

THE LYMPHANGIOGENIC ROLE OF MAST CELLS IN PREMALIGNANT AND MALIGNANT LESIONS OF UTERINE CERVIX

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Summary

The role of mast cells in lymphangiogenesis, wasn't determined till now, this way, this article has the purpose to launch a theory which could demonstrate the correlation between the number and function of mast cells and the lymphangiogenesis in different types of uterine cervix lesions. The study's results have marked a considerable correlation between the number of mast cells and the number of lymphatic vessels from the superficial stroma of SIL and from the peritumoral areas from invasive squamous carcinomas.

Rezumat

Rolul limfangiogenic al mastocitelor în leziunile premaligne și maligne ale colului uterin

Rolul mastocitelor în limfangiogeneză nu este cert cunoscut până în prezent. Drept urmare, lucrarea dată are ca și scop punerea în evidență a posibilei implicări a mastocitelor, prin corelarea dintre numărul lor și densitatea limfovaculară la diferite etape ale neoplaziei de cervix uterin. rezultatele noastre au evidențiat o corelație semnificativă între numărul mastocitelor și densitatea vaselor sangvine din stroma superficială satelită leziunilor scuamoase intraepiteliale, cât și cancerului invaziv.

Introduction

The lymphangiogenesis is the process of “de novo” formation of lymphatic vessels, or from preexisting lymphatic vessels, being determined by the specific proliferative factors of the lymphatic endothelium. Among the major lymphangiogenic factors, can be named: PROX-1(Prospero homeobox gene-1) transcription factor, LYVE-1 (Lymphatic vessel-1), endothelial growth factors, synthesized also in lymphatic vessels: VEGF-A, VEGF-C and VEGF-D , which are included in VEGF group, which contains VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, integrine- α 9, FGFR-3, neuropiline-2; other factors, which have a lymphangiogenic role, are bFGF, angyopoiethyne-2, PDGF(Platelets derived growing factor). Prox-1 transcription factor is implicated in the initial lymphangiogenesis step, as it activates genes transcription for VEGFR-3 and FGFR-3, this factor is being activated as a response at cell's interaction with IL-3 and IL-7. VEGFR-3 is a receptor with tyrosine-kinase action and has VEGF-C and VEGF-D ligands, the last being a dispensable one, and VEGF-C is an essential factor in physiological lymphangiogenesis. After the complete maturation realized by limited proteolysis, VEGF-C and VEGF-D, become factors of angiogenesis. In the uterine cervix, these lymphangiogenic factors are synthesized by different cell groups: epithelial cells of intermediary layer of stratified squamous epithelium, which elaborates and secrete VEGF-C, VEGF-C VEGF-D macrophages, fibroblasts secrete specific fibroblast growing factors. The tumoral lymphangiogenesis has its specific aspects, being determined especially by VEGF-C, which is overproduced by malign cells from the invasion front.

Nowadays, it was demonstrated the statistically significant relation between the angiogenic tumoral profile, and the vascular micro-density. In comparison with angiogenesis, the knowledge about normal and pathological lymphangiogenesis (tumoral, lymphatic edema, chylothorax, inflammatory, Milroy syndrome), are deficient not only in what concerns the blood vessel formation, but also the complicated potential implicated in this process. Tumoral cells dissemination is controlled by mechanisms which include the local invasion, lymphosanguine spread, and the direct invasion in different cavities of the body. In almost all the gynecological malign tumoral invasions, the major way of malign cell spreading, is the lymphatic one(we know

as well that in the cancer of the uterine cervix, the lymphatic node estate, is a major factor in what concerns the diagnose and the therapeutic approach of the case). A sufficient amount of studies is accumulated, which demonstrates the tumoral cell dissemination, and their metastasis in regional lymphatic nodes (Gombos and collabs., 2005).

A possible lymphangiogenic role in the endothelial specific cytokines synthesis can be held by mast cells implicated in vasodilatation processes, the augmentation of vascular permeability, of proliferation and cellular motility. The mast cells, which are specific for the uterine cervix, are triptase-positives, and chimase-negatives, and are considerably bigger than mast cells which are associated to pulmonary stroma, or mammary gland's stroma. In several malign tumors, mast cells represent the principal source for VEGF (Chang and collans., 2006), but we should mention that these dates are correlated only at the angiogenic role of the mast cells.

This article is an author's tentative to elucidate, the eventual parallels between the lymphangiogenesis, and the mast cell density in LGSIL (Low Grade IntraEpithelial Lesions), HGSIL (High Grade IntraEpithelial Lesions), CIS (Carcinoma in situ), microinvasive carcinoma, and invasive carcinoma localized in the uterine cervix. This way, we used the immunostaining with D2-40 antibody, lymphatic vessels, and the mast cells marked with anti-mast cell's triptase antibody (anti-MCT). The results that were obtained were statistically analyzed, in order to evaluate the correlation between the mast cells and lymphatic vessels number.

The D2-40 antibody connects specifically with podoplanin proteine. Primarily identified as T1a/Aggrus/PA2.26/E11 in osteoblasts, osteocytes, renal tubes epithelium, keratinocytes fibroblasts, podoplanin is a transmembranous mucin-like glycoproteine, formed by 161 amino acids, 9 of them which form the intracellular domain, the extracellular being O-Glycosilated with syalic acid linked with galactose in the α -2,3 position, which form the biggest part of the protein. Recently, it was demonstrated the stimulating effect of podoplanin, in the malign tumor development by the introduction of trombocyte aggregate associated with tumoral stroke formation, which contributes to the epitheliomesenchymal transition, the last one being responsible of cellular migration, invasion, and metastasis. In addition, podoplanin alterates intercellular junctions, bringing its direct contribution to the cellular migration. Being synthesized in the endothelium of lymphatic vessels, and not synthesized in vascular endothelium, podoplanin is largely used like a specific marker for lymphatic endothelial cell identification.

The anti-MCT antibody is specific for MCT or MCP-6, a neutral protease, which is specific for mast cells and almost all the human basophiles, alike with tripsine. Triptase derives from serine-proteases group, has a 134 kD molecular mass, and it's stocked in mast cells in a form of granules, including more than 25% of the dry mass of the cell. MCT is able to stick with heparin and it's secreted in complex with it. Tripase has a proteasic action for the basal membrane's proteins surrounding blood capillaries endothelium, fibrinogen, collagenase, and on a big number of proteinases precursors, which take part of the group of matrix metalloproteinases (MMP). The lesion of the basal membrane's proteins by proteolysis, is on of the major lymphangiogenic factors. Besides the protease effect of mast cell's tripase, its mitogenic effect for fibroblasts, was also identified. Because of the fact that in tissues MCT is an enzyme that is specific only for mast cells, it's used like a specific marker for those immunomarcation.

Materials and methods

In the research cases of biopsies of uterine cervix resulted from patients with manifested clinical signs, were included. The specimens were obtained by laparotomy, conisation or biopsy puncture. After fixation in buffered formalin 10% for 24 hours, tissues were included in paraffin using standard methods. To establish the histopathological diagnosis, 5 μ m sections resulting from each case were colored using the hematoxilin-eozin method. From each case additional sections for imunocoloration, were selected. We used polyclonal antibodies F VIII (Dako

Cytomation, RTU), to mark endothelial cells from blood vessels, antibodies anti-D2-40 (Clone D2-40), for revealing endothelial cells from lymphatic vessels, and antibodies for mastocyte deriving triptase (Clone AA1), to visualize mastocytes. The antigens were exposed by warming in a microwave in citrate buffer pH=6.0 using the method of intermittent warming 6 times for 5 minutes, during 30 minutes, after blocking the endogenous peroxidase and incubation with primary antibody for 30 minutes, the LSAB+/HRP work system followed by the visualization with 3, 3'-diaminobenzidine. In a double immunocoloration case the second chromogen (D2-40,MCT) mastocytary triptase was colored in red by ethylaminocarbazol (EAC). The Counterstain was realized with Lille's modified hematoxiline. All histochemical steps were executed in an automatic system using DacoAutostainer. Morphological and immunohistochemical preparations were examined using a NIKON ECLIPSE E600 microscope equipped with camera, that allowed taking microscopic images. The analysis of the photographs and statistics was accomplished by using the Lucia G program.

Results

After the histopathological diagnostics of the preparations colored with hematoxilin-eozine, there were identified 15 cases of squamous metaplasia, 14 cases of cervical intraepithelial neoplasia (SIL), 3 cases of carcinomas "in situ" (CIS), 7 cases of microinvasive carcinoma, and 13 cases of invasive squamous carcinoma.

The immunomarked preparations analysis by immunohistochemical methods, has validated the presence of isolated mast cells in the agglomerations of epithelial tumoral cells from the squamous invasive tumors, in all the metaplastic squamous epithelium layers, but also in that neoplastic of the cervix, also in tumoral strokes from the lymphatic vessels of the peritumoral areas. In several cases, the cells from the peripheral tumoral zone, are D2-40 positive, alike with the immunophenotype of basal cells from the normal composed epithelium. The epithelial component from SIL often doesn't express podoplanin, those areas being visualized like areas of discontinuity of coloration with D2-40, positive in normal state, for the basal layer of the squamous stratified epithelium. D2-40 negative, are also the majority of cells from the tumor. A big number of mast cells disposed in a form of nests, were identified near lymphatic vessels in SIL cases, but also in invasive and microinvasive squamous carcinomas. The biggest part of the lymphatic vessels from the intratumoral stroma, but also the lymphatic vessels from above the CIN affected areas, or affected by microinvasive carcinoma and in situ carcinoma, are small, a part of them having a lumen, the others being collapsed. In these zones, in perfused vessels, frequently we can observe big lymphocytes agglomerations. In peritumoral areas, we can observe vessels with a well-formed lumen, having often a tumoral stroke in it. The bigger lymphatic vessels from the intratumoral stroma, are halfopened or totally collapsed and are disposed in a form of horseshoes surrounding tumoral cells or they are strongly apophisated and have no lumen.

In comparison with deep stroma of SIL, the microinvasive, and invasive squamous carcinomas, where mast cells don't present the degranulating reaction, in superficial stroma, the degranulating reaction is much more frequent, and the mast cells and lymphatic vessels are more numerous. The number of mast cells and intratumoral lymphatic vessels can be relationned to the size of malign tumoral cells conglomerates and to the quantity of stroma in the intratumoral area of invasive squamous carcinoma. The stromal component, being in a direct proportional relation with mast cell infiltration level and of penetration into the intratumoral area of lymphatic vessels, the size of tumoral areas being indirectly proportional. The immunostained preparations study has elucidated the numeric and percental proportionality between mast cells and lymphatic vessels of each type of lesion (Tab. 1). The maximal number of mast cells (n=283) is identified in microinvasive carcinoma, and the maximal number of vessels in peritumoral area of squamocellular invasive carcinoma (n=31).

Table 1

Lesion type	Average number of lymphatic vessels	Average number of mast cells	lymphatic vessels,%	Mast cells,%
SM	6.73	87.99	11,88	18,40
SIL	16.08	104.81	28,39	29,92
CIS	8.33	78.17	14,71	16,35
MIC	15.71	120.14	27,74	25,13
ISC	9.78	87.03	17.27	18,20

As we estimated the correlation between the number of mast cells and lymphatic vessels in each case from a lesion in Lucia G. program, we can mention that the level of correlation by Pearson's model, is significant in SIL lesions where the correlation is 0.858 and $p=0.001$, and in invasive squamous carcinomas of 0.894 with $p=0.001$.

Discussions

The results of this study, have demonstrated that in premalignant lesions of the cervix, in the superficial stroma of it, the big number of mast cells, is associated with a big number of small lymphatic vessels, and in the stroma of malignant lesions and the epithelium with squamous metaplasia, there is a low level of mast cells and lymphatic vessels, the last being in conformity with the dates from the bibliography of specialization. The situation of mast cells in different layers of the metaplastic epithelium, from the epithelium from SIL and among the epithelial malign cells of the invasive squamous carcinoma, can serve as proof of the proteolytic function of MCT. Instead of that, the function of the mast cells situated among the epithelial cells, isn't elucidated. The significant correlation between the number of lymphatic vessels and that of mast cells from the peritumoral areas, in invasive carcinomas, in comparison with the squamous metaplasia, and with the carcinoma "in situ", may generate the supposition that mast cells have a limphangiogenic role in this kind of lesions. The correlation between a big number of mast cells and lymphatic vessels can be noticed in many sources, but these notes weren't correlated.

It's unquestionable the importance of the basal membrane lesion for the tumoral cell to invade the vessel. One of the factors which initiates and guides this process, is MCT, this fact being demonstrated during "in vivo", but also in experimental models of angiogenesis(). In the obtained results, we have identified mast cells with a degranulated pattern in the stroma that corresponds to the premalignant lesions of the cervix. Taking into consideration the fact that in tumoral affections of the cervix, the lymphangiogenetic switch, appears at SIL stage (), we'll consider naturally this functional activation of mast cells by degranulation, particularly in this case of lesions.

Conclusions

For lack of sources of information which could affirm or negate the implication of mast cells in lymphangiogenesis, this work represents the first step in the tentative of finding an eventual implication of mast cells in the process of lymphangiogenesis connected with lesions of cervix, by the correlation of the number of mast cells with the number of lymphatic vessels. This study is more a constataion of facts at the observation level of several processes which couldn't be described and explicated in the bibliography of specialization, till this moment, there are phenomena which include: the presence of mast cells among the tumoral strokes of the epithelial cells or the epithelial compartment from SIL, squamous metaplasia or CIS.

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ADAPTATION OF THE HUMAN BODY TO MECHANICAL IMPACT

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Summary

The action of mechanical factors on the human body is reflected, whether it occurred spontaneously or was used deliberately by people from certain social reasons, such as habits of some people or for commercial purposes. Attention is pointed on the necessity of advocacy among population for prevention and timely treatment of diseases caused by the action of mechanical factors on the human body.

Rezumat

Adaptarea corpului uman la acțiunea mecanică

În articol se reflectă acțiunea factorului mecanic asupra corpului uman, indiferent dacă acest a apărut spontan sau a fost folosit de către oameni în mod conștient, din anumite motivele sociale, cum ar fi obiceiurile la unele popoare sau în scopuri comerciale. Se accentuează atenția la necesitatea lucrului de iluminare sanitară cu populația pentru prevenirea și tratamentul unor maladii cauzate de acțiunea factorului mecanic asupra corpului uman.

Novelty of Theme

News of the problem is that doctors need to use more capacity to modify the human body by mechanical action in order to correct developmental mistakes and to create conditions for social and psychological comfort to people.