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Review

Mechanisms governing metastatic dormancy in breast cancer

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ABSTRACT

Breast cancer is a systemic disease characterized by early dissemination of tumor cells to distant organs. In this foreign environment, tumor cells may stay in a dormant state as single cells or as micrometastases for many years before growing out into a macrometastatic lesion. As metastasis is the primary cause for breast cancer-related death, it is important to understand the mechanisms underlying the maintenance of dormancy and dormancy escape to find druggable targets to eradicate metastatic tumor cells. Metastatic dormancy is regulated by complex interactions between tumor cells and the local microenvironment. In addition, cancer-directed immunity and systemic instigation play a crucial role.

1. Introduction

Breast cancer is the most frequent cancer among women worldwide [1]. It is a heterogenous disease which can be subgrouped into different immunohistochemical and molecular subtypes [2]. The classical immunohistochemical analysis allows the distinction of three major subtypes, the estrogen receptor α (ERα)-positive, the human epidermal receptor 2 (Her2)-positive and the triple-negative breast cancer (TNBC). Each subtype is associated with specific prognoses and responses to anti-cancer drugs. Though treatment of breast cancer has been much improved leading to a decline in breast cancer mortality in developed countries like the United States [3], breast cancer is still a leading cause of cancer-related death among women [1]. The reason is that breast cancer is a systemic disease characterized by early tumor cell dissemination [4]. Disseminated tumor cells often withstand systemic therapies and can give rise to lethal metastasic lesions [5]. Because metastasis formation is a very inefficient process [6], this may take years or even decades during which the disseminated tumor cells are in a state of metastastic dormancy [7].

Metastatic dormancy can either be induced by the failure of cancer cells to proliferate in the foreign environment (cellular dormancy) or by the establishment of an equilibrium between proliferation and apoptosis (balanced proliferation). Balanced proliferation can be the consequence of the lack of an angiogenic network (angiogenic dormancy) or the result of a tumor-active immune defense which keeps metastatic growth at bay (immune system-induced dormancy) [8,9]. Computer simulations and experimental data are more compatible with the idea of metastatic dormancy being the result of cellular quiescence [10]. One major reason for cellular dormancy is the foreign microenvironment which poses a challenge to the tumor cells regarding survival and proliferation [11,12]. For a tumor cell to escape dormancy the dormancy-permissive microenvironment needs to be changed to a dormancy-restrictive microenvironment. Such a process can be triggered by the tumor cells themselves, as they are able to reshape the microenvironment to better serve their own needs [13]. Co-evolution of tumor cells and their microenvironments is a well-known phenomenon that drives tumor progression. A good example is the development of leukemia where premalignant haematopoietic cells co-evolve with abnormal osteoprogenitor cells to remodel the haematopoietic stem cell (HSC) niche such that it allows for efficient leukemic cell production [14]. Of note, the HSC niche is also a preferential place for disseminated breast cancer cells to lodge within the bone [15].

Angiogenic dormancy is induced by a limited availability of nutrients and oxygen. In this case, the tumor cells are able to proliferate and may have already formed micrometastatic lesions while awaiting stimulation of angiogenesis for further outgrowth (angiogenic switch) [16]. Angiogenesis can be either locally induced or triggered by systemic instigation, a term used to describe the ability of a tumor at one location to stimulate the growth of a tumor at another site [17,18]. In immune system-induced dormancy, cancer cells are eliminated by the immune system in the same rate as cancer cells are generated by proliferation [9].

In order to find ways to eliminate solitary dormant cells and dormant micrometastasis, we need to understand which mechanisms are responsible for metastatic dormancy and what processes trigger dormancy escape. This review intends to summarize the current knowledge on the mechanisms underlying metastatic dormancy in breast cancer. The preferred metastatic sites for breast cancer are bone,

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lung, liver and brain, whereby the frequency by which breast cancer form metastasis in these organs strongly depends on the breast cancer subtype [19,20]. Each organ poses a specific challenge to the tumor cells for their survival and proliferative activity. For example, a specific requirement for metastatic outgrowth in bone is the induction of osteoclastic bone degradation [21].

2. Factors involved in metastatic dormancy

2.1. Cellular dormancy

2.1.1. MAPK, Src kinases and AKT

Early studies on cellular dormancy had been performed with the Hep3 head and neck cancer cell line. Aguirre-Ghiso and co-workers showed that the proliferation status of Hep3 cells is determined by the balance between the activities of the mitogen-activated protein kinases (MAPKs) ERK1/2 and p38 [22,23]. A switch toward ERK1/2 phosphorylation favors proliferation, while predominant phosphorylation of p38 leads to quiescence. This was also shown *in vivo* by inoculating these cells into mice or chick embryos [24]. The activities of these kinases were found to be driven by a complex interaction of urokinase-type plasminogen activator receptor (uPAR), α5β1 integrin, fibronectin, focal adhesion kinase (FAK) and Src-kinase [23]. High expression of uPAR leads to activation of α5β1 integrin and subsequent activation of FAK and Src resulting in ERK1/2 phosphorylation. Phospho (P)-ERK1/2 levels relative to the P-p38 level can be further increased by uPA, the ligand of the uPAR, and by fibronectin which activates α5β1 integrin. In the contrary, p38 phosphorylation is favored when uPAR expression is lost and fibronection is absent. The expression of uPAR and α5β1 integrin also determined growth of these cells when inoculated into chick embryos [25]. In an analysis to determine the genes p38 is targeting to induce cellular quiescence, Aguirre-Ghiso's group found 16 transcription factors [26]. Among these were the proto-oncoproteins c-Jun and fork head box protein (FoxM1), which were downregulated by p38, and the transcriptional repressor basic helix-loop-helix protein 3 (BHLHB3), whose expression was increased by p38. Importantly, either downregulation of c-Jun or FoxM1 or upregulation of BLHLB3 induced cellular quiescence. More recently, dormancy was shown to be induced by transforming growth factor β2 (TGFβ2) which activates p38 in cancer cells disseminated to bone [27]. This effect coincided with upregulation of the proliferation inhibitor p27 and downregulation of cyclin-dependent kinase 4. Similarly, cancer cells whose dormant state was induced by hypoxia (see Section 2.1.6) showed high expression of TGFβ2 and p27 [28]. There is also evidence that the upregulation of p38 in dormant cells is linked to endoplasmic reticulum stress response [29,30]. The chaperone BiP (Grp78), the translation initiator factor 2α kinase RNA-dependent protein kinase like endoplasmic reticulum kinase and the transcription factor ATF6α are upregulated upon p38 activation which coincides with the inhibition of the pro-apoptotic protein Bax. Consequently, downregulations of these factors reduced survival of dormant cells and rendered them more sensitive to drugs. Interestingly, breast cancer cells disseminated to bone show increased expression of BiP [31].

The involvement of p38, ERK1/2, Src and integrin β1 in regulating proliferation and metastastic growth was also shown for breast cancer cells (Table 1). One study showed that inhibition of the lysophosphatidic acid receptor 1 induced metastatic dormancy of 4T1 and MDA-MB-231T breast cancer cells in Balb/c mice [32]. This effect coincided with decreased ERK1/2 and increased p38 activities. By using a Her2-transgenic mouse model, Harper and co-workers recently showed that expression of Her2 plus downregulation of p38 not only leads to early dissemination of breast cancer cells to lung and bone, but also to the formation of metastatic lesions [33]. In addition, patient-derived Her2-positive cancer cells which became dormant after entering the brain of immunocompromised mice showed low activity of p38 and high activity of ERK1/2 [34]. In another study, a 3D-environment that consisted of collagen, endothelial cells and either bone marrow-derived osteoblasts or mesenchymal stem cells (MSCs), was shown to be dormancy-permissive for ERα-positive and −negative breast cancer cells *in vitro* and *in vivo* [35]. This effect was dependent upon factors secreted by the stromal cells. Inhibitors of p38 or of the TGFβ1 receptor released breast cancer cells from the mitogenic block suggesting that p38 and TGFβ were involved in keeping cells in a quiescent state.

Osteoblasts and MSCs may also promote dormancy escape. In a recent study, Wang and co-workers showed that, under the experimental conditions they have used, these stromal cells rather stimulated breast cancer proliferation in 3D culture and induced outgrowth in the bone [36]. They demonstrated that, by direct binding to the breast cancer cells *via* heterotypic E-cadherin/N-cadherin complexes, osteogeneic cells form niches for breast cancer cells and induce the activation of mammalian target of rapamycin (mTOR) and AKT. Disruption of this cadherin-based cell-cell adhesion inhibited the formation of micrometastases. Osteogeneic cells may therefore play a dual role in metastatic dormancy in the bone. On the one hand, they may promote dormancy by secreting anti-proliferative factors, on the other hand, they may suppress dormancy by directly adhering to breast cancer cells.

Src and Src-related kinases were shown to a play a role in metastatic outgrowth of breast cancer cells in lung, liver and bone. El Touny and co-workers demonstrated that the ability of murine D2.03 breast cancer cells to form metastatic lesions in the fibrotic lung of CD1*nu/nu* athymic mice was strongly reduced by Src knock-down or by inhibition of Src kinases, each of which induced the activation of cell cycle inhibitor p27 [37]. In liver, breast cancer cells were found to interact with hepatocytes through claudin-2 [38] whose expression is positively regulated by the Src kinases Yes and Fyn and inhibited by the Src kinase Lyn [39]. Claudin-2 is only expressed by liver-seeking murine 4T1 breast cancer cells and shown to be only important for the formation of breast cancer metastatic lesions in the liver. In bone marrow-seeking MDA-MB-231 breast cancer cells, Src acts as a pro-survival factor by inhibiting apoptosis and by stimulating the activity of phosphoinositol-3-kinase (PI3K)/AKT pathway [40]. A highly active PI3K/AKT pathway was found to be essential for bone metastatic outgrowth of MDA-MB-231 cells. Interestingly, the PI3K/AKT pathway was shown to negatively regulate the activity of p38 in MCF-10-Her2 breast cancer cells [33], suggesting that the increased p38 activity in cells expressing low levels of AKT may contribute to keep breast cancer cells in a low-proliferating or quiescent state. A high AKT activity is also linked to the proliferative activity of low-metastatic MCF-7 breast cancer cells *in vitro*. Dey-Guha and co-workers demonstrated that MCF-7 cells undergo asymmetric division to generate one highly proliferating daughter cell with an integrin β1/FAK-induced high AKT activity and one slowly growing cell with low AKT activity [41,42]. Suppression of AKT signaling promoted the generation of slowly growing daughter cells. The slowly growing cells showed high levels of the stemness marker HES1 and were more resistant to chemotherapy *in vitro*. Notably, cells that survived chemotherapy in patients, as measured by comparing primary breast cancer biopsies before and after treatment, expressed low AKT levels. Also, breast cancer cells residing in bone of *non*-metastatic breast cancer patients show low AKT activity [43].

2.1.2. Factors regulating stemness

Given the concept of cancer stem cells (CSCs) being responsible for metastasis [44], regulation of stemness should play a role in metastatic dormancy and dormancy escape. This notion is supported by a study showing that BMP4, secreted by lung resident cells, induces dormancy of murine 4TO7 breast cancer cells by keeping stemness-relevant genes, such as Nanog and Sox2, at a low level [45]. When breast cancer cells gain the ability to express Coco, a BMP antagonist, they can escape from dormancy which coincides with higher expression of stemness genes. Interestingly, the mechanism does not apply for breast cancer dormancy in bone and brain, probably because disseminated cancer cells inhabit niches in these organ which do not contain BMP4. Two other