Cancer Letters xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

The role played by the microenvironment in site-specific metastasis

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Metastasis
Micrometastasis
Dormancy
Microenvironment

ABSTRACT

Cancer cells that disseminate to metastatic sites may progress to frank metastasis or persist as dormant micrometastasis. Significant progress has been made in defining the genetic and phenotypic cancer-cell-autonomous determinants of metastasis and in the understanding of the cross-talk between metastasizing tumor cells and the metastatic microenvironment.

However several questions remain open, in particular the identity of microenvironmental factors that keep micrometastatic cells in a state of dormancy and those that promote survival, proliferation and progression of such cells. Significantly more information is available on the latter factors than on microenvironmental cells and molecules that restrain micrometastasis. This mini-review summarizes findings suggesting that:

- The interactions between the metastatic microenvironment and cancer cells metastasizing to this microenvironment are unique to each metastatic microenvironment.
- cells or molecules in the metastatic microenvironment could act as "double agents" exerting pro- or anti-malignant functions.
- In the early phases of tumor progression, the interactions between cancer cells and the metastatic microenvironment are inhibitory whereas in later stages such interactions promote progression towards metastasis.

In view of the above, it is not unlikely that metastases residing in different microenvironments may require ‘‘individualized’’ treatment modalities.

1. The tumor microenvironment takes part in shaping the malignant phenotype of tumor cells

Metastasis is the major killer of cancer patients. One of the major tasks of cancer researchers should be to find ways to prevent, halt or cure metastasis. In order to achieve these goals it is essential to thoroughly understand the molecular and cellular mechanisms leading to metastasis. Significant progress has been made in defining the genetic and phenotypic cancer cell autonomous determinants of metastasis, in the understanding of the interrelationships of metastasizing tumor cells with cells and molecules in the metastatic microenvironment and in deciphering metastasis-associated processes such as epithelial to mesenchymal transition, targeted migration, or dormancy [1–17]. There are, however several open questions that need to be accurately pinned down: What attracts tumor cells to specific metastatic microenvironments? What sustains the survival of disseminated tumor cells in a particular organ site? What induces tumor cells to proliferate at the metastatic site? Are metastases that originate in a particular tumor and develop in different organ sites similar or different? Are the survival and growth factors for metastasis in a particular metastatic microenvironment similar or different from such factors in a different metastatic microenvironment?

It is well established that by employing intricate signaling pathways, tumors and their microenvironments regulate and shape each other's phenotype.

The interaction of tumor cells with the unique microenvironment of the primary cancerous lesion provides signals that confer upon the tumor cells traits that promote their progression including migratory and invasive capacities. Similarly, signals originating in the microenvironment of organ sites favored by a particular tumor (site-specific metastasis) regulate the targeted migration of the tumor cells to these specific organ sites and their survival and propagation in these sites [18–24].

These tumor-microenvironment interactions are bidirectional and each interaction partner regulates and shapes the phenotype of the other [2,4,25–27].

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http://dx.doi.org/10.1016/j.canlet.2013.08.029

The interactions of cancer cells with components of their microenvironment are crucial determinants of progression towards metastasis. Both the non-tumor cells in the tumor microenvironment as well as the tumor cells themselves are accessories in tumor progression towards metastasis. However the tumor is undoubtedly the original perpetuator. On the one hand it evolves into an increasing malignant entity and at the same time alters, conditions, or educates cells and molecules in its microenvironment making them its accomplices in the act of promoting progression towards metastasis [28–30]. Signals delivered by such corrupted non-tumor cells may direct the tumor towards one or several possible molecular evolution pathways many of which lead to metastasis [4].

According to the present state of art of the Tumor Microenvironment field, many, if not most, of the interactions between cancer cells metastasizing to a specific organ site and the metastatic microenvironment at that site enhance tumor progression and promote metastasis.

Below are a few examples to illustrate the point that the targeted migration of tumor cells to metastatic sites, their survival in these sites and their further progression depend upon their interaction with the metastatic microenvironment.

Chemokines are present in essentially every organ and chemokine receptors are expressed by many types of tumor cells. It is now well established that chemotactic and other interactions between the chemokines in specific organ sites and chemokine-receptors expressed by tumor cells are responsible for the targeted migration of tumor cells to these sites [31–47].

The bottom line of these findings and of many others is that cancers expressing receptors for specific chemokine ligands will have a tendency to migrate to organs in which these ligands are expressed in an appropriate fashion.

The survival of cancer cells in metastatic microenvironments and their further progression is regulated by complex interactions with non-tumor cells residing in or infiltrating into the tumor microenvironment such as endothelial cells, inflammatory cells and other immunocytes, bone marrow derived stem cells, adipocytes, and fibroblasts [48–55]. This regulation is mediated through intricate signaling networks involving cytokines [56–71], chemokines [31–36,38,43,44] and other molecules [72–78].

The microenvironment of solid tumors is characterized by stressful conditions [79]. For example a state of hypoxia contributes greatly to tumor progression by influencing both the tumor cells as well as the tumor microenvironment [80].

Similarly chemotherapy or other forms of cancer therapy regulate gene expression patterns in cancer cells thereby influencing their malignancy phenotype [81–84]. Unpublished Results obtained in our laboratory showed that human melanoma cells with the BRAF V600E mutation that were rendered, in vitro, resistant to Vemurafenib exhibited an increased malignant phenotype in xenografted nude mice.

Tremendous efforts are invested in the development of targeted therapies for the personalized treatment of human cancers [85,86]. These new therapies may target pathways critical to tumor development or specific driver mutations in cancer cells, in particular protein kinases. An increased understanding of the specific molecular pathways and the identification of driver mutations critical to cancer cell growth have allowed the development of these advanced therapeutics. By using advanced sequencing technologies the ability to predict response to such targeted therapies has progressed rapidly allowing the characterization at single nucleotide resolution of tumor DNA and RNA. Such studies indicated an enormous complexity and evolution between primary tumor and distant metastasis [87]. While a high response rate to a given targeted therapy was associated with a "favorable" profile of genetic markers, a substantial percentage of patients expressing such a profile did not respond to the treatment. Exhaustive high-resolution genetic profiling of these cases failed to find any additional genetic lesions that could account for this unresponsiveness. Recent studies [88,89] suggested that this discordance may be attributed to microenvironmental influences, which cannot be inferred from the genotype of the tumor cells.

2. The metastatic microenvironment controls micrometastasis dormancy

Cells disseminating to secondary organs may develop into overt metastasis but may also persist in harboring organs as dormant cells for prolonged periods of time [90]. These dormant tumor cells are referred to as disseminated tumor cells or micrometastases. The formation of micrometastasis is now recognized as an integral phase of the metastatic cascade [90–93]. Micrometastatic cells remain as solitary cells or as small, steady state cell clusters, either due to a balance between proliferation and apoptosis or due to cell cycle arrest [94,95]. Such dormant micrometastases could progress to overt metastasis [90,91,96].

Regional lymph nodes and bone-marrow are major target sites for disseminating tumor cells [96]. If micrometastasis indeed progresses towards frank metastasis in a given organ site, it is logical to assume that this progression would take place at that particular organ site. However, with some exceptions [97], the experimental evidence to support this assumption is rather limited. A possible reason for that is that detection of micrometastsis represents a great technical challenge [90,98].

Employing the xenograft models of human neuroblastoma lung [99] and melanoma brain metastasis [100] developed in our laboratory, we generated local and metastatic variants with an identical genetic background. In further studies we detected the presence of micrometastases that formed spontaneously in lungs and brain following an orthotopic inoculation of neuroblastoma [101] and melanoma [100] cells respectively. The micrometastatic cells of these two tumor systems grew in vitro and formed local tumors when implanted in the orthotopic sites, demonstrating that the intrinsic autonomous proliferative capacity of these cells remained intact except in the corresponding metastatic microenvironments.

Are there factors in metastatic microenvironments that inhibit proliferation of micrometastasis residing in these microenvironments?

Many microenvironmental cells such as macrophages [102,103] fibroblasts [104–107] or Treg cells [108], as well as numerous types of molecules such as cytokines, chemokines, transcription factors, angiogenic factors, adhesion molecules, and many others [109–113] are known to enhance tumor progression. In contrast, relatively little is known regarding inhibitory microenvironmental cells and molecules with a few notable exceptions such as immunocytes and their products [114] or granulocytes [115].

Some factors will serve as "double agents", exerting pro- or anti-malignant functions in different stages of tumor progression [4,116].

The inhibitory effects exerted by microenvironmental factors are reminiscent of observations, reported almost 50 years ago [117] and confirmed by several groups [118–120], that normal cells have the capacity to restrain the proliferation of tumor cells. Klein et al. proposed that Microenvironmental Control [121] is one of several non-immunological mechanisms that protect organisms against neoplasia, being responsible for the fact that we do not "all die of cancer at an early age" [121–125].

We hypothesize that microenvironmental control accounts also for the dormancy of micrometastases in metastatic microenvironments. Such a mechanism may restrain the proliferation of

Please cite this article in press as: A. Klein-Goldberg et al., The role played by the microenvironment in site-specific metastasis, Cancer Lett. (2013), http://dx.doi.org/10.1016/j.canlet.2013.08.029