===================== HYPOTHESIS =====================

Preniche as Missing Link of the Metastatic Niche Concept Explaining Organ-Preferential Metastasis of Malignant Tumors and the Type of Metastatic Disease

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Abstract—Here we attempt to supplement the metastatic niche concept with a stage of "preniche" that determines the site of development of a premetastatic niche and of a subsequent metastasis. The "preniche" includes all cellular and molecular events in the site of a prospective metastasis preceding the entrance of myeloid progenitor cells. The "preniche" integrates an activation of vascular endothelium of the microcirculatory vessels of target organs in the site of a future metastasis under conditions of chronic persistent productive inflammation that can be induced by cytokines from the primary tumor and independently of it. The endothelium activation is responsible for adhesion and clustering of the recruited myeloid progenitor cells and also for the retention of cells of malignant tumors. The preniche easily arises in organs enriched with organ-specific macrophages (lungs, liver, brain, etc.) where the endothelium is predisposed for intensive recruiting of myeloid progenitor cells of macrophages, especially under conditions of inflammation. Introduction of the "preniche" concept allows us to avoid difficulties associated with the development of the metastatic niche concept, especially concerning the problem of organ-preferential localization of metastases, and to make some predictions for experimental verification and potential approaches for preventing metastasizing in some oncologic patients.

It seems that the abundance of special information about mechanisms of tumor progression and the role of various molecules in metastasis obtained with different model systems of human blastomas rather prevents than promotes understanding of carcinogenesis and especially the control of tumor growth. There are different approaches to systematizing such information. We think that searching for prototypes of physiological reactions among pathological processes can be a rather promising approach. This approach is now not very popular in the case of metastasis of malignant tumors, which often appears to be a cascade of molecular processes as if created by Nature purposefully to generalize malignancies. But the "physiological approach" allows us not only to remove this apparent uniqueness of processes associated with tumor progression but also to subordinate different mechanisms involved in metastasis; this approach can also reveal yet unknown aspects of this process and pathways to control tumor dissemination.

The recently proposed concept of metastatic niches [1-6] can be very helpful in searching for physiological prototypes of metastasis [1-6]. This concept allowed us to

Abbreviations: HPC, hematopoietic progenitor cells; ICAM1, intercellular adhesion molecule 1; LOX, lysyl oxidase; MIF, macrophage migration inhibitory factor; MIP2, macrophage inflammatory protein 2; MMP9, matrix metalloproteinase 9; SAA3, serum amyloid A3; SDF1, stroma cell factor 1; TGFβ, transforming growth factor β; Ti+, tumor producing a powerful pool of proinflammatory cytokines; Tmh, hybrid cell clone resulting of fusion of macrophage and tumor cell; TNFα, tumor necrosis factor α; VAP1, vascular adhesive protein 1; VCAM1, vascular cell adhesion molecule 1; VEGFA, vascular endothelium growth factor A; VEGFR1+, vascular endothelium growth factor receptor 1; VLA4, integrin receptor "very late antigen 4".

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quite otherwise elucidate many problems associated with metastasis and explain experimental data that could not be interpreted earlier. Although the concept of metastatic niches still has many blank spots, its development (especially on searching for a probable physiological prototype of the metastatic niche) can be very promising for comprehension of such problem of oncology as organ-preferential localization of metastases. But it must be stipulated beforehand that in the present work, first, it is admitted that metastasis of all, or at least the majority, of carcinomas and melanomas can be described by the concept of metastatic niches (although the available experimental and clinical data concern only a limited range of studied tumors) and, second, mesenchymal tumors will be deliberately not considered because by now about them there are no data necessary for the theory of niches. A clear subordination of the metastasizing stages is also emphasized — in the present paper we shall speak only about processes preceding formation of micrometastases leaving aside the problem of formation of a macroscopic node of a secondary tumor.

ORGAN-PREFERENTIAL LOCALIZATION OF METASTASES

Although there is no organ which would be absolutely protected against development of metastases of malignant tumors, such metastases are relatively often developed in a rather limited number of "typical" localizations: regional (with respectively to the primary tumor location); lymphatic nodes (lymphogenous metastases); lungs, liver, bone marrow, and brain (hematogenous metastases); peritoneum and pleura [7-9]. Much less often hematogenous metastases are found in kidneys, gonads, spleen, subcutaneous fat tissue, and extremely seldom in the walls of the gastrointestinal tract, uterus, heart, and skeletal muscles. It should be noted that in the overwhelming majority of cases metastasis into atypical locations is associated with the generalization of the process affecting many organs and tissues. However, the spleen is an interesting exception. This organ is rarely damaged by macrometastases, except for the cases of generalized tumors (especially melanomas), but it has been shown earlier that micrometastases in the spleen occur rather often, whereas is muscles micrometastases are virtually not found [10].

There is no doubt that localization of metastases is partially associated with specific features of the lymph and venous blood outflow from the region of the primary tumor location. Just this determines the development of lymphogenous metastases into the regional lymph nodes and of hematogenous metastases of abdominal cavity organ tumors (stomach and pancreas carcinomas, colorectal cancer) into the liver. However, it is impossible to explain the localization of metastases only by specific features of the vascular system responsible for delivery of tumor cells to the site of metastasis. Thus, the bone marrow and liver are usual sites for hematogenous metastasis of kidney tumors, although these organs are not located on the pathway of venous outcome from the liver. Mechanisms responsible for differences in organ vulnerability are intensively discussed in the literature, but there is still no integral concept describing the causes of organ-preferential metastasis.

CONCEPT OF METASTATIC NICHES: ITS STRENGTH AND WEAKNESS

The presence in blood of circulating tumor cells not always leads to development of macro- and micrometastases in target organs [11-14], and experimental works have shown the absence of a direct and constant correlation between the ability of endothelial cells to constitutively express selectins halting tumor cells and the adhesion of these cells on the endothelium, on one hand, and the sites of preferential localization of metastases, on the other hand [15]. Even more interesting is the discovery of a phenomenon of "inefficient metastasis" when immigrated tumor elements are present in the target organs but fail to produce metastases [12, 13]. These findings clearly suggest that formation and localization of micrometastases are more likely determined not by the presence of tumor elements in the blood flow but rather by some specific features of target organs (including those arising under the influence of the primary tumor) that are responsible for occupation of a suitable organ by blastoma cells and formation from them of a micrometastasis [16, 17]. The same findings also show that the halting of tumor cells in the target organs without some additional conditions is yet insufficient for development in them of metastases. This so-called "seed and soil" hypothesis was proposed by Stephen Paget in the beginning of the last century [1, 18], but only recent data filled it with concrete content. Researchers of D. Lyden's group have established that the development of micrometastases in target organs is preceded by the accumulation in them of cells immigrated from the bone marrow and creating a stromal microenvironment that is adequate for the tumor and determines the development of metastases [2, 19-21]. To describe this process, the concept of "niche" was proposed, borrowed from hematology where it was used for description of microenvironment that regulates the proliferation, homing, and differentiation of stem cells [22, 23]. Lyden's concept suggests the formation and step-by-step changes in the site of a future metastasis of the following forms of microenvironment: a premetastatic niche with bone marrow precursor cells without tumor elements; a micrometastatic niche characterized by the presence of a cluster of immature bone marrow and tumor cells; a macrometastatic niche with angiogenesis added to the preceding processes [1, 24].

According to Lyden's theory [1, 5], the formation of a micrometastatic niche is determined by several processes:

— first, the primary tumor cells capable of secreting the cytokine VEGFA mobilize from the bone marrow hematopoietic progenitor cells (HPC) (VEGFR1$^+$) into the peripheral blood flow; on the surface of these cells there is an integrin VLA4 interacting with fibronectin and thus promoting the homing of HPC [20, 25-28];

— second, fibronectin is accumulated in the sites of future metastases. This fibronectin is synthesized *in situ* by fibroblasts and seems to be also produced in the primary tumors, released in the blood flow, and accumulated in the target organ [26, 29];

— third, VEGFR1$^+$ HPCs due to the VLA4$^+$ receptor migrate into the sites of fibronectin accumulation where they form a cluster of immature cells. Note that the phenotype of these cells is significantly overlapped with the phenotype of macrophage series cells with different maturity. Thus, a premetastatic niche is formed. Formation of the premetastatic niche is also promoted by other factors that are secreted by the primary tumor and *in situ* (LOX, MIP2, MMP9, KIT-ligand, TGFβ, TNFα) [1-4, 30, 31];

— fourth, tumor cells and macrophages are recruited into the produced cell cluster (into the premetastatic niche) due to chemokines (serum amyloid component SAA3, chemokines S100A8, S100A9, and SDF1) synthesized by these cells [1-4, 32]. These cells are also supplemented with elements of the fibroblast series, and this results in formation of a full micrometastatic niche capable of providing for survival and proliferation of tumor cells.

Due to introduction of HPCs as a new messenger, the concept of metastatic niches allows us to remove the contradictions enumerated in the beginning of this section. This concept also opens great prospects for control of metastasis. However, the metastatic niche theory is still not a completed concept. In particular, it remains unclear what specific events trigger the formation of a metastatic niche, i.e. lead to accumulation of fibronectin and changes in the endothelium favorable for HPC homing. The formation of a metastatic niche is usually described as a process depending on the primary tumor [1-6]. However, it is well known from experimental oncology that intravenous injection of cells of some tumors can induce development of metastases in internal organs, and in some cases with a rather specific (mono-organ) location. This indicates that metastatic niches can be formed due to processes independent of the development of the primary tumor node, but due to some other physiological or pathophysiological reaction. Note that the theory of metastatic niches describes the formation of metastases in general not considering the question why metastases are formed mainly in particular "typical" sites and why the location of metastases and the type of metastatic disease

vary in different patients. The known factors synthesized by the tumor and regulating the development of the niche (LOX, MIP2, VEGFA, TGFβ, TNFα) are not organ-preferential [33-35]. Nevertheless, the groups of D. Lyden and of Y. Maru have attempted to study this problem experimentally [1, 3]. Mice with grafted Lewis lung carcinomas were injected with conditioned medium from melanoma B16 cells characterized by generalized non-selective metastasis, and as a result metastatic niches and micrometastases developed in various organs and tissues, including such atypical locations as the oviducts [1]. However, the molecular mechanisms underlying the phenomenon observed by D. Lyden's group are still unclear.

We think that the questions presented above might be answered taking into account the events preceding the formation of a premetastatic niche as it is and to suppose that these events should be based on a physiological or pathophysiological process more or less independent of the development of the primary tumor node. The overall *in situ* conditions that precede the formation of a premetastatic niche (recruiting HPCs into the target organ) and determine the localization of a future metastasis is reasonable to term as a "preniche". It is important to note that we think the preniche plays a key role in HPC homing and also is essential for emigration of tumor cells from the blood flow.

PRENICHE: PHYSIOLOGY AND PATHOPHYSIOLOGY

Considering the most frequent locations of metastases, it becomes evident that they have one feature in common: they have a large pool of organ-specific macrophages (Kupffer cells in the liver, alveolar macrophages in lungs and microglia in the brain, peritoneal and bone marrow macrophages, lymph node macrophages) [36, 37]. This feature is not characteristic of the heart, gastrointestinal tract organs, skeletal muscles, kidneys, or gonads — and in these organs solitary metastases occur relatively seldom. Obviously, the endothelium of these organs has to be adapted to an active immigration into them of macrophage precursors under both normal and inflammation conditions when the need for restitution of the physiological macrophage pool is especially strong [38].

It seems very likely that regeneration of specialized macrophages should occur not only due to mature monocytes of peripheral blood but mainly due to myeloid progenitor cells that are present in the blood circulation [17, 39-43] and capable of specific differentiation under the influence of specific microenvironmental factors in the correspondent organs. And just these cells can form a premetastatic niche. It seems that under normal conditions they can emigrate from the blood flow at a low frequency, but this emigration can markedly increase on

development in an organ of a chronic persistent inflammation. If the mechanism of restitution of specialized macrophages corresponds to that described above, the endothelium of these organs has to constantly express certain adhesive molecules providing for the recruiting of myeloid progenitor cells; and under conditions of inflammation in the sites of "typical" metastasizing the endothelium reaction has to qualitatively and/or quantitatively differ from the reaction of the microcirculatory vessels, e.g. of the heart.

In fact, some observations confirm that such organs as the liver and lungs have leukocyte recruiting mechanisms other than those of "usual" tissues [44]. On the liver and bone marrow endothelium such molecules as ICAM1, VAP1, SDF1, and P- and E-selectins are presented constitutively, and they can be important at certain stages of HPC homing [39-41, 44-48]. Activation of HPC homing under conditions of inflammation in organs with "typical" metastasizing seems to be caused by an additional expression of VCAM1 type proteins that together with fibronectin are major molecules interacting with integrin VLA4 on the surface of HPCs [49, 50]. Thus, the innate increased ability of the microcirculation vessels for HPC homing seems to be the first physiological component of a preniche. However, the ability of the endothelium for intensive selective adhesion of HPCs in organs containing specialized macrophages is insufficient for formation of preniches because HPCs normally must rapidly differentiate into macrophages and are not accumulated in the tissue. We supposed that for development of premetastatic niches the prerequisite should be the formation of a cluster of immature HPCs capable of being committed by the tumor cells and of creating, under their influence, a microenvironment that would be adequate for the development of metastasis. We think that this occurs due to another already pathophysiological component of the preniche due to development of a persistent chronic productive inflammation when the formation of a cell cluster is due to their accumulation within the inflammatory infiltrate under the influence of MIF-type factors. Consequently, the niche formation in organs with "typical" metastasizing seems to depend, first, on the adhesion molecules constitutively synthesized by the endothelium and increased ability of the microcirculation vessels for homing and accumulation of HPCs; and, second, on development of persistent chronic productive inflammation providing conditions necessary for HPC cluster formation. A full value preniche can arise when these two conditions are combined.

We consider it very important that under conditions of inflammation immature myeloid cells can be recruited as material for commitment into organ-specific macrophages and replenishing the pool of local macrophages only in organs where such pool is rather large. Therefore, under conditions of adequate inflammation, HPCs can be recruited only in these organs. For metastasis in atypical locations, such as the heart or kidneys, as well as during generalized metastasis, a superphysiological activation of the endothelium in corresponding organs is necessary, which would result in appearance of a receptor phenotype (and in saturation of the interstitium with fibronectin) similar to that in the organs with "typical" metastasis under conditions of adequate inflammation. This can occur either during a long-term local productive inflammation [51-53] or as a result of systemic cytokine stimulation similar to that, which is observed on development of the syndrome of systemic inflammatory response [54].

In such cases, under conditions favorable for HPC accumulation and clustering, a full value preniche is also produced, and under conditions of systemic cytokine activation any site of the organism's microcirculation vessels can become a preniche. Obviously, HPCs must be constantly present in a certain amount in peripheral blood and also be mobilized from the bone marrow under the influence of VEGFA, which is known to be synthesized in inflammation foci [18].

The extreme importance of a persisting chronic inflammation for the preniche formation is supported by many indirect data. First, organs with typical metastasizing, such as lungs, liver, lymphatic nodes, and serous membranes, often contain so-called cold lymphohistiocytic infiltrates even in patients and SPF-laboratory animals without clinically detectable signs of disease (in the brain where such morphological findings are relatively rare the role of "cold infiltrates" can be due to microglial reactions) [55-57]. These infiltrates can be caused by a persisting infection, by an alteration due to a transient ischemia, etc. Second, histologically metastatic niches are formed in peribronchial zones of the lungs or in periportal zones of the liver [1, 5] where inflammatory infiltrates of these organs are usually developed [38]. Third, it has been known for long that tumor metastasis into atypical regions often coincides with the presence in these organs of a long-standing chronic inflammation ("metastasis into scars") [51-53, 58]. Fourth, all molecules known to participate in the niche formation are factors involved in development of inflammatory reactions that occur in the absence of any tumorigenesis (e.g. in psoriasis [59]). Fifth, non-steroid anti-inflammatory drugs are shown to be successful in inhibiting tumor metastasis into lungs (e.g. Lewis carcinomas) [60].

It should be emphasized that not all foci of a chronic productive inflammation possess a complete set of features necessary for formation of a preniche. Thus, in some cases the infiltrate can be a result of an effector immune reaction either of the Th1-type or of the Th2-type with a corresponding set of cytokines [61-63]. Participants of chronic inflammation can be M1- or M2-type macrophages, etc. [64-67]. It is reasonable to expect that such essential differences can influence the ability of the inflammatory infiltrate to function as a preniche.

Such speculations are appropriate as the comparison of organs with constitutive macrophages sharply different in probability of development of hematogenous metastases (liver, lungs, bone marrow on one hand; spleen on the other). Later on it will be necessary to more precisely determine the cell composition and intercellular relations within inflammatory foci (including organs with a constitutive pool of macrophages) that limit the formation of preniches and niches.

marrow, which is frequently affected by metastases, constitutively expresses both E-selectin and SDF1 (which is important for the cell rolling change-over to stable adhesion to the endothelium), i.e. the whole receptor apparatus required for homing tumor elements [48, 74]. Having in mind that the bone marrow is a source of HPCs, it can be considered to be a persistently acting as a constitutive premetastatic niche that does not need inflammation for arising.

PRENICHE AND RECRUITING CIRCULATING TUMOR CELLS

The metastatic niche concept suggests that HPC clustering should precede the formation of a micrometastasis. But this does not mean that tumor cells cannot be recruited into tissues before the formation of premetastatic niches. On one hand, tumor cells are known to enter the circulation long before the formation of a clinically detectable metastasis, but on the other hand there are many adhesive molecules capable of retaining circulating tumor elements in a particular organ. These molecules can be both constitutive and induced first of all due to development of an inflammatory reaction. Both constitutive and activated (by proinflammatory cytokines, metalloproteinases, hypoxic factors) expression of P- and E-selectins [68, 69], VCAM1, ICAM1 [70], and SDF1 is well known to promote the adhesion and homing of tumor cells [38-40, 43-47, 54-58]. There are very interesting observations that E-selectin responsible for the initial stage of adhesion and expressed only on the endothelium retains its activation also in foci of chronic inflammation [71]. And due to coincidence of some participating molecules, the tumor elements can be retained just in the sites with a preformed preniche (a chronic persistent inflammation in a site of "typical" or "atypical" metastasis) or in sites with conditions favorable for its arising (constitutively expressed adhesive molecules in sites of "typical" metastasis) [72, 73].

Thus, not only HPCs but also tumor cells can be accumulated in the sites of the future metastasis due to arising in them of preniches. Certainly, not every locus containing adhesive molecules for tumor cells can also recruit HPCs. Therefore, we think that the retention of tumor cells in the site of development of an acute inflammation in an organ "atypical" for metastasis or due to constitutive ligands in the absence of inflammation will not lead to metastasis. Nevertheless, such a locus can retain tumor cells ("inefficient metastasis") in the G_0 phase of the cell cycle for an indefinitely long time, until conditions suitable for recruiting HPCs develop in this place. This phenomenon, at least in some cases, seems to underlie the so-called late metastases observed tens of years after the extirpation of the primary tumor. Note also that the microcirculatory system of such organ as bone

PRENICHE AND PRIMARY TUMOR

For the "preniche" concept under consideration it is important that in some cases conditions determining the development and localization of future metastases do not depend on influences of the primary tumor node. In fact, chronic persistent inflammation underlying the formation of a preniche and then of a premetastatic niche can arise long before the development of the primary tumor. Just the presence of preexisting inflammatory foci can be an explanation of arising of metastatic nodes in the lungs and liver of laboratory animals injected with a suspension of tumor cells without "preconditioning" by the primary tumor. But this does not mean that the preniche cannot be initiated under the proinflammatory influence of the primary tumor. We think that in the above-cited experiment by D. Lyden [1, 2] the injection of the medium from the melanoma B16 cell culture (a tumor characterized by wide and nonselective metastasis) has demonstrated just a possibility of formation of a preniche (and later also of a niche) under the influence of factors secreted by the primary tumor. Some data confirmed that the presence of a systemic inflammatory response determined by the C-reactive protein level in oncological patients was associated with an increased probability of tumor dissemination and bad prognosis [75]. But because proinflammatory cytokines are not organ-preferential, they cannot induce the formation of solitary or multiple metastases in a certain organ but can be responsible only for development of "generalized" preniches and nonselective generalized metastasis.

Thus, the "secretory" phenotype of the primary tumor can determine only a general type of metastatic disease according to the "all or nothing" principle − the disseminated metastasizing of tumors capable of secreting proinflammatory cytokines (it is reasonable to term them "inflammatory tumor", Ti+) or the absence of such metastasizing in tumors unable to systemically activate the endothelium (Ti−). Note that the inflammatory activation of the endothelium and formation of preniches seem not only to arise due to the distant secretion of cytokines but can be also provoked *in situ* by Ti+ cells occurring at sites of the future metastases owing to adhesion and homing on interaction with the constitutive receptors or simply because of a mechanical "sticking" in

the microcirculatory system. The formation of solitary and multiple mono-organ metastases is determined only by local processes of persisting chronic inflammation independently of proinflammatory cytokine influences of tumor cells.

"NICHE AS IT IS" IS A MACROPHAGE "HYBRIDOMA"

We were considering the metastasizing processes taking as axiom that clustering HPCs should be a key condition for survival of tumor cells and formation of a micrometastasis. However, there is at least one exception when a tumor cell seems to have no need in a classical premetastatic niche and, consequently, also in a preniche. More and more evidences are accumulated that a tumor cell can *in vivo* form hybrids with macrophages or with immature cells of the macrophage series, and that this process can significantly influence tumor progression [76-78]. In fact, it is reasonable to suppose that such tumor—macrophage hybridoma (Tmh) should be able to express surface molecules and soluble factors inherent in macrophages and, respectively, to perform their functions as a regulator of the stromal microenvironment. It seems very likely that hybridization with the tumor cell committed the resulting hybridoma for requirements just of the tumor parenchyma. Thus, first, Tmh as a cell of the macrophage series has a broad ability for homing and can occupy both typical target organs and various inflammation foci in the sites of "atypical" metastasis; second, not needing HPCs, Tmh can independently form multiple metastases in these organs (but without generalized nonselective metastasis). If such tumor has the Ti+ phenotype, it will inevitably take the pathway of nonselective generalization of tumorigenesis.

PRENICHE AND FEATURES OF FORMATION OF METASTASES

On describing the essence of the "preniche" concept, we have already mentioned different clinical variants of metastatic disease. To more clearly demonstrate how different variants of metastasis follow from the preniche concept, we shall attempt to present them briefly and generally.

Local and multiple metastases arise in several situations:

— first, in all cases of productive (subacute and chronic) inflammation in organs which possess specialized macrophages. Metastases are formed in "typical" sites (liver, lungs, brain) and, depending on localization and spreading of the inflammation and also on the intensity of mobilization of HPCs into the blood flow, can be solitary or multiple, mono-, two-, three-organ, etc.

Although metastases into lymph nodes are formed first of all due to lymphogenous dissemination of tumor cells, the mechanism of preniche formation in them can be similar to that which acts in other organs possessing a specialized pool of macrophages;

— second, metastasis occurs on development of productive (subacute and chronic) inflammation in the sites of atypical localization of hematogenous metastases ("metastasis into scars"), adhesion in them of Ti– cells and a pronounced activation of the endothelium recruiting HPCs from the blood flow, and also in the case of VEGFA secretion in the tumor or in the inflammatory focus mobilizing HPCs from the bone marrow. In such cases metastases are limited only by the inflammatory focus location or by the bone marrow where metastases seem to always occur;

— third, local metastases are formed at the primary adhesion of TmhTi– in foci of productive (subacute and chronic) inflammation. In such cases a micrometastasis can be formed in the absence of a preceding cluster of myeloid progenitor cells. The clinical consequence is a formation of metastases in "typical" sites and in every focus of chronic inflammation.

Generalized nonselective metastasis is developed on secretion of proinflammatory cytokines and cytokines recruiting myeloid progenitor cells in the primary tumor Ti+ with an inflammatory activation of the microcirculatory vessels of all organs or at the primary disseminated adhesion of TmhTi+.

The absence of metastases may be declared only by convention, because micrometastases always seem to form in the bone marrow and individual vagabond tumor cells can be retained for a long time in other organs. Clinically detectable metastases can appear from these foci first of all depending on angiogenesis. However, the absence of clinical manifestation of extramedullary metastases can also be associated with a "metastatically inefficient" state of the tumor cells inside a target organ until a full-value niche is formed in the site of their localization.

PREDICTIONS FOR EXPERIMENTAL TESTING OF THE PRENICHE CONCEPT

The preniche concept allows us not only to explain some important observations of clinical and experimental oncology but also to utter some speculative predictions that would promote the testing and refining of this hypothesis:

— myeloid progenitor cells should be regularly detected in foci of chronic inflammation in the liver, lungs, lymphatic nodes, serous membranes, and in foci of microglia reaction in the brain, but should be virtually absent in acute myocarditis, myositis, or nephritis;

— injection of individual cytokines or of conditioned medium from stimulated cells involved in chronic inflam-

mation should induce in the organism a generalized formation of premetastatic niches and abolish the organ-preferential metastasis;

— a certain number of myeloid cells that are niche precursors should be found in the blood flow, especially if a chronic inflammation focus is present in the organism;

— the adhesion of myeloid progenitor cells onto the microcirculatory channel endothelium of the main target organs of metastasis under conditions of a long-term chronic inflammation should occur much more actively than onto the endothelium of such organs as the heart and skeletal muscles;

— the adhesion of macrophages onto the endothelium of different organs altered under the influence of a chronic inflammation should occur much more intensively than the adhesion of immature myeloid cells;

— the elimination of chronic inflammation foci under conditions of low secretion of proinflammatory cytokines by the tumor should lead to suppression of metastasis, and, on the contrary, the presence of chronic hepatitis or peribronchitis should promote the formation of metastases in these organs. (But note that in such studies prednisolone must not be used because it mobilizes myeloid cells from the bone marrow and, therefore, can promote metastasis).

Thus, in general, adaptation of tumor elements in the sites of metastasis seems to include the following stages: a preniche that is a totality of cellular and molecular events developed in the site of the future metastasis development previously to entrance into it of myeloid progenitor cells; a premetastatic niche mainly characterized by the presence of a cluster of myeloid progenitor cells without tumor elements; a micrometastatic niche characterized by the presence of a cluster of myeloid progenitor cells with tumor elements; a macrometastatic niche possessing characters of the micrometastatic niche plus the presence of myeloid progenitor cells of the endothelium and initial manifestations of angiogenesis (the second to fourth stages are the essence of the concept of D. Lyden et al.).

Thus, the search for elements of pathophysiological reaction, i.e. an organ-specific response of the endothelium to chronic inflammation involved in the development of tumor progression and the "preniche" stage proposed on its basis allowed us to complete the concept of metastatic niches: to explain the sites of the most frequent localization of solitary metastases, the formation of metastases in laboratory animals injected intravenously with tumor cells, the cause of different types of metastasis (solitary metastases, multiple metastases in the same organ, or a generalized dissemination throughout the whole organism), and to determine the role of the primary tumor and of the target organ in these processes. Moreover, this hypothesis allowed us to propose some concrete ideas for experimental testing. If the preniche concept is confirmed, it will lead to creation of schemes of anti-inflammatory therapy preventing development of metastases (at least in patients bearing tumors with a low ability for secretion of proinflammatory cytokines), and this will be important for practice.

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