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ROLE OF MAST CELLS IN TUMOR PROGRESSION

Nadejda Stratan, Victor Cazac
(Academic adviser: Vitalie Mazuru – Senior lecturer)
Department Histology, Cytology and Embriology
State Medical and Pharmaceutical University "Nicolae Testemiţanu"

Summary

The aim of this article is to show the key points regarding the crucial role of mast cells in supporting and promoting tumor progression. The article reveals important facts about both the immunosuppressive function of mast cells, that greatly enhances the tumor progression, as well as the changes in intercellular junctions induced by mast cell activity. Additionally, the article focuses on blood and lymphatic vessels growth provided by mast cell activity, an essential prerequisite necessary for tumor growth.

Rezumat

Rolul mastocidelor în progresia tumorală

Scopul acestui articol este de a prezenta momentele cheie privitor la rolul crucial al mastocitelor în promovarea progresiei tumorale. Articolul aduce informaţii importante atât despre funcţia imunosupresivă a mastocitelor, care considerabil duce la accelerarea progresiei tumorale, cât şi despre modificările joncţiunilor intercelulare induse de activitatea mastocitelor.

Deasemenea, articolul descrie angiogeneza şi limfangiogeneza promovată de activitatea mastocitelor, o premisă necesară pentru creşterea tumorală.

1. Mast cells (physiology)

Human mast cells are divided into 2 major subtypes based on the presence of tryptase (MC_T cells) or tryptase and mast cell-specific chymase (MC_{TC} cells), each predominating in different locations [27]. There is also a third subtype of mast cells but it is the less frequent - MCc containing only chymase and being found in the intestinal submucosa and nasal mucosa. MC_T cells are the prominent mast cell type within the mucosa of the respiratory and gastrointestinal tracts and increase with mucosal inflammation. MC_{TC} cells are localized within connective tissues, such as the dermis, submucosa of the gastrointestinal tract, heart, conjunctivae, and perivascular tissues.

Mast cells are KIT (CD117) positive - receptor for stem cell factor (SCF) and FcεRI positive - a high-affinity receptor for the Fc region of IgE; they express other cell-surface receptors, depending on their location and stage of differentiation and activation. Mast cells express the activating IgG receptor FcγRIIa (CD32a) in the resting state and, in the presence of IFN-γ, the high affinity activating FcγRI (CD64). Inhibitory G protein–coupled receptors can also be expressed on mast cells, including the β_2-adrenergic receptor, the adenosine receptor A2B, and the prostaglandin (PGE_2) receptor EP_2. Mast cells might also express the following receptors: C3a and C5a receptors, IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, GM-CSFR, IFN-γR, CCR3, CCR5, CXCR2, CXCR4, nerve growth factor receptor, and Toll-like receptors (TLRs), among others [8]. Human mast cells arise from $CD34^+$ pluripotent progenitor cells. Mast cell precursors circulate in the blood and then home to tissues, where they mature. Maturation of precursors in the tissues is dependent on SCF expressed on the surface of fibroblasts, stromal cells, and endothelial cells through binding to KIT on mast cells. Mast cell phenotype and behavior is altered by cytokines, such as IL-4, IL-5, and IFN-γ. For example, IL-4 upregulates expression of FcεRI, IL-5 promotes proliferation in the presence of SCF, and IFN-γ decreases mast cell numbers.

Human mast cells contain two types of serine protease, tryptase and chymase. Tryptase is a trypsin-like enzyme, and chymase is a chymotrypsin-like enzyme. Human mast cells are divided into the tryptase-positive mast cells (MC_T), which contain tryptase but not chymase, and the tryptase- and chymase-positive mast cells (MC_{TC}), which contain both tryptase and chymase.

In patients with cancer, a significant correlation between mast cell number and angiogenesis has been reported, and patients in the high-count mast cell group showed a significantly worse outcome than those in the low-count group. In mast cell-deficient mice, it was found that there was a decreased rate of tumor angiogenesis, and that hematogenous metastasis was also reduced .

In 1863, Rudolf Virchow critically recognized the presence of inflammatory cells infiltrating neoplastic tissues and first established a causative connection between the "lymphoreticular infiltrate" at sites of chronic inflammation and the development of cancer [6]. Tumor cells are surrounded by an infiltrate of inflammatory cells, namely lymphocytes, neutrophils, macrophages and mast cells (MCs). These cells communicate via a complex network of intercellular signaling pathways, mediated by surface adhesion molecules, cytokines and their receptors. Inflammatory cells cooperate and synergize with stromal cells as well as malignant cells in stimulating endothelial cell proliferation and blood vessel formation. The most aggressive human cancers, such as malignant melanoma, breast carcinoma and colorectal adenocarcinoma, are associated with a dramatic host response composed of various inflammatory cells, especially macrophages and MCs. However these inflammatory cells, including mast cells, have potential effects that might either benefit the tumor or contribute to tumor resistance or rejection.

2. The immunosupressive function of MCs

In recent years, the role of MCs in innate immune responses has received increased attention and it is now well established that the mast cell is an important player in the elicitation of optimal host responses to a variety of pathogens. Mcs are considered to induce the suppression of the immune system of human body.

Nevertheless, to what extent are MCs able to limit, suppress or terminate immune responses is an intriguing question. It is known that several MC mediators, including TGF-β, IL-4, IL-10 and histamine, can negatively regulate biological responses, and MCs could therefore be involved in suppressing immune responses. The activation of MCs could result in immunosuppression [25]. It is determined that MC-derived histamine contributes to the immunosuppressive actions of ultraviolet-B (UV-B) irradiation on the expression of contact hypersensitivity in mice [29], while IL-10, is known to have immunosuppressive functions, its increased production being shown to correlate with the presence of MCs . MCs have the ability to suppress, at least in some settings, the development or the magnitude of adaptive immune responses [21].

It is established that T-regulatory cells (Treg) and mast cells are abundant in tumors. MCs have an intricate interaction with Treg that regulates the functions of both cell types in a reciprocal manner [26]. Interaction between the two is known to promote immune suppression or loss of Treg functions and autoimmunity. The outcome of this interaction is the generation of potently immune suppressive but proinflammatory Treg (ΔTreg). These Treg switch from suppressing to promoting MC expansion and degranulation. [10]. This change is also brought about by direct coculture of MC and Treg, or culture of Treg in medium containing IL6 and IL2. MC, in return, induce Treg to switch function and escalate inflammation without losing T-cell–suppressive properties.

3. MCs and intercellular junctions (EMT)

Twenty hours after BrX-537A(mast cell degranulation activator) administration, several typical colonic epithelial alterations were observed, such as the presence of submucosal oedema, disorganisation of the epithelium with an increase in immunocyte presence and lymphoid follicles, and an increase in luminal mucus.

Structural observations of TJs were performed 72 hours after stress or BrX-537A administration. In most of the samples, alterations not seen in control conditions were observed. The percentage of opened TJs reached 14.3% and 13.2% 72 hours after BrX-537A or stress session, respectively, this percentage being more than doubled compared with vehicle (6.4%).

At three days after a single stress session, expression levels of TJ(tight junction) proteins were affected. Indeed, expression of mRNAs encoding occludin and ZO-2 were decreased by 60% and 43%, respectively, compared with controls. Expression of ZO-1 mRNA was unchanged [13].

Tryptase cleaves PAR2(protease-activated receptor 2) within the extracellular N terminus at Ser-Lys-Gly-Arg-Ser^{34}↓Leu-Ile-Gly-Lys-Val (human, ↓ = cleavage site) to expose a tethered ligand domain (SLIGKV) that binds to and activates the cleaved receptor.

In enterocytes, PAR2 interacts with the multiadaptor protein β -arrestin (βARR), which mediates receptor endocytosis and recruits activated extracellular signal-regulated kinases 1/2 (ERK1/2) to endosomes. Cytosolically retained ERK1/2 may regulate the cytoskeleton to control paracellular permeability. Claire Jacob and colleagues hypothesized that mast cells signal to colonocytes by release of tryptase and activation of PAR2, and that PAR2 couples to ERK1/2 by a βARR-dependent mechanism to regulate TJ assembly, perijunctional filamentous actin (F-actin), and paracellular permeability.

Alterations in the localization and expression of TJ proteins and perijunctional F-actin are associated with changes in paracellular permeability of epithelial cells. Optical sections in the x-y plane revealed that immunoreactiveZO-1, occludin, and claudin-1 were prominently localized to the perimeter at the apical pole in cells treated with reverse peptide (100 μm, 24 h).

Projections in the plane of stacks of optical sections confirmed the prominent localization of ZO-1, occludin, and claudin-1 in the apical region of the cell, consistent with the presence of these proteins in TJs. Lower levels of these proteins were detected in the basolateral membrane beneath the tight junctions and in the cytosol [4].

4. Angiogenesis (matrix remodeling and endothelial proliferation)

Angiogenesis plays a crucial role in the cancerogenesis, growth and progression of human solid and haematological tumours. New blood vessels provide them with a gateway through which to enter the circulation and metastasize distant sites.

MCs are an abundant source of angiogenic factors. [17] In various tumor models, MCs appear at the edges of invasive tumors, where they facilitate angiogenesis by releasing preformed mediators or by triggering proteolytic release of extracellular matrix-bound angiogenic compounds. Human MCs release preformed fibroblast growth factor-2 (FGF-2) from their secretory granules. Human cord blood-derived MCs release vascular endothelial growth factor (VEGF) upon stimulation through FcεRI and c-kit while bone marrow–derived mast cells produce VEGF-A on activation with different stimuli. Both FGF-2 and VEGF have also been identified by immunohistochemistry in mature MCs in human tissues. It has been shown that human MCs are a potent source of VEGF in the absence of degranulation through activation of the EP2 receptor by prostaglandin E2 [31]. This angiogenic activity is partly inhibited by anti-FGF-2 and VEGF antibodies, suggesting that these cytokines are involved in the angiogenic reaction.

In addition, MCs store large amounts of preformed active serine proteases, such as tryptase and chymase, in their secretory granules. Tryptase, a potent proangiogenic factor in malignancies, stimulates the proliferation of human vascular endothelial cells, promotes vascular tube formation in culture and also degrades connective tissue matrix to provide space for neovascular growth. Tryptase also acts indirectly by activating latent matrix metalloproteinases (MMPs) and plasminogen activator (PA), which in turn degrade the extracellular connective tissue with consequent release of VEGF or FGF-2 from their matrix-bound state [30].

Chymase is a chymotrypsin-like enzyme that is stored in the secretory granules of mast cells. Human chymase in vascular tissues converts angiotensin I to angiotensin II, which is a potent angiogenic factor via the induction of vascular endothelial growth factor (VEGF) thus enhancing neovascularization and increasing blood flow in ischemia-induced angiogenesis. Therefore, chymase-generating angiotensin II induces VEGF expression and results in the generation of angiogenesis. Chymase also activates promatrix metalloproteinase-9 to generate matrix metalloproteinase (MMP)-9, which is well known to be associated with tumor cell invasion, stromal remodeling and tumor-induced angiogenesis. MCs have also the potential to synthesize and release MMP-9. [16] MMP activity is generally balanced by the tissue inhibitor of metalloproteinase (TIMP), and tissue degradation is dependent on an imbalance between MMP and TIMP. Besides, MC-derived chymase degrades extracellular matrix components and therefore matrix-bound VEGF could be potentially released.

Other MC-specific mediators with angiogenic properties include histamine and heparin. Histamine, the major preformed mediator stimulates new vessel formation by acting trough both H1 and H2 receptors. [15] Heparin, the main glycosaminoglycan constituent of MC granules, may act directly on blood vessels or indirectly by inducing release of FGF-2 from the extracellular storage site.

In addition, other cytokines produced by MCs, such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), nerve growth factor (NGF) [9] and interleukin-8 (IL-8), have been implicated in normal and tumor-associated angiogenesis. Recently, MCs from human uterine leiomyomas has been found to contain leptin, a peptide mainly secreted by adipocytes which, besides its involvement in obesity development, has also been found to express angiogenic activity.

Angiogenesis and lymphangiogenesis, the formation of new blood and lymphatic vessels, are important aspects of tumor growth and inflammatory diseases. Both angiogenesis and lymphangiogenesis rely on the production of vascular endothelial growth factors (VEGFs), which are the most potent proangiogenic mediators. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). VEGF-A and VEGF-B are key regulators of blood vessel growth, whereas VEGF-C and VEGF-D regulate primarily lymphangiogenesis. VEGFs signal through 3 VEGF receptors (VEGFRs): VEGFR-1, VEGFR-2, and VEGFR-3.

PGE_2 and adenosine are 2 relevant mediators of inflammation and tumor growth [1]. PGE_2 is a lipid mediator that regulates key aspects of inflammation and immunity and exerts pro-oncogenic effects by enhancing tumor angiogenesis.

Adenosine is an endogenous purine nucleoside acting on specific membrane receptors. This molecule is produced in high concentration during tumour growth and it has been implicated in promoting angiogenesis. Interestingly, the human MC line HMC-1 expresses A_{2A}, A_{2B} and A_3 adenosine receptors. Selective stimulation of the A_{2B} receptor increases VEGF and IL-8 secretion by HMC-1 MCs whilst the A_3 receptor increases the expression of angiopoietin-2 mRNA. Increasing concentrations of NECA - 5′-(N-ethylcarboxamido) adenosine, a metabolically stable analog of adenosine, induced mRNA expression of $VEGFA_{165}$, VEGFC, and VEGFD. So, adenosine acts in a functional fashion to promote tumour angiogenesis by a cooperative paracrine mechanism involving A_{2B} and A_3 receptors on infiltrating MCs that, in turn, secrete angiogenic factors.

Thus, PGE_2 and NECA play different roles in angiogenesis by promoting the production of different VEGFs and these factors produced by mast cells could intervene at different times to regulate the angiogenic process [12]. One of the major proinflammatory effects exerted by VEGF-A on cells expressing VEGFRs is chemotaxis. Thus, the VEGF/VEGFR system is emerging as a fundamental regulator of angiogenesis and lymphangiogenesis in allergic, inflammatory, and neoplastic disorders.

MCs efficiently release VEGF-A on stimulation with PGE_2 and NECA, and their supernatants induce an angiogenic response *in vivo*. On the other hand, expression of VEGFR-1 and VEGFR-2 enables mast cells to be attracted by different VEGFs. Therefore mast cells might represent a source and a target of VEGFs during inflammatory and neoplastic processes.

There are three novel areas of mast cell interactions with tumor cells [35]:

1. mast cell effects on tumor angiogenesis,
2. mast cell-mediated tissue remodelling and
3. mast cell-dependent immune regulation via recruitment of immune cells and immunosuppression.

Mast cells migrate toward supernatants from a number of tumorigenic cell lines, but not from primary cells or non-tumorigenic cell lines, suggesting tumor-intrinsic factors in mast cell recruitment across a range of tumor types [36]. More recently, stem cell factor (SCF) produced by tumor cells in vivo has been implicated in mast cell accumulation at the periphery of developing tumors [39] and [2]. SCF over-expression in developing mammary tumors increased mast cell accumulation at local sites of tumor growth, whereas inhibition of SCF expression resulted in decreased mast cell accumulation and decreased angiogenesis.

Peripheral mast cell localization suggests that recruitment occurs either from:

a) resident mast cells migrating from neighboring healthy tissue or,

b) de novo recruitment of mast cell progenitors via healthy vasculature close to the tumor site

Angiogenesis is integral to tumor development, providing nutrients necessary for cell growth [14] and is required for tumor growth > 1 mm^3. In addition, angiogenesis is a necessary step for metastasis of solid tumors to distal sites in the body. Neovascularization requires mast cell granular components, while the presence of mast cell membranes alone is insufficient. Several studies have also suggested that SCF, which attracts mast cells into the tumor

environment, also increases mast cell release and production of both VEGF and bFGF. Mast cell infiltration into a developing tumor triggers an "angiogenic switch" - the passage from the preangiogenic phenotype to the angiogenic phenotype and allows the formation of a neovasculature that is indispensable for tumour growth and metastatic dissemination..

In addition to producing pro-angiogenic compounds, mast cells are major sources of proteases, which act on the surrounding extracellular matrix (ECM), and have been demonstrated to play a role in tissue remodeling [28]. It has been suggested that in the context of developing tumors, the ability of mast cells to remodel tissues is subverted, disrupting surrounding ECM and increasing tumor spread. In addition to providing room for tumor spread, the disruption of local ECM leads to the release of matrix-bound factors including SCF and FGF-2, and thereby increases endothelial cell migration and proliferation and likely promotes angiogenesis, tumor spread and growth.

Release of mMCP-4 (chymase) and mMCP-6 (tryptase) by mast cells were both demonstrated to play roles in tissue remodelling. mMCP-6 was found to stimulate dermal fibroblasts, inducing DNA synthesis in quiescent cells and also increased α-1 collagen production in MC-rich areas in vivo. [18] In addition, mMCP-4 was shown to activate progelatinase B (MMP9) early in tumor development, further inducing ECM remodelling. In turn, mMCP-4 and MMP9 activity led to release of bound pro-angiogenic compounds from the ECM, highlighting the tight link between tissue remodelling and angiogenesis.

Besides, there is a study that illustrates a clear feedback loop whereby mast cell recruitment by SCF can stimulate localized tissue remodelling, which in turn results in further SCF release and ultimately in modulation of local immune responses. Tumor-derived SCF both recruits mast cells to the tumor environment and also activates them. Thus, there is an additional requirement for SCF to activate recruited cells following their migration to the tumor site. SCF-stimulated mast cells release active MMP9 into the local environment, disrupting ECM and release further matrix-bound SCF, acting as a positive feedback loop on mast cell activation within the tumor [2].

Within the developing tumor environment, mast cells do not act alone. Mast cells have been recognized for their ability to recruit eosinophils and neutrophils and to activate adaptive T and B cell responses.

Angiogenic factors released by mast cells: Vascular endothelial growth factor (VEGF), Fibroblast growth factor-2 (FGF-2), Transforming growth factor-β (TGF-β), Tumour necrosis factor-α (TGF-α), Interleukin-8 (IL-8), Matrix metalloproteinase-2 (MMP-2), Matrix metalloproteinase-9 (MMP-9), Nerve growth factor (NGF), Platelet-derived growth factor (PDGF), Angiopoietin-1, Leptin, Adrenomedullin, Tryptase, Chymase, Histamine, Heparin [7].

c-Kit plays a critical role in the development survival and function of mast cells. c-Kit is capable of inducing hyperplasia, suppressing apoptosis, and increasing activation by Fc- RI-dependent mechanisms. c-Kit deficient mice lack mast cells.

It is important to note that in different tissues transferred mast cells may change their protease expression profile. Indeed mast cells phenotype is variable, profoundly altered according to their tissue localization. In line with this diversity, their effects on tumors may vary. Responses of tumor cells even to similar stimuli may differ.

Unlike many lymphocytes, mast cells are long-lived cells. Mast cells originate from the bone marrow, yet their final differentiation takes place in tissues and is influenced greatly by their interactions with those tissues [5].

So, mast cell phenotype can change markedly under different conditions. The *in vitro* activity of mast cells might be substantially different from *in vivo* activity, depending on specific environmental cues.

Mast cell-increased density has been demonstrated during the progression toward malignant melanoma. Thus, transformation of common nevi, penetration into the dermis, and subsequent metastasis formation in melanoma has been correlated to increased mast cell number. A study of endometrial cancer demonstrated a correlation between angiogenesis, measured as

microvessel counts and MC tryptase-positive cell counts and showed that these parameters increased with tumor progression.

The net effect on tumor growth is likely to be the result of multiple complex interactions between the various components of mast cell granules and adjacent stromal cells such as vascular endothelium and fibroblasts [22].

5. Lymphangiogenesis

The lymphatic system has traditionally been overshadowed by the greater emphasis placed on angiogenesis. In recent years there has been discovered a small number of potential lymphatic-specific markers [24]. These include: LYVE-1, a lymphatic endothelial receptor for hyaluronan, Prox1, a homeobox gene product involved in regulating early lymphatic development, podoplanin, a glomerular podocyte membrane mucoprotein which is also found on lymphatic endothelium, but not in blood vessels, 5'-nucleotidase, an enzyme whose activity is very high in the lymphatic capillaries, but much lower in the blood capillaries, and the vascular endothelial growth receptor-3 (VEGFR-3) which is a transmembrane tyrosine kinase receptor predominantly expressed on the lymphatic endothelium .

It is known that VEGF-C is synthesised as a prepropeptide which undergoes proteolytic maturation to a varying degree. These shorter peptides bind to VEGFR-3, but only the fully processed form can bind to VEGFR-2. In addition to promoting lymphangiogenesis, it has now been shown that VEGF-D promotes lymphatic metastasis [34].

The lymphatic vasculature is essential for tissue fluid homeostasis and cancer metastasis. Lymphatic vessels seem to originate from a subset of venous endothelial cells, which commit to the LEC(lymphatic endothelial cell) lineage. Tightly regulated and intricately interconnected signalling pathways control the directed proliferation, sprouting and migration of ECs that occur during lymphangiogenesis. At the heart of this signalling network are several factors which are instrumental.

Lymphangiogenesis relays on the interplay of several growth factors and receptors. Recent studies have shown that inflammatory cells, such as mast cells are recruited to tumors to stimulate lymphangiogenesis by a wide range of tumor cell–derived cytokines and growth factors, including VEGF-C and VEGF-D. Members of the vascular endothelial growth factor (VEGF) family, VEGF-C and VEGF-D, are thus far the best characterized lymphangiogenic factors that bind the VEGF receptor-3 (VEGFR-3) specifically expressed on LEC, and transduce proliferation and differentiation responses. In addition to VEGF-C and VEGF-D, it is also considered that VEGF-A stimulates lymphatic growth and the activation of its receptor VEGFR-2 seems to be required for LEC organization into functional capillaries. Indeed, although VEGF-A is considered a pro-angiogenic factor, several studies have reported that VEGF-A overexpression induced enlargement of lymphatic vessels and stimulation of lymphangiogenesis in experimental animals [3] and [11]. Furthermore, in an animal model of skin cancer, VEGF-A induced lymphangiogenesis and promoted lymphatic metastasis. As we can see, vascular endothelial growth factors and their endothelial tyrosine kinase receptors are central regulators of lymphangiogenesis [19].

VEGF-C expression during embryonic development is seen where the first lymph sacs develop and in regions of lymph vessel sprouting. Expression of VEGF-D, present in two splice isoforms, is detected in various locations in embryos, but is strongest in the heart, lung, skeletal muscle, colon and small intestine. The binding affinities of VEGF-C and VEGF-D for their receptors are regulated by proteolytic processing of the propeptides, with their affinity for VEGFR-3 increasing with processing, and only the mature forms binding VEGFR-2 [38]. Deletion of VEGF-C in mice leads to a complete absence of lymph vessels and embryonic lethality; the first lymphatic endothelial cells fail to migrate and proliferate to form the lymph sacs, but blood vasculature appears to develop normally [23]. VEGF-D, on the other hand, seems to be largely dispensable for the development of the lymphatic system[20], [33], [37]. In a recent study using a mouse model of lymph node removal, VEGF-C therapy was shown to regenerate

mature, functional collecting lymph vessels that could fuse to transplanted lymph nodes. In adult human tissues, VEGFR-3 is specific to the lymphatic endothelium, with the exception of some fenestrated and discontinuous blood capillary beds. Stimulation of VEGFR-3 protects lymphatic ECs from apoptosis and stimulates their proliferation and migration *in vitro.*

A recent study showed that VEGFR-2 signals result only in circumferential growth of lymph vessels. However, transgenic expression of the VEGFR-3-specific engineered ligand VEGF-C156S in embryonic skin leads to lymphatic hyperplasia with widened lymph vessels but seemingly much less sprouting than induced by VEGF-C in the same model, indicating that VEGFR-2 may have a role in activating lymphatic sprouting during embryonic development.

In both experimental and human tumours, VEGF-C and/or VEGF-D expression correlate with lymph vessel and lymph node involvement, distant metastasis and, in some cases, poor clinical outcome. In a recent study, the expansion of lymphatic networks within the lymph node was stimulated by VEGF-C overexpression even before the onset of metastasis, probably facilitating the spread of tumour cells [32]. The pressure gradient and lymph vessels situated at the tumour margin may be more important in spreading tumour cells than intratumoural lymph vessels. Lymphatic ECs send long filopodia towards the tumour cells which results in opening of the vessel lumen, allowing facilitated access of tumour cells. VEGF-C induces widening of the collecting lymphatic vessels, draining fluid from the immediate tumour environment and probably allowing for easier transit of aggregates of metastatic tumour cells.

6. Concluding remarks

Based on the discussed below, one could easily state that mast cells are not just peaceful bystanders in tumor progression. As shown, a pathogenetic loop of events leads to the progressive rise of mast cell number in the tumor focus, which once activated provide the nourishment resource necessary for the continuously growing tumor population by promoting vascular and lymphatic proliferation. Matrix remodeling and intercellular junction alteration induced by mast cells has lead to the idea that their activity adds to the chain of events leading to tumor invasion and metastasis. On top of that, the suppression T regulatory cell activity makes a potentially effective mechanism such as the immune response switch to a pathogenetic chain promoting mast cell activity. Their intricate role in different stages of tumor growth and progression may prove a starting point for tumor growth suppression, based on the idea that the block of one ore more of their effects via therapeutic methods might slow tumor progression, which in turn, would lead to a better outcome in clinical cases viewing neoplastic syndromes.

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DINAMICA EXPRESIEI FACTORULUI DE CREŞTERE AL ENDOTELIULUI VASCULAR-C ÎN LEZIUNILE PRENEOPLAZICE ŞI NEOPLAZICE DE CERVIX UTERIN

Vitalie Mazuru
Catedra Histologie, Citologie şi Embriologie USMF „Nicolae Testemiţanu”

Summary

Dynamics of the Vascular Endothelial Growth Factor C expression during the preneoplastic and neoplastic lesions of the uterine cervix

The spreading throughout the body of neoplastic cells, using different ways, is a characteristic hallmark for malignant tumors. The main importance of the lymphovascular route for metastasizing is well known for a long period of time. Discovering of the lymphatic vessels markers allowed scientists to understand more detailed the mechanisms of this phenomenon. It has been well established that VEGF-C (vascular endothelial growth factor-C) can induce the proliferation of lymphatic endothelial cells. *The aim*: to study the features of the VEGF-C expression during the progression of the uterine cervix neoplasia. *Material and methods*: the study was performed on 22 cases of squamous metaplasia, CIN I – 14; CIN II – 12; CIN III – 6; microcarcinoma – 15; invasive carcinoma – 32 cases. PT-Link LSAB+ immunohistochemical method was performed using anti VEGF-C (clone SC9047, Santa Cruz, USA, dilution 1:100) as a primary antibody. *Results*: the less numerous cases of positive reaction for VEGF-C were found in squamous metaplasia (27.28%). The numbers of positive anti VEGF-C cases increasing were dependent of stage of cervical lesion. In the invasive carcinoma stage all cases were positive for VEGF-C. The intensity of the VEGF-C expression was heterogeneous, but in the area of the invasive edge was strong in 100% cases. *Conclusions*: the intensity of the VEGF-C expression correlates with the stage of uterine cervix neoplasia progression. Vascular invasion is strongly associated to intensive expression of VEGF-C.

Rezumat

Răspândirea la distanţă, prin diverse căi, a celulelor neoplazice este o trăsătură caracteristică tumorilor maligne. Importanţa rutei limfovasculare în cadrul metastazării era cunoscută demult, însă odată cu depistarea markerilor imunohistochimici a fost posibilă studierea mai detaliată a fenomenului în cauză. Este bine cunoscut faptul că VEGF-C (factorul de creştere al endoteliului vascular-C) este mitogenul de bază pentru celula endotelială limfatică. *Scopul*: evidenţierea particularităţilor de expresie a VEGF-C în cadrul progresiei neoplaziei de cervix uterin. *Material şi metodă*: în studiu au fost incluse 22 cazuri metaplazie scuamoasă, CIN I – 14; CIN II – 12; CIN III – 6; carcinom microinvaziv – 15; carcinom invaziv – 32 cazuri. Studiul imunohistochimic a fost făcut prin metoda LSAB+, utilizând în calitate de anticorp