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Cross-Talk between Tumor Cells and the Microenvironment at the Metastatic Niche

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Abstract

This review presents recent information about the cross-talk between the tumor cells and the microenvironment in the target organ of metastasis at the premetastatic and metastatic stages.

The development of metastatic foci is driven not only by the tumor cells intrinsic properties, but also by the interplay with resident and foreign cells located at particular niches in the target organ. The primary tumor modulates the metastatic target through the production of soluble factors that mobilize cells from distant organs like the bone marrow, which in turn localize in the metastatic niche. There is also strong evidence indicating that some primary tumors induce a fertile ground for the tumor cell at the target organ even before the arrival of the disseminated tumor cell (premetastatic niche).

The relationship between the players of the metastatic setting is dynamic and shows a high degree of plasticity. Tumor cells change through the acquisition of genetic and/or epigenetic alterations that provide adaptive advantages and the metastatic niche is remodeled by incoming cell types or newly secreted soluble mediators, as a result a reciprocal dialogue is established that invokes new levels of molecular and cellular complexity.

Unraveling the mechanisms that sustain the metastatic niche will allow a better understanding of the biology of the disseminated tumor cell, the design of new therapeutic approaches and, hopefully, the improvement of cancer patients' survival.

Keywords: bone marrow derived cells, metastatic niche, microenvironment, premetastatic niche, seed and soil, target organ.

Introduction

It is highly accepted that cancer is a systemic disease, since it entails abnormalities that extend beyond the local phenomena of the primary tumor. Metastasis is the process by which tumor cells from a primary tumor spread to remote organs. It is well known that metastasis mainly account for the morbidity and death of cancer patients. Consequently, further insight into the mechanisms of metastasis is critical to improve the prognosis and treatment of malignant disease.

Metastasis can be described as a cascade of multiple sequential steps, each one implying different biological properties that the tumor cells have to accomplish [1]. Following oncogenic transformation, the incipient primary tumor can grow progressively until a limited size and after that, it relies on the induction of angiogenesis to support its metabolic requirements and further growth. The first step away from the site of origin is achieved by the process of invasion, which, in the case of singe cell invasion, requires the detachment of individual tumor cells from the tumor mass. However, the tumor can maintain the cell- cell adhesion and push forward as a whole in the collective cell invasion strategy [2]. In general, distant organs can be reached by malignant cells only by the circulation through lymphatic or blood vessels, thus intravasation is mandatory. Dissemination succeeds if once inside the vessels, malignant cells survive while they circulate, reach the target organ and extravasate into the new microenvironment. Different organs are not equally prone for the development of metastasis, a fact that has intrigued researchers since early years. In 1889, Stephen Paget queried about the distribution of metastasis throughout the body as a matter of chance. In his study of fatal cases of breast cancer, he noticed that the liver had a strong propensity to be seat of secondary growth compared to any other organ. From this and other careful observations, he proposed the 'seed and soil' hypothesis. Just as the "seeds are carried in all directions", the tumor cell can reach any organ through the circulation; but the seeds "can only live and grow if they fall on congenial soil" and the tumor cell will develop into fully overt metastasis solely in an appropriate microenvironment provided by a specific organ [3]. Built on this concept in addition to emerging data, the "metastatic niche" model has been described. This hypothesis suggests that the microenvironment of the destination sites of future metastasis changes significantly as a result of tumor secreted factors, and this "premetastatic niche" evolves into a metastatic niche following tumor cell engraftment [4]. However, it is uncertain to which extent this hypothesis is applicable to the diverse tumor types. Furthermore, alternative models, such as the mechanistic theory, have explained consistently the patterns of metastasis distribution in particular tumors [5]. In the present review we will discuss

evidences about the changes that take place in the target organ of metastasis under influence of the primary tumor in a premetastatic phase, the mechanisms exploited by the tumor cells for homing to the metastatic site and the influence of the microenvironment on the disseminated cancer cell until it develops into a metastatic lesion.

1. Priming the secondary organ: The premetastatic niche

It could be thought that the organ specificity for metastasis is solely determined by intrinsic properties of the tumor cell and the destination site, either by providing the receptive microenvironment for a specific tumor cell type and/or by means of matched patterns of gene expression [6]. However, the metastatic preference can also be considered as a tumor-driven setting, where tumor derived factors would prime the target organ to become conducive for tumor seeding before the arrival of tumor cells. These non-intrinsic, newly acquired properties of the soil under the influence of the seed vary from extracellular matrix proteins, soluble mediators and, furthermore, the recruitment of specific accessory cell types.

For example, Hiratsuka *et al* [7] reported the upregulation and enhanced activity of the matrix metalloproteinase 9 selectively in endothelial cells and macrophages from the premetastatic lung. MMP9 induction was via vascular endothelial growth factor receptor 1 (VEGFR1) [8] signaling, since it was not observed in VEGFR1 tyrosine kinase $^{-/-}$ mice. This early change in the target organ microenvironment favored the subsequent tumor cell recruitment and invasion preferentially to the lung tissue. *In vitro* lung culture experiments pointed towards VEGF and placental induced growth factor (PIGF), both VEGFR1 known ligands, but not the VEGFR2-specific ligand VEGF-E, as the factors triggering the induction of MMP9.

In 2005, Kaplan *et al* [8] reported that a primary tumor could elicit an influx of bone marrow derived cells (BMDCs) that lodge in and prepare the destination site for future metastasis. This specialized tumor-supportive microenvironment was termed the "premetastatic niche". In particular, the recruited BMDCs were VEGFR1+ and further characterization revealed the expression of the progenitor antigens CD133, CD34 and CD117; thereby suggesting that these cells are hematopoietic progenitors (HPCs). Based on these findings, it was proposed that the premetastatic niche acts as a functional peripheral niche, just as the physiological BM niche [9]. The premetastatic niche was established as early as 12 days after tumor implantation and preceded the arrival of tumor cells. Inoculating mice with antibodies against VEGFR1 blocked VEGFR1+ HPCs mobilization and clustering in the target organ and prevented metastasis. In

addition, the niche formation and the increment in lung metastasis could be reproduced in the absence of tumor cells by challenging mice with tumor-conditioned media (CM) before intravenous administration of tumor cells, indicating that the process is driven by tumor-derived factors. The tumor-induced clustering of BMDCs at distant sites was proven to be related to the tumor cell type. In this way, B16 melanoma tumor cell dictated premetastatic niches at lungs, liver, spleen and kidney while Lewis lung carcinoma (LLC) cells induced premetastatic niches only in the lungs. Moreover, LLC metastasis could be redirected to B16 melanoma common metastatic sites by administration of B16 CM before intravenous LLC implantation. Among the possible mechanisms mediating the premetastatic niche, a key role for the fibronectin receptor very late antigen 4 (VLA-4) on HPCs, and the expression of MMP9 and inhibitor of differentiation 3 (Id3) in the clusters was demonstrated, as assessed by inhibiting VLA-4 expression with anti integrin α4 antibodies or studying cell cluster formation in MMP9 and Id3 knockout mice.

The expression of VLA4 in VEGFR1+ cells would be important in the adhesion to the premetastatic site where, consistently, an upregulation of fibronectin was observed from day 3 after tumor implantation.

Once the VEGFR1+ cells are recruited, clustered in the target organ and have prepared a receptive microenvironment by direct or indirect mechanisms, it is time for tumor cell arrival. Here, the stromal derived factor 1 (SDF-1)/CXCR4 axis was suggested to promote tumor cell adherence and growth [8]. Remarkably, several of the proposed mechanisms denote that cancer cells hijack physiological homing mechanisms [9].

As it has been suggested, these findings point towards VEGFR1 signaling inhibition as a potential therapeutic approach to interfere with the premetastatic niche formation, in addition to the multi anti-tumor effects already described for this pathway in the regulation and promotion of tumorigenesis, angiogenesis, inflammation and metastasis. Nevertheless, several on-target toxic effects have been described for both the small molecule inhibitors and the neutralizing antibodies, and this has restricted the current approved indications for this treatment [10,11]. On the other hand, VEGFR1 assessment could be clinically useful as prognostic factor, since VEGFR1 expression was associated with a worse prognosis or disease progression in primary solid tumors, such as lung [12,13] and colorectal [14] carcinomas, gastric cancer [15], intracranial schwannomas [16]; or hematogenous tumors like acute myeloid leukemia [17]. A work with 810 samples from both bone marrow and peripheral blood from gastric cancer patients found that the combined presence in the bone marrow of isolated tumor cells (ITC) and high VEGFR1 expression was a better predictor for hematogenous metastasis compared with the evaluation of VEGFR1

or ITC status alone. In a multivariate analysis, high expression of VEGFR1 was the strongest independent factor predicting lung and liver metastasis in ITC bone marrow positive patients. These findings indicated that the simultaneous presence of ITC and VEGFR1 expression at ectopic sites is clinically significant for disease progression [18].

The description of the premetastatic niche stimulated the search for tumor-derived factors that would account for its establishment [19-21]. For instance, the gene expression profile of lungs, derived from malignant tumor bearing mice (B16, LLC and 3LL), revealed a strong induction of the chemoattractants S100A8 and S100A9 compared with normal or benign tumor bearing mice [19]. The expression of both genes was detected in the endothelial cells as well as Mac1+ myeloid cells in the premetastatic lungs. Besides, the recruitment of myeloid Mac1+ cells in the lungs was induced by the primary tumor. Tumor necrosis factor (TNFα), transforming growth factor β (TGFβ) and VEGFA were partially responsible for the upregulation of S100A8/A9, as assessed by antibody interference experiments *in vitro* and *in vivo*. Consistently, the solely injection in normal mice of TNFα or VEGFA alone or in combination could enhance the recruitment in the lungs of LLC cells injected intravenously. S100A8/A9 induced the migration of macrophages and tumor cells, the latter showing a substantial morphological change including the development of invadopodia. Interestingly, LLC maximal migration was achieved when lung tissue was treated *in vitro* with S100A8/A9 and the resulting lung CM was used in the assays. Similar results were obtained with separate or mixed CM of S100A8/A9-treated endothelial cells and Mac1+ myeloid cells derived from the target organ of tumor bearing mice. This indicated that S100A8/A9 could promote the migration of tumor cells both directly and indirectly, by inducing the secretion of migratory stimulating factors, which remained to be definitely identified. Remarkably, neutralizing antibodies against S100A8/A9 were effective in the blockade of tumor cell dissemination. Focusing at the metastatic phase, the antibodies were shown to block the spontaneous metastasis of the highly metastatic 3LL tumors, while at the premetastatic phase these antibodies decreased the recruitment of myeloid cells to lungs of mice harboring non-metastatic LLC tumors and diminished the number of tumor cells in the lung after intravenous administration. Mechanistically, the migration to the lungs of myeloid and tumor cells was proven to use a common signaling pathway, through the p38 mitogen-activated protein kinase (MAPK) cascade.

Gaining further insight in the mechanism by which S100A8 elicits cell accumulation at the destination site of a primary tumor, it was described that this chemoattractant stimulates the paracrine secretion of

SAA3, another chemotactic protein. SAA3 was proven to activate NFκB through TLR4 signaling and to support further secretion of SAA3. In experiments with mice harboring non-metastatic LLC tumors and treated with antibodies against SAA3 the mobilization of BMDCs was suppressed as well as the colonization of lung by Mac1+ cells and tumor cells after intravenous delivery, while no effects were observed in TLR4 $^{-/-}$ mice [20].

In a recent study, S100A4, another member of the S100 family of calcium binding proteins, was implicated in the induction of premetastatic changes in the target organ of metastasis. The metastatic ability of CSML100 mammary carcinoma cells, which is suppressed in S100A4 $^{-/-}$ mice [22], could be restored by intravenous administration of S100A4 $^{+/+}$ mouse embryonic fibroblasts (MEFs). Moreover, at a pre/early metastatic phase, S100A4 $^{+/+}$ MEFs facilitated the attraction of T cells to the lungs, where they concentrated around blood vessels. It was suggested that T cells could in turn attract myeloid cells to generate the premetastatic niche [23]. In another preclinical study, it was shown that the antiangiogenic agent TSU68 modulates the microenvironment in the liver before the formation of metastasis in an orthotopic model of colon cancer. Microarray analyses from premetastatic livers showed an upregulation of CXCL1 in tumor bearing mice compared with controls, which was suppressed by TSU68 treatment. An increment in the number of neutrophils and CD11b+ cells was also observed in the livers from tumor bearing mice, and it was suggested that elevated levels of CXCL1 protein in peripheral blood would account for its mobilization. Accordingly, the use of an antibody against CXCR2, the CXCL1 receptor, resulted in less metastatic foci in the liver [24].

In a work focused on the vascular changes in the premetastatic phase that facilitate subsequent lung metastasis, pulmonary vasculature destabilization and enhanced permeability was described. Of note, the extent of the changes correlated with the metastatic potential of the primary tumor, being more dramatic with highly aggressive tumor cell lines. Angiopoietin 2 (Angpt2), MMP3 and MMP10 were upregulated in the premetastatic lungs and contributed to the pulmonary vascular destabilization, myeloid cell infiltration and spontaneous lung metastasis development [25].

Together with the increment of fibronectin [8] the accumulation of the enzyme lysil oxidase (LOX) secreted by hypoxic primary tumor cells was reported by Erler and coworkers [26]. LOX was shown to contribute to the premetastatic niche formation by providing a crosslinked basement membrane at sites of future metastasis, which would support the recruitment and adhesion of myeloid cells in the lungs, and promote their production of MMP2. MMP2 activity would facilitate lung tissue invasion and further

BMDC influx due to production of chemoatractant collagen IV peptides, getting the microenvironment ready for the arrival of tumor cells.

Recently, in a study comparing the highly metastatic rat pancreatic adenocarcinoma cell line BSp73ASML ($ASML^{wt}$) with a selective CD44v knockdown ($ASML^{kd}$), the CM of tumor cells was dissected in order to assess the contribution of the soluble fraction and the exosomes to the premetastatic organ niche preparation. $ASML^{wt}$ cells metastasize from the footpad through the lymphatics to the lung but do not grow locally, while $ASML^{kd}$ cells poorly metastasize. Intrafootpad administration of $ASML^{wt}$ CM before tumor cell inoculation could support $ASML^{kd}$ dissemination to the draining lymph nodes and the lungs and promoted lymphocytes expansion/recruitment. A mixture from $ASML^{wt}$ soluble fraction and $ASML^{kd}$ exosomes showed cooperation to promote metastatic spread. Then, it was suggested that exosomes are the main actors in the (pre)metastatic niche preparation but they relay on the cooperation of the soluble fraction [21].

Taken together, these evidences (summarized in Table 1) suggest that the preconditioning of the target organ of metastasis by the primary tumor is necessary for the subsequent homing and engraftment of disseminated tumor cells, at least for certain tumor models.

Table 1. Changes that take place in the target organ of metastasis at the premetastatic phase.

Primary tumor (target organ)	**Main premetastatic change**	**Reference**
Lewis murine lung carcinoma (lungs)	MMP9 upregulation	Hiratsuka, S. *et al* (2002)
B16 murine melanoma (lungs, liver, spleen and kidney)	VEGFR1+ cell recruitment Fibronectin upregulation	Kaplan, R. N.*et al* (2005)
	S100A8, S100A9 upregulation Mac1+ cell recruitment	Hiratsuka, S. *et al* (2006)
TK-4 human colon carcinoma (liver)	CXCL1 upregulation	Yamamoto, M. *et al* (2008)
B16 murine melanoma (lungs, liver, spleen and kidney) MDA-MB-231 human breast cancer (lungs)	Vascular destabilization and increased permeability Angiopoietin 2, MMP3 and MMP10 upregulation	Huang, Y. (2009)
MDA-MB-231 human breast cancer (lungs) 4T1 murine mammary tumor (lungs)	Lysil oxidase accumulation	Erler, J. T. *et al* (2009)
BSp73ASML rat pancreatic adenocarcinoma (lymph nodes, lung)	CD49c, CD49d, CD54, urokinase-type plasminogen activator receptor (uPAR), VEGFR1 and VEGFR2 upregulation	Jung, T. *et al* (2009)
CSML100 mammary carcinoma (lungs)	T cell recruitment	Grum-Schwensen, B. *et al* (2010)

2. Traffic and homing to the secondary organ

Tumor cells have the ability to leave the primary lesion and travel to an ectopic environment although the majority will not grow into overt metastases. It has been widely discussed whether the acquisition of metastatic ability occurs early or late during malignant progression. In a recent report, Klein has summarized current evidences, supported mainly on human data, in two models of metastatic dissemination. The linear progression model predicts that the tumor cells acquire a fully malignant phenotype within the primary tumor microenvironment and only, after selection and late-stage clonal expansion, the cells are capable to metastasize. On the other hand, the parallel progression model states that tumor cells depart from the primary lesion before the acquisition of a fully malignant phenotype to undergo somatic progression and metastatic growth at a distant site [27]. Nowadays, it is possible to compare whole genome studies of extravasated cells, before the development of metastasis, with those of the primary tumor [28]. In this way, Stoecklein *et al* found that primary esophageal tumors diverged for most genetic aberrations with single lymphatic and hematogenous disseminated cancer cells. The region comprising HER2 (Cr17q12-21) was the most frequent gain in disseminated tumor cells that were isolated from both ectopic sites. Moreover, survival analysis demonstrated that HER2 gain in disseminated tumor cells, but not in primary tumors, conferred high risk for early death [29]. Thus, we may assume that in the case of linear progression the metastatic founder cell must show overlapping traits with the primary tumor, whereas parallel progression would suggest genetic/epigenetic divergence of accumulative mutations. However, it is also possible for the metastatic cell to acquire at distant organs identical solutions than those of the primary tumor. A novel hypothesis was suggested by the challenging work of Podsypanina *et al* who showed that even normal cells might establish at target organs. Untransformed mouse mammary cells engineered to express the inducible oncogenic transgenes MYC and mutant Kras or polyoma middle T administered intravenously in mice could bypass transformation at the primary site and develop into metastatic pulmonary lesions upon immediate or delayed oncogene induction. Therefore, “premalignant cells” may disseminate during the first stages of tumor progression and undergo malignant transformation at ectopic sites such as the premetastatic microenvironment [30]. Hence, additional studies are necessary to determine which model is the appropriate for each tumor type.

Whatever the timing of the tumor cell exit from the primary tumor, there is no doubt that seeding can occur to multiple organs, but metastases may develop only in one or a few. As discussed above, several

reports have indicated that the creation of an immunosuppressive prometastatic permissive microenvironment before the arrival of the circulating tumor cell, the premetastatic niche, is essential to promote the engraftment of the tumor cell. However, other authors [4] have questioned whether the niches preexist as 'inducible niches' or whether they are newly initiated, in view of the fact that following experimental intravenous injection of malignant cells, a minority will successfully engraft in certain sites, suggesting that the preparation by the primary tumor is not required. Alternatively, it could be argued that the intrinsic properties of the metastatic cell are more important determinants of metastasis than any contribution of the host microenvironment. For instance, the loss of metastasis-suppressor genes impaired metastasis without affecting primary tumor growth [31], microRNA, such as miR-335, behaved like a metastasis suppressor gene by downregulating SOX4 and tenascin C, reducing cell migration and invasion [32], and the expression of particular sets of genes can mediate metastasis to a specific organ [33]. In the same way, it was demonstrated that the simultaneous targeting of genes involved in the increment of lung metastasis (such as the genes encoding the epidermal growth factor receptor ligand epiregulin, the cyclooxygenase COX2, MMP1 and MMP2) impairs tumor cell outgrowth in the target organ [34,35]. Furthermore, it was suggested that cells with self-renewal and tumor initiation capacities have a crucial role during the metastatic process, topic that is addressed in other report of this journal. Therefore, tumor cells might condition their own metastatic microenvironments, creating metastatic niches in a paracrine fashion. Whatever, both this theory and the premetastatic niche model are compatible with the generally accepted assumption that metastasis occurs in a stepwise fashion.

Once tumor cells reach the circulation, they further take advantage from local cells. Thus, platelets can promote the survival of circulating cancer cells, acting as shields that protect them from the cytotoxic effects of TNFα, facilitating adhesion to the endothelium and/or enhancing tumor embolization in the microvasculature, features that enable engraftment at secondary sites [36,37]. On the other hand, the extravasation of tumor cells could be modulated by the endothelia of different tissues, as it is known that every vascular bed expresses specific adhesion molecules or chemokines, having its own specific molecular "address", a fact that tumor cells can exploit to home to specific tissues, if they express the corresponding receptors. For example, the expression of the α2,6-sialyltransferase ST6GALNAC5 in breast cancer cells enhances their adhesion to brain endothelial cells and their passage through the blood-brain barrier [33] and the tumor cell surface protein metadherin facilitates the adhesion specifically to lung vessels [38]. This findings may be therapeutically valuable, for instance, antibodies reactive to the

lung-homing domain of metadherin and siRNA-mediated knockdown of metadherin expression in breast cancer cells inhibited experimental lung metastasis, strengthening that tumor cell metadherin mediates localization at the metastatic site [38]. Tumor cells may coopt the mechanism used by leukocytes to promote, via IL-1 and TNFα, the endothelial expression of selectins which in turn promotes the attachment of leukocytes to specific areas of the endothelium [39]. In a human colorectal model of metastasis, it was reported that E Selectin expression by endothelial cells mediates the arrest of cancer cells in the liver. Moreover, *in vivo* pretreatment of nude mice with antisense oligonucleotides that abrogated E-selectin induction, in response to intrasplenic/portal inoculation of human colorectal carcinoma CX-1 cells, reduced the number of liver metastases by 86% relative to controls, suggesting that the inhibition of tumor-induced, hepatic microvessel E-selectin expression may provide a useful strategy for the prevention of hepatic metastasis [40]. In addition, the angiogenic factor VEGF, abundantly expressed by most cancer cells, increases the permeability of the endothelium and thus facilitates extravasation [41].

Recently, employing dual channel in vivo imaging systems, it was determined that after arriving at target organs, tumor cells remain inside the blood vessels where they proliferate, exhibit MMP activity and then develop into micrometastases [42]. Therefore, the cancer cell/endothelium interaction could represent a novel microenvironment and a unique therapeutic target to inhibit the subsequent development of macrometastases.

3. Growing in the secondary organ

After cell engraftment in the target organ, the tumor cell may have different fates. Extravasated single tumor cells can reside in the target organ in a state of dormancy (non-proliferative state) for a prolonged period of time [43]. The individual disseminated cell, might be modulated by external signals from the microenvironment, regardless of a gene mutation [44]. Cells could remain quiescent whereas cell division is minimized, cell death is evaded and/or differentiation is prevented. In this case, it is also probable that the “niche that houses the extravasated cells” protects them from chemo or radiotherapy. In this sense, Liu *et al* found that CD133 positive cancer cells, isolated from human glioblastoma display a strong capability on tumor's resistance to chemotherapy [45]. This resistance is probably due to the higher expression of drug resistance genes such as BCRP1 and DNA-mismatch repair genes such as MGMT, as

well as the anti-apoptosis protein and inhibitors of apoptosis protein families by the CD133 positive cell [45].

In an elegant work, Darrase-Jéze and coworkers [46] studied the response triggered at the first encounter between the immune system and the tumor cells. The authors found that at this time point, a crucial battle between self-specific memory regulatory T cells (Tregs) and tumor-specific naïve effectory T cells (Teffs) takes place that profoundly affects tumor fate, where a subset of self-reactive CD44hi Tregs is responsible for early induction of tumor immunity. The balance between effector and regulatory responses did not depend on the number of Tregs and Teffs, but rather on their relative activation speed, so the final immune response is driven by the memory status of the players. Future studies will indicate whether in the parenchyma of the target organ a similar "battle" takes place, determining the outcome of the extravasated tumor cell.

Dormancy can also antagonize the expansion of a tumor mass, through an active immune system[47]. The mechanism of immunosurveillance leads to an equilibrium between the immune system and the tumor [48].

Another mechanism of tumor mass dormancy is the lack of a supportive vascular network, which results in micrometastases that do not progress further. Only upon the formation of new vessels, tumor proliferation is reinitiated. This phenomenon is termed "angiogenic dormancy". Here, there is a balance of cell proliferation and apoptosis [49,50]. The inability to recruit blood vessels is likely caused by the lack of expression of angiogenic factors such as VEGF, and/or high expression of angiogenesis inhibitors such as thrombospondin [51]. Tumor cells from many types of carcinoma usually home to the bone marrow (BM) [52]. It can be speculated that the BM might also be an important reservoir of dormant tumor cells, from which they could re-circulate to other distant organs where better growth conditions may exist. The fact that tumor cells are detectable in the peripheral blood of patients with breast cancer months to years after complete removal of the primary tumor indicates that these cells might re-circulate between metastatic sites. As in some tumors cell dissemination is an early event in tumor progression; tumor classification systems should take account of the concept of disseminated tumor cells.

Among the mechanisms that have been associated with dormancy, Aguirre-Ghiso determined that an impaired or reduced ligand-dependent signaling through adhesion molecules such as α5β1 or other β1 integrin heterodimers and adhesion signal transducers such as focal adhesion kinase (FAK) are observed

in dormant tumor cells [53]. A previous work, demonstrated that a low ERK/p38 signaling ratio predicts for dormancy while the opposite ratio predisposes to active proliferation [54]. Therapeutic approaches modulating this pathways could induce the state of dormancy, for instance the blockade of EGFR with Gefitinib was shown to inhibit ERK and activate p38 [55].

Several reports support the concept of an epigenetic role of the microenvironment in reprogramming the metastatic ability of the disseminated tumor cell [56]. In this sense, it was shown that aggressive type tumor cells, such as melanoma, express genes associated with various cell types, including progenitor cells, while concomitantly downregulate genes specific to their parental melanocytic lineage [57]. Consequently, these cells can acquire a multipotent plastic phenotype, which provides enhanced adaptation and survival. For instance, in the process of "vasculogenic mimicry" the tumor cells can assemble into an extravascular fluid-conducting network that allows a better adaptation to hypoxic conditions [58,59]. When these cells were placed in an embryonic microenvironment, the reversion of the metastatic phenotype was observed. Similar results were obtained with metastatic breast and prostate cancer cells. Therefore, disseminated tumor cells may be inefficient to survive in a particular organ due to the impairment of its metastatic potential imposed by the microenviroment reprogramming.

Whether the primary tumor can modulate the microenvironment of the target organ affecting the growth of already engrafted cells is a question that remains unanswered, giving the conflicting results that have been reported. McAllister *et al* demonstrated that human breast carcinomas promote the growth of otherwise-indolent tumor cells, micrometastases, and human tumor surgical specimens located at distant anatomical sites. The authors demonstrated that the systemic instigation is accompanied by incorporation of bone-marrow cells (BMC) into the stroma of the distant, once-indolent tumors and that the secretion of osteopontin by the instigating tumors is necessary for BMC activation [60]. On the other hand, it is well known that after the surgical removal of the primary tumor, the micrometastatic lesions grow rapidly to form macrometastases [61]. Here, it is clear that the primary tumor exerts some kind of restriction on disseminated cells.

At the target organ of metastasis, the tumor cells establish dynamic and reciprocal interactions with non-tumor cells, not only by cell-cell contact but also through the release of several cytokines that reach local and distant cells, such as fibroblasts, BMDC and inflammatory cells [62]. Besides, tumor cells unable to proliferate in the target organ may prepare the "field" for the engraftment of more invasive cell subtypes.

The strategies by which the metastatic cell modulates the microenvironment resemble those that have been already exploited by the primary tumor. For instance, cells from distant organs can be recruited and, upon arrival, establish a dialogue with the tumor cell. As a result of the secreted soluble factors the local homeostasis may be disrupted [62]. The transition from micrometastases to angiogenic macrometastases requires the recruitment of endothelial progenitors and their accompanying supportive cells, to the metastatic niche, in order to facilitate blood vessel settlement and/or stabilization. Hypoxia-induced expression of angiogenic growth factors, such as VEGFA, can promote the recruitment of VEGFR-2+ bone marrow-derived endothelial progenitor cells (EPCs) and pericytes [63]. EPCs also express a variety of angiogenic molecules, suggesting that their recruitment further promotes local angiogenesis and subsequent metastatic tumor growth. Gao *et al* identified bone EPCs as critical regulators of the angiogenic switch in a mouse model of pulmonary metastasis. They showed that tumors induced the expression of the transcription factor Id1 in the EPCs and that suppression of Id1 after metastatic colonization blocked EPC mobilization, caused angiogenesis inhibition, impaired pulmonary macrometastases, and increased the survival of tumor-bearing animals [63]. Moreover, Gille *et al* suggested that the combined blockage of both the inflammatory and the VEGFR-2-dependent angiogenic response are necessary to effectively inhibit solid tumor growth and formation of lung metastasis by B16 melanoma cells [64]. Finally, it was demonstrated that the quantification of circulating EPCs may be a potential biomarker to monitor cancer progression and, in certain cases, treatment response [65,66].

Other players in the premetastasic/metastatic microenvironment that may promote tumor growth and progression include the macrophages, tumor-associated fibroblasts and bone marrow-derived mesenchymal stem cells (MSC) [67,68]. MSC are non-hematopoietic stem cells, characterized by the expression of a large number of adhesion molecules and stromal cell markers (CD73, CD105, CD44, CD29, CD90) in the absence of hematopoietic and endothelial markers [69]. They are precursors of tumor stromal fibroblasts and produce a plethora of cytokines, growth factors and extra cellular matrix proteins (*e.g.* FN and laminin) [70]. MSC were shown to promote tumor growth directly or indirectly, stimulating tumor vessel formation by the production of pro-angiogenic factors or moreover, (trans) differentiating into endothelial-like cells [68,70,71]. The inhibition of MSC and other BMDC was explored as a strategy to reduce tumor growth. In this sense, authors have used several strategies like suicide genes or neutralizing antibodies, in order to modulate the biological activity of these cells [72,73].

In addition to seeding *de novo* metastasis, invasive tumor cells can also contribute to primary tumor growth: disseminated cells can return to their original site leading to progressive accumulation of aggressive cells and to local recurrence, a self-seeding hypothesis supported by several experimental and clinical observations, as proposed by Norton and Massagué [74].

Supported in the concept that cancer could be considered a stem cell disorder, Lin *et al* revisited the "seed and soil" theory. They proposed an integrated tumorigenesis model involving three interdependent stem cell compartments: circulating EPC, MSC and cancer stem cells (CSC). Targeting the CSC/EPC/MSC compartments may be a therapeutic option [75].

The different emerging models that summarize all these data should be instructive for a better understanding of the biology of metastasis.

Conclusion

Metastatic disease persists as the main concern of malignant progression. Despite the overwhelming amount of research on metastasis, solutions that directly improve cancer patients' quality of life remain elusive. However, the understanding of the mechanisms underlying metastasis has improved substantially and a holistic view has emerged, underlining the importance of the microenvironment as well as the temporal course as pillars of this complex process. Therefore, we are optimistic that these findings might yield new therapeutic strategies to suppress the metastatic phenotype.

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