Hematogenous Metastasis

Metastatic cells often use the bloodstream to colonize distant target organs. To do this, tumor cells must detach from the primary tumor, gain access to blood vessels, and survive and manage the unique conditions in the vasculature. In this environment, tumor cells are confronted with plasma proteins, erythrocytes, leukocytes and platelets. The metastatic cells are further exposed to shear forces that are generated by blood flow and physically oppose cell attachment. It is generally accepted that fairly large numbers of cells from a primary tumor enter the circulation. However, only few will give rise to metastases [1]. To colonize their target organs successfully, tumor cells must attach to vascular endothelial cells or components of the vessel wall. This is mediated by specific adhesive functions of tumor cell receptors, including integrins. In the live host, it is not clear whether tumor cells attach directly to the endothelium. They may require crosslinking plasma protein ligands and support by platelets and/or leukocytes to adhere to the vessel wall in the presence of flow dependent shear forces [2]. Tumor cells that fail to attach are rapidly cleared from the circulation. Metastatic cells that manage to arrest within microvessels of their target organs either extravasate, or start to proliferate at the attachment site [3]. Cells that cannot proliferate within the vasculature undergo rapid apoptosis [4]. Non-proliferating extravasated cells may remain dormant for extended periods of time [5]. Failure to proliferate within target organs of metastasis is mediated, at least in part, by metastasis suppressor genes [6]. Interestingly, these genes do not affect primary tumor growth [7]. Their mechanism of action and relation to the functionality of adhesion molecules are still unclear [8]. However, integrin supported adhesion of circulating tumor cells, and their interaction with platelets within the vasculature not only represents the first critical step toward target organ colonization, but also contributes to tumor cell survival and proliferation at the secondary site [9] (fig. 1).

Tumor Cell Arrest

The first critical step in tumor cell anchorage within the vasculature is shear resistant attachment to the endothelium or to components of the vessel wall. Under these conditions, tumor cell integrin αvβ3 plays a special role, because it uniquely mediates tumor cell arrest under dynamic flow conditions [2]. In flowing blood, αvβ3 supports tumor cell interaction with adherent platelets and leukocytes. This results in shear resistant tumor cell arrest [10]. To mediate tumor cell binding to platelets, αvβ3 must be activated, and the interaction requires multivalent plasma protein ligands as crosslinking bridges [10]. In these respects, αvβ3 resembles leukocyte integrins of the β4 and β2 families and platelet integrin αIIβ3. In breast cancer cells, integrin αvβ3 can exist in a constitutively activated or a non-activated state.
expressed in a constitutively activated form, as shown for metastatic tumor cell phenotype. In breast cancer cells, these functions, that are critical for post arrest events, promotes an interaction with platelets during blood flow and further expression of an integrin adhesion receptor that supports and invasion, while to the fact that platelet receptor [10, 11]. Transfection of melanoma cells with either tumor cells strongly inhibits colonization of target organs sis, and that inhibition of expression of in vivo studies showing that in certain tumor cell types for successful metastasis. This concept is supported by under blood flow conditions, provides a selective advantage for successful metastasis. This concept is supported by in vivo studies showing that in certain tumor cell types expression of αvβ3 is required for hemotogenous metastasis, and that inhibition of αvβ3 function on circulating tumor cells strongly inhibits colonization of target organs [10, 11]. Transfection of melanoma cells with either αvβ3 or platelet receptor αIIbβ3 indicated that both receptors mediate tumor cell arrest during blood flow, but only integrin αvβ3 supported hemotogenous metastasis. This is likely due to the fact that αvβ3 further mediates tumor cell migration and invasion, while αIIbβ3 fails to do so [2]. Therefore, expression of an integrin adhesion receptor that supports interaction with platelets during blood flow and further functions, that are critical for post arrest events, promotes a metastatic tumor cell phenotype. In breast cancer cells, these functions are strongly enhanced when the integrin is expressed in a constitutively activated form, as shown for αvβ3 [10].

**Migration and Invasion**

To analyze post arrest events and specific contributions of activated breast cancer cell integrin αvβ3, we compared in vitro generated and in vivo selected variants of a human breast cancer cell model. These variants express either constitutively activated or non-activated αvβ3. We included a variant that lacks αvβ3. To test a clinical relevance of activated αvβ3, we further analyzed primary metastatic cells isolated from blood samples of stage IV breast cancer patients. These primary metastatic cells express a strongly platelet-interactive phenotype, which is mediated by integrin αvβ3. Based on this criterion and further functional tests, αvβ3 is constitutively activated on patient derived circulating metastatic breast cancer cells.

We found that metastatic, activated αvβ3 expressing breast cancer cells produce a soluble factor, which strongly promotes αvβ3 dependent migration. We identified this factor as metalloproteinase MMP-9 [12]. MMP-9 and several other MMPs are secreted as latent pro-enzymes by tumor cells, host stromal cells, leukocytes and platelets [13–15]. It is still unknown how the latent protease is targeted to the surface on invading cells. However, it is clear that the latent enzyme must bind to the cell surface where it is converted to the active enzyme, which then degrades proteins in the pericellular space [16]. We found that metastatic breast cancer cells, which express activated integrin αvβ3, convert pro-MMP-9 into enzymatically active MMP-9 [12]. This is true for pro-MMP-9 produced by the tumor cells or when exogenously added. In contrast, poorly metastatic cells, which express non-activated αvβ3, may produce pro-MMP-9 or receive it from another source, but they are unable to convert the enzyme into active MMP-9. It is only active MMP-9 that promotes αvβ3 mediated breast cancer cell migration. The enzyme does so by modifying the tumor cell surface and by degrading proteins in the microenvironment of the cell [12].

Other MMPs, such as MMP-2, are known to promote endothelial cell invasion during angiogenesis. MMP-2 can interact with endothelial integrin αvβ3 [17, 18]. The integrin is involved in MMP-2 maturation in non-metastatic breast cancer cells [19]. However, neither pro- nor active MMP-2 promoted αvβ3 dependent migration in our breast cancer cell model [12]. Importantly, the activation state of tumor cell integrin αvβ3, which profoundly affects metastatic activity, had no effect on MMP-2 processing [12].

Thus, our results suggest a new pathway, where activation of breast cancer cell adhesion receptor integrin αvβ3 triggers activation of metalloproteinase MMP-9 at the tumor cell surface, and thereby initiates a cascade of events that promote breast cancer spreading (fig. 2).
Fig. 2. Functional consequences of the integrin αvβ3/MMP-9 activation pathway. Breast cancer cells expressing constitutively activated integrin αvβ3—but not those expressing non-activated αvβ3—can convert latent metalloproteinase MMP-9 into enzymatically active MMP-9. Active MMP-9 strongly promotes αvβ3 mediated breast cancer cell migration by modifying the tumor cell surface, and by degrading matrix and other proteins in the pericellular space.

Survival and Proliferation at the Perivascular Site

Considering the arrest of circulating tumor cells in the vasculature an initial critical step, and appreciating the interaction between arresting tumor cells and platelets as a mechanism that supports this step, the following concept emerges. A malignant event renders integrin αvβ3 constitutively activated at the tumor cell surface. The activated integrin supports arrest of circulating tumor cells within the blood stream by promoting tumor cell binding to vascular cells, likely via interaction with activated platelets. In close contact or surrounded by activated platelets, the arrested tumor cells are exposed to a multitude of platelet released factors at a high local concentration. Such factors include integrin ligands, growth factors, and proteases such as MMP-9. In this microenvironment, activated tumor cell integrin αvβ3 can support conversion of pro-MMP-9 to active MMP-9. This triggers matrix degradation and strongly promotes tumor cell migration and invasion. During this process, vascular permeability factors are released and allow platelet and leukocyte derived growth factors to permeate into the perivascular space where they support the proliferation of invading metastatic cells (fig. 1).

References