground" deployment of pathological processes or participate actively in carrying out these processes. In inflammatory process microenvironment is directly involved in the initiation, conduct and "stopping" inflammatory response. In case of the tumoral growth, microenvironment become tumoral micro-environment, actively involved in tumor development [8].

Stem cells microenvironment. A particular microenvironment is the microenvironment formed around stem cells in various organs of the body, the bulge region, located in the hair follicle, hematopoietic cells niche, the niche cells from the intestinal crypts level, dental pulp [9]. These specific micro-environments have the ability to maintain stem cells in G0 of the cell cycle stage (undifferentiated stem cell) keeping a balance between stem cells and cells that enter into differentiation. Niche stem cells are maintaining a low metabolism, are protected by genes mutation accumulation. In essence, the stem cell micro-environment consists of stromal cells, the extracellular matrix and soluble factors. The difference from the other types of micro-environments is the soluble factors. The composition of growth factors has some specificity according to the type of stem cells [9].

There is a complex interaction between stem cells and the cellular microenvironment niche. The main mechanism is the integrins especially β1 integrins that can activate or inhibit a number of kinases (adhesion kinases) which controls the activity of stem cells [9].

The most well-known and well-defined anatomical "niche" is the bulge region, in the hair

follicle, composed of melanocytic stem cells and epidermal stem cells, separated from each other by a seria of proteins like tenascin-C protein, collagen VI, collagen XVIII, fibrillin, all are better expressed than in differentiated keratinocytes [10].

Tumor microenvironment

Tumor development is the result of the gradual accumulation of genetic and epigenetic changes that cause cancer cells to acquire a number of characteristics that distinguish them from normal cells (unlimited proliferation, poor response to suppressive factors, resistance to apoptosis, avoiding destruction by the immune system, stimulating angiogenesis, metastasis and exacerbation of the tumor due to inflammation) [11]. All these features would not be able to express and ultimately lead to the formation of solid tumors without a microenvironment favorable to the process. Thus, as the tumor progresses, it produces its own microenvironment transforming the normal microenvironment in tumoral microenvironment (TME) .

A characteristic of the tumor microenvironment is that TME does not contain new elements different from normal microenvironment (extracellular matrix, cells, cytokine) but normal microenvironment acquires protumoral important properties which promote tumor growth [12].

Tumor microenvironment is formed in the case of solid tumors, of extracellular matrix with a number of non tumoral cells present in matrix (cancer associated fibroblasts CAF), activated adipocytes, stem cells, (mesenchymal, myofibroblasts), infiltrate cells with immune functions (platelets, mast cells, neutrophils, monocytes, myeloid marrow derived suppressor cells macrophages, T $CD8^+$ lymphocytes, NK cells, $CD4^+$ T lymphocytes, B lymphocytes) [13].

Depending on the type and location of tumor, tumor microenvironment components can vary both in structure and actual importance in tumor development.

Tumor extracellular matrix. Because signals from stromal tumoral cells, fibroblasts, myofibroblasts secrete a number of cytokines, chemokines, growing factors that deeply modify the extracellular matrix. There is a metabolic reprogramming, transcription activation and alteration of reparatory protein synthesis[12].

Extracellular matrix contains a fibrillar network of collagen, laminin, fibronectin, proteoglycan, hyaluronic acid, in a specific organization [14]. In the course of tumor growth the ECM network of fibrils undergo condensation, remodeling and realignment. It has been demonstrated that the collagen fibers are very dense near the tumor (tumor-associated collagen signature) (Tacs 1) large collagen fibers surrounding the tumor (Tacs 2) and the normal collagen fibers aligned with the edges of the tumor (Tacs3). There is an intimate interaction in early stages of tumor, between the tumor cells and dense collagen fibers [15].

Taken in its entirety, ECM abnormalities that occur during the tumor growth were termed desmoplasia and represents an increase in the number of collagen, fibronectin and proteoglycans, tenascin C fibers. In most cancers desmoplasia is associated with increased tumor aggressiveness [16].

The constituent cells of the tumor microenvironment. Tumor microenvironment contains many non tumor cells (stromal cells, immune, vascular) the function of which is converted in promoting tumor growth. On the other hand, the interaction of the tumor cells creates tumor microenvironment.

Fibroblasts are basic connective tissue cells located in the matrix, having the ability to produce and secrete the elements constituting the cellular microenvironment. By activating the tumor cells, the fibroblasts become cancer associated fibroblasts (CAF), which are different from normal fibroblasts. CAF present ability to remodel the extracellular matrix and turn it into tumor microenvironment by secretion of factors (VEGF, TGFβ, CXCL12) secretion of growth factors, cytokines, inducing immunosuppression, inflam-matory cell recruitment, activation of transcription factors YAP, NF/kB as general activating factors release (TGFβ, CX-CL12) or vascular factors.

Fibroblast activation and converting them into CAF, occurs by cell to cell communication (tumor cell-fibroblast?) growth factors, adhesion molecules, microRNAs [17]. An important factor in the operation of a CAF is the transmembrane

serine protease, fibroblast activatory protein (FAP). In normal tissues, FAP is slightly expressed. FAP is expressed in stromal fibroblasts, in large scale carcinomas and in addition to the role of the CAF it activates proteases with role in promoting the metastasis [18].

CAF differs significantly from normal fibroblasts. As opposed to normal fibroblasts that are stopping tumor growth, CAF supports tumor growth both in vivo and in vitro [19]. CAF derive mainly from fibroblasts but by endothelial mesenchymal transition can be derived from endothelial or epithelial cells [18].

The endothelial cells. The formation of new blood vessels is essential for tumor development. Tumor vascularisation requires cooperation between the microenvironment of tumor cells including endothelial cells, pericytes and cells derived from bone marrow [18].

The main stimulus for tumor neovascularization is the hypoxia. To this was added soluble factors that are in TME as VEGF, FGF, platelet derived growth factors (PDGF). These and other proangiogenic factors are produced by the cooperation of tumor cells with cells in the tumor microenvironment: CAF, tumor associated macrophages, mesenchymal stem cells [18].

The tumor lymphangiogenesis presents the same pattern as tumor angiogenesis. Activated macrophages as well as myeloid cell populations acting on lymphatic endothelial cells via growth factors VEGF-C and VEGF-D. Note that the VEGF (or VEGF-A) is produced by both tumor cells and inflammatory cells [18,19]. The production of new blood vessels in the tumor is based on the development and recruitment of preexistent endothelial cells, activated endothelial cells and progenitor cells from the bone marrow [20].

Adipocytes. Adipose tissue contains two types of cells (adipocytes) or white adipocytes, (univacuolars) and brown adipocytes (plurivacuolar). The brown color of adipocytes is given by the large number of mitochondria in the cytoplasm.

Generally both types of adipocytes are associated with tumor development as cancer associated adipocytes (CAAs) present especially in the tumor invasion front. CAAs secret many protumoral factors involved in matrix remodeling and induce epithelial mesenchymal transition [21]. In addition to these mature adipocytes there are adipocyte stem cells that, in terms of tumor growth, can be considered associated with tumor growth. These cells contribute to the remodeling of the tumor microenvironment can differentiate the cancer stromal cells and can promote epithelial mesenchymal transition [22].

Immune competent cells. There are cells involved in innate immunity and adoptive immunity, transient in tumor microenvironment, the most important being: macrophages, NK cells, Langerhans cells, T cells and B cells, T regulatory.

Macrophages. They are cells resident in tumor microenvironment or come from peripheral blood. They have multiple roles in inflammatory response such as: phagocytosis, secretion of cytokines and tissue homeostasis.

They have the ability to function as antigen presenting cells. There were identified till now two subsets of macrophages. First subset (M1) includes classical activated macrophages which secrete proinflammatory cytokines (IL-12, IL-23, TNF) and have antigen-presenting activity and anti-tumor role. Second subset, tumor-associated macro-phages (TAM) or (M2) produce anti-inflammatory cytokines (IL-10, TGFβ) present protumoral activity [22]. TAM also produce VEGF, EGF (acting on tumor cells, activating NF-kB, to enhance secretion of exosomes).

In tumors, M2 macrophages, recruit monocytes, have the ability to promote angiogenesis, lymphangiogenesis and metastasis [18]. Switching from state M1 to state M2 appears to be due to hipoxya from tumor microenvironment [22].

NK cells. These cells have the ability to kill tumor cells, especially when these are in general circulation. Inside solid tumors NK cell cytotoxic activity is greatly reduced even NK cells may be seen in the tumor microenvironment as in the tumor. Cytotoxic activity of NK cells is dependent on the degree of their activation by various cytokines (eg. IL-2) which is reduced in the tumor microenvironment due to immunosuppressive factors or cells action (tumor-associated fibroblasts) [23,24].

T lymphocytes. Within the tumor, several subpopulations of lymphocytes may be present: $CD8^+$ cytotoxic T lymphocytes, memory T lymphocytes ($CD8^+$ $CD45^+$ RO), Th1 and Th2 lymphocytes, T regulatory ($CD2^{5+}$ $FoxP3^+$). The large number of regulatory T-lymphocytes is associated with a poor prognosis [19,25]. The functions of T-lymphocytes and cytotoxic T-lymphocytes in particular are reduced by the action of tumoral microenvironment. Decreased tumor antigens immunogenicity as the inappropriate presentation of tumor antigens determine the inefficiency of anitumoral response.

Some immunosuppressive mechanisms were determined. Indoleamine-2,3 dioxygenasis (IDO), PD-L1 / B7-H1, recruitment of regulatory T cells [26,27]. All of these mechanisms with tumoral microenvironmental involvement determine, in case of solid tumors, the suppression of T-cells activity [26,27].

Regulatory T lymphocytes. It is a subset of T lymphocytes expressing the transcription factor FoxP3 which plays an important role in the maintenance of immune homeostasis, controlling autoimmunity, inflammation and tumor immunity. They have the ability to release cytokines with suppressor role (TGFβ, IL-10, IL-35), controlling a number of chek points of the cell cycle and receptors with suppressor roles, suppress the presentation of tumor antigens by dendritic cells as well as cytotoxic function the T lymhocytes.

In the tumor microenvironment these cells are found in large numbers and have the ability to suppress antitumor immune response and contribute to tumor progression. In murine models TGFβ and IL-10 contributes essentially to the transformation of $CD25^+$ $FoxP3^+$ lymphocytes in suppressor cells of antitumoral response [19,28].

MDSC cells. MDSC cells are a category of cells derived from bone marrow (BMDC) with a strong role in the inhibition of anti-tumor immune surveillance mechanisms but actively promoting the proliferation of tumor. MDSC cells (myeloid derived suppressor cells) migrate from the bone marrow to tumor where it is converted into stromal cells components of tumor microenvironment [29].

Several tumors secrete chemokines which recruit these cells and reach the tumor. Immunosuppressive activities of these cells is performed at different levels of anti-tumor immune response: antigen presentation, T activation, inhibition of cytotoxic T-cells, inhibition of NK and macrophage TAM transformation. The achievement of these effects is via cytokines and chemokines within the tumor microenvironment. The immunosuppressive action of MDSC is conjugated with T regulatory cells action this being one of the most important mechanism of "escape" of the tumor.

Microenvironment in tumor metastasis. Metastasis is the detachment of tumor cells from the primary tumor and their location at other sites, forming new tumor masses. It is a multistage process which includes local invasion, circulation passage, survival of detached cell in circulation and transition in tissue and colonization itself. Tumor microenvironment participates in almost all these phases. By its cell components (TAM, CAF) and humoral components (VEGF-A, TGFβ, TNF) it promotes proliferation of the tumor, local invasion, endothelial cell activation and adhesion of myeloid cells derived from the bone marrow to vascular endothelium, crossing the endothelium and the formation of the premetastatic niche (pretumoral niche or protumorigenic niche).

Premetastatic niche is a site where detached cells may locate [18] The site is formed by secretion of extracellular factors which are formed in the cellular microenvironment. Thrombospondin 1 is the most important and is secreted by the cells derived from the bone marrow, theory of "seed and soil" Paget(cancer cell / tissue favorable soil) (Paget 1889 cited by Bahrami A [45]. It can be said that the primary tumor is preparing the site for metastasis by “seed-and-soil” –premetastatic niche [29,30].

A second hypothesis, assume that the stem cells colonize new metastatic sites induce periostine in local fibroblasts that ultimately restore tumor microenvironment [31]. Composition of premetastatic microenvironment differs from tumor microenvironment itself by the fact that there are many more VEGFR1 + cells derived from bone marrow. The microenvironment of the

premetastatic niche by its various factors maintain the metastatic cells in the G_0 phase of the cell cycle for a limited period [12,18].

Innate and adoptive immunity in tumoral microenvironment. In addition to the ability to promote the proliferation, tumor micro-environment may suppress innate and adoptive immune response, but also present a supportive role in antitumor immune responses. In most cases immunosuppressive mechanisms predominate [30].

Tumors express antigens on their surface which may be recognized by the immune system leading to the development of a specific immune response involved in destroying tumor cells. Tumor microenvironment has many mechanisms that oppose adoptive immune response at all levels. So, tumor cells loose or "hide" tumor antigens that may be recognized by immune cells, decrease the activity of antigen presenting cells, and even remove the tumoral cells from T cytotoxic lymphocytes.

Through various soluble factors (eg. Th2 Cytokines) cells become tumor-associated stromal cells (macrophages, fibroblasts, adipocytes) secrete protumoral factors having also immunosuppressive action [32].

The mechanisms of inhibition of anti-tumor immune response in the tumor micro-environment are due to the presence of an increased number of negative regulatory factors at this level such as PD-L1, IDO, TregFoxP3$^+$cells.

PD-L1 and IDO are induced by IFNα and Treg are recruited by chemokines and CC222, both produced by activated $CD8^+$ effector T lymphocytes [33]. On the other hand the oxygen and potassium have immunosuppressive action on T lymphocytes [34].

In innate immunity the tumor micro-environment converts antitumoral mechanisms in protumoral mechanisms as in adoptive immunity. The secretion of metalloproteinase 2 (MMP-2) secreted by melanoma cells and stromal cells cleaves R1 IFNα receptor, stimulate TLR2 on dendritic cells making them to promote the differentiation of Th2 lymphocytes that secrete proinflammatory cytokines [35].

The response to the aggression of pathogens in the case of innate immunity is the direct destruction of the pathogen, inflammation, cell recruitment and stimulation of adaptive immunity.

Elements of innate immunity (NK cells, macrophages, antigen presenting cells) recognize a group of molecules on the surface of the pathogen called "PAMP" (Pathogen Associated Molecular Pattern) followed by the onset of innate immune response. Recognition of PAMP is done by specialized receptors (PRR, Pathogen Recognition Receptor) the most important being TLR (Tall-Like Receptor). TLR receptors are a family of transmembrane proteins (TLR1-TLR10), which after coupling with PAMP is associated with a cytoplasmic adapter molecule MyD88 and finally activate NF-kB [36].

TLR activation plays an important anti-tumoral role. It enables the production of IFN (TLR3) induction of pro-apoptotic signals (TLR4) stimulate dendritic cells (TLR7) stimulate the adoptive immune response (TLR9). TLR are found on many cell types including tumor cells [37]. The tumor microenvironment converts also adaptive antitumoral immunity mechanisms in protumoral mechanisms. Thus, TLR ligands increase the production of immunosuppressive cytokines (IL-10, TGFβ) block tumor cell apoptosis, activate Treg, inhibit tumor cell recognition by the adoptive immune system. However, the agonists of TLR7 have a strong anti-tumor activity and are used in therapy (Imiquimod) [37].

In addition to binding to receptors on the surface of pathogens (PAMP) receptors in innate immunity (TLR, NOD-like receptors) recognize and series of molecules that appear in the cell destruction or in the tumor growth (proteins from the extracellular matrix, components of tumor-associated antigens (TAA) heat shock proteins. Recognition of these molecules by cells carrying TLR or NLR results in the stimulation or innate immunity, but which promotes tumoral proliferation by tumor microenvironment [38].

Tumor infiltrating lymphocytes (TIL). Cells that infiltrate the tumor: macrophages, neutrophils, dendritic cells, NK cells, naive and memory T lymphocytes, B lymphocytes, cytotoxic T lymphocytes, that leave the blood stream and localize within the tumor [31].

Viewed from the point of view of the microenvironment, which contains fibroblasts, endothelial cells, fundamental substance, TIL can

be considered a component of the tumor microenvironment. Clinical observations have shown that the presence of an abundant infiltration is associated with a good prognosis, after immunotherapy or surgical excision [39].

Thus, cells composing TIL have the ability to reach near tumor cells [40]. However, it is not ruled out further action of microenvironment to protect tumor proliferation, as it is not excluded the initiation of adaptive immune response in the microenvironment, independently from the secondary lymphoid organs [41].

Therapeutic perspectives

Understanding the fact that tumor microenvironment is an important part of physiology, structure, and tumor growth, made to appear, at least in theory, new approaches in tumor therapy.

Apart from tumor proliferation, TME is involved in suppression of the host defense biological mechanisms as well as resistance to antitumor therapeutic means. Basically TME forms a biological barrier surrounding the tumor by reducing the number of anti-tumor immune cells and limiting the action of anti-tumor medication [32, 42, 43].

Inside the biological barriers or TME formed around the tumor, the number of cytotoxic T cells or NK cells is low, antigen stimulation is reduced both by the poor presentation of tumor antigens as well as through direct effects of T cells via cytokines, finally resulting in their exhaustion [32, 43, 44].

TME antitumor therapeutic methods aimed primarily the conversion of the tumor microenvironment in normal microenvironment. In this way it is hoped to a normal status of the malignant cells, and of the extracellular matrix which permit antitumor mechanisms of the host to intervene effectively destroying or restricting the proliferation of tumor cells and metastasis [43].

The main research targeted immunotherapy. Thus it tried to block the immunosuppressive checkpoints respectively through the chimeric antigen receptors, or T-cells that express tumor-specific antigens. Monoclonal antibodies were directed against these checkpoints or changing the profile of cytokines (chemokines) in TME [45]. Another theoretical way targeted non-tumor cell component of TME. Thus it was attempted TAF destruction by blocking tumor associated FAP (serine protease that promote tumor growth and metastasis).

The induction of apoptosis has also been attempted in the TAM (tumor associated macrophages). TAM was attempted to be destructed by zoledronic acid or a cysteine proteaze- legumain- that generate a response against TAM via $CD8^+$ T cells [44, 45].

Other cells in the tumor microenvironment such as pericytes or adipocytes, both are therapeutic targets.

Studies, so far, suggest that antitumor therapy should not focus only on the direct destruction of tumor cells and the destruction of the factors that maintain the proliferation and metastasis of tumor. The results obtained so far, experimental and clinical, in therapy directed against TME elements shows that this therapy is absolutely necessary for the destruction of the tumor or its transformation into a „chronic disease".

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Conflict of interest
NONE DECLARED

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