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Review

Metastasis mechanisms

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ABSTRACT

Metastasis, the spread of malignant cells from a primary tumor to distant sites, poses the biggest problem to cancer treatment and is the main cause of death of cancer patients. It occurs in a series of discrete steps, which have been modeled into a "metastatic cascade". In this review, we comprehensively describe the molecular and cellular mechanisms underlying the different steps, including Epithelial–Mesenchymal Transition (EMT), invasion, anoikis, angiogenesis, transport through vessels and outgrowth of secondary tumors. Furthermore, we implement recent findings that have broadened and challenged the classical view on the metastatic cascade, for example the establishment of a "premetastatic niche", the requirement of stem cell-like properties, the role of the tumor stroma and paracrine interactions of the tumor with cells in distant anatomical sites. A better understanding of the molecular processes underlying metastasis will conceivably present us with novel targets for therapeutic intervention.

Contents

The classical view on the metastatic cascade, starting from a primary, epithelial, neoplastic lesion includes: 1. EMT and breach of the basement membrane barrier; 2. dissociation of tumor cells from the bulk tumor; 3. invasion of the neighboring tissue; 4. intravasation into pre-existing and newly formed blood and lymph vessels; 5. transport through vessels; 6. extravasation from vessels; 7. establishment of disseminated cells (which can stay dormant for a prolonged period of time) at a secondary anatomical site; and 8. outgrowth of micrometastases and macrometastases/secondary tumors (for review, see [1–4]) (see Fig. 1 for an overview of the different steps of metastasis). Each step creates one or more physiological barriers to the spread of malignant cells. To successfully metastasize, tumor cells have to overcome all of those barriers [1,5]. In the following, the principles of metastasis are summarized, explaining at the molecular and cellular levels how tumor cells are enabled to complete each and every step of the metastatic cascade.

Recent insights have suggested yet another step, to be added at the beginning of the cascade (and therefore designated as step "0" in Fig. 1): the creation of a "premetastatic niche" at the target site, before the first tumor cells arrive at this distant location. The Lyden group showed in an elegant mouse study that bone marrow-derived cells

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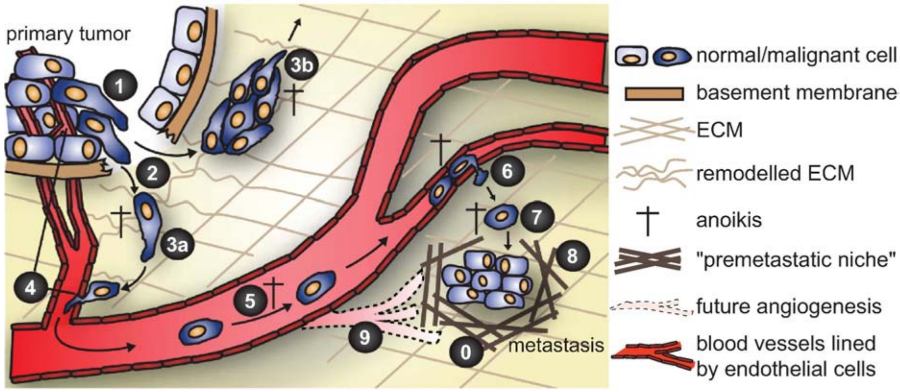

Fig. 1. The metastatic cascade. 0) Being induced by a distant tumor and mediated by bone marrow-derived cells, a "premetastatic niche" forms before metastasis becomes evident. 1) Cells in the primary tumor undergo Epithelial–Mesenchymal Transition (EMT) and acquire invasive properties. 2) Degradation of basement membranes and remodeling of the ExtraCellular Matrix (ECM) by proteinases facilitate tumor cell invasion. 3) Tumor cells invade surrounding tissue as single cells (3a) or collectively (3b). 4) Intravasation of tumor cells into newly formed vessels within or nearby the tumor. 5) Tumor cells are transported through the vasculature and arrest in a capillary bed where they extravasate (6). 7) Extravasated tumor cells can stay dormant for years. 8) Eventually, some disseminated cells grow out to a secondary tumor / macrometastasis, requiring ongoing ECM remodeling and angiogenesis (9). Cells outside their normal microenvironment undergo anoikis ("detachment-induced apoptosis"). Anoikis could hamper metastasis at several steps of the cascade, as indicated in the scheme. Not all steps of the metastatic cascade necessarily occur in a linear way. For example, premalignant tumors can already be vascularized while the timing of induction of the premetastatic niche remains elusive.

are mobilized owing to the presence of a distant, intradermal tumor. As a consequence, these cells accumulate as clusters in the lungs, where they change the local microenvironment into a niche suitable for the establishment of secondary tumors. At a later stage, tumor cells arrive at these sites and co-localize with the bone marrow-derived cell clusters [6]. The precise timing (whether at the premalignant stage or after local invasion has been initiated) of the establishment of the premetastatic niche is unknown. In line with the findings from the Lyden group, Hiratsuka and colleagues reported that distant tumors induce elevated levels of the pro-inflammatory chemokines S100A8 and S100A9 in the lungs of tumor-bearing mice [7,8]. These chemokines then attract tumor cells to the lungs via a serum amyloid A3 (SSA3)/toll-like receptor 4 (TLR4)-mediated positive feedback loop inducing NFκB. [9]. Currently, it is unclear how well these findings translate to human cancer progression. However, this could raise possibilities to therapeutically interfere with the endocrine and paracrine signaling networks required for the establishment of a premetastatic niche, thereby preventing the establishment and outgrowth of distant metastases.

1. Tumor cell dissemination and epithelial–mesenchymal transition

Epithelial tissues, representing the origins of most solid tumors, form relatively rigid sheets of cells. They are separated from the stroma by a basement membrane and are highly organized by lateral belts of cell–cell adhesion complexes. During the progression from a tumor in situ to an invasive carcinoma, epithelial tumor cells are released from their neighbors and breach the basement membrane barrier. The process underlying this phenomenon has often been suggested to involve EMT [10,11]. During EMT, initially polarized, epithelial cells acquire attributes reminiscent to those of mesenchymal cells, thereby inducing cellular invasion into neighboring tissues. Similar processes have been observed in embryogenesis, for example during gastrulation or migration of neural crest cells [12].

EMT is characterized by loss of cell polarity and downregulation of epithelial proteins, most prominently E-cadherin, but also occludin, claudins, cytokeratins or catenin proteins [10,13]. Cadherins and catenins participate in regulating cell–cell adhesion. Additionally, cells that have undergone EMT often acquire a spindle-shaped morphology, enhance cell migration and induce mesenchymal proteins like N-cadherin, vimentin, tenascin C, laminin β1 or collagen type VI α, as well as various proteinases [10,13]. The key signaling pathways and molecules inducing EMT include Receptor Tyrosine Kinases (RTKs), the transforming growth factor β (TGFB) superfamily, WNT, NOTCH, hedgehog pathway [14,15] and NFκB [16]. For example, concomitant RAS and TGFB signaling induces EMT in vitro [17] and is associated with metastasis and an EMT phenotype in a mouse skin carcinoma progression model [18]. Many of the EMT-inducing pathways play prominent roles in development and stem cell self-renewal [19,20]. Eventually, transcription factors of the snail family (SNAI1/snail, SNAI2/slug) and ZEB family (ZEB1, ZEB2), as well as TWIST1, TWIST2 and E12/E47, control the EMT transcriptome program [14,21–25]. The microRNA (miR) 200 family regulates ZEB levels, further contributing to EMT regulation [26].

EMT is likely to contain several intermediate steps, including "cell scattering" [27]. Furthermore, differences in stimuli and cell systems lead to variations in the extent of regulation of the various epithelium- and mesenchyme-specific proteins involved, as well as in the reversibility of these changes. Because of this variability and the fact that the EMT markers described in vitro await consistent confirmation in histological studies of human tumors, the role of EMT in tumor progression is still incompletely understood [28,29]. The current model proposes that EMT occurs both locally, that is, at the invasive front of tumors, and transiently, under influence of factors present in the tumor microenvironment, like TGFB [20]. Following dissemination, the reverse process, Mesenchymal–Epithelial Transition (MET), could be involved in establishing secondary tumors with an epithelial appearance, similar to the primary one [11].

EMT can promote metastasis in several ways. First, the loss of cell–cell adhesion allows for tumor cell invasion, as has been shown in E-cadherin knockout mouse models [30,31]. Invasion of tissues and vessels could be aided by a second property of cells that have undergone EMT, namely the commonly observed secretion of protein-degrading enzymes like matrix metalloproteinases (MMPs) [13]. Consistently, MMPs are often overexpressed in tumors and especially in the tumor stroma [32]. These molecules are able to remodel the ExtraCellular Matrix (ECM) within the tumor microenvironment (see below), thereby releasing and processing mitogenic and angiogenic factors sequestered by the ECM. Cleavage of ECM components like laminin 5 or collagen IV exposes cryptic sites that stimulate cell migration [33] or angiogenesis [34]. Third, E-cadherin provides not only adhesion but also functions in intracellular signaling, for example via its interacting partner δ-catenin (p120CTN). δ-catenin released from the dissolved E-cadherin complex affects the activity of small GTPases [35], corresponding to regulators of cell migration and adhesion implicated in metastasis [36]. Moreover, E-cadherin activity