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Research paper

The role of microRNAs in bone metastasis

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ABSTRACT

The skeleton represents a common site of metastases for osteotropic cancers such as prostate and breast tumors and novel therapeutic targets and new markers for the monitoring of bone lesions are urgently needed. The formation of bone metastases is a complex process that starts at the level of the confined tumor and that is characterized by a dynamic crosstalk between the primary cancer and the future metastatic site, the bone. Factors released by the primary tumor contribute to prepare a fertile "soil", where a "pre-metastatic niche" is established prior to future colonization by cancer cells. When the primary cancer progress from the confined disease to its invasive phase, tumor cells will acquire an invasive phenotype, enter into the circulation and colonize the previously prepared site where they will establish a "metastatic niche". Among the variety of molecules that participate in the metastatic cascade, microRNAs are a class of small non-coding RNA that play an important role in the development of metastatic bone lesions. Many studies have addressed the role of small non-coding RNAs (miRs) in metastasis in osteotropic cancers and have highlighted the role of miRs as oncogenes (oncomiRs) or tumor suppressor miRs.

In this review we present describe the role of miRs in the processing of the supportive bone microenvironment prior and after the bone colonization by cancer cells. Finally, future therapeutic strategies and perspectives are also discussed.

1. Introduction

The skeleton represents a common site of metastases for prostate and breast cancer with approximately 70% of the patients dying of these cancers showing evidence of metastatic bone disease at autopsy. In addition, carcinomas of thyroid, kidney and lung cancer metastasize to bone, albeit at lower frequency (30% to 40% of the cancer deaths at autopsy) [1]. One of the major challenges in oncological research is the identification of new therapeutic targets and the discovery of new markers for the monitoring the development of bone lesions, particularly at the early stage and for their treatment.

The notion that molecular factors might be involved in the specific bone tropism of some cancer cells was for the first time postulated by Sir Stephen Paget who introduced the "Seed and Soil" hypothesis in which he compared the bone metastatic breast cancer cells to the seed of plants, capable of growing only in a fertile soil, the bone marrow [2]. Even more, tumor cells localized at the primary site are known to prepare this fertile soil for future tissue/organ colonization through the establishment of the so called "pre-metastatic niche" [3]. The formation of bone metastasis is characterized by a complex number of sequential events, that occur at the level of the primary tumor, when the cancer progress from the confined disease to its invasive phase. For skeletal metastasis to occur, osteotropic tumor cells must acquire an invasive phenotype by various modes of migration, either as single cells or as multicellular cluster also known as collective migration [4]. For instance, several carcinomas may undergo the so-called epithelial-to-mesenchymal transition (EMT) and acquire invasive characteristics. These cells switch from a sessile/epithelial to an invasive/mesenchymal phenotype, invade the extracellular matrix and the surrounding stroma and, subsequently, may enter the lymphatic or blood circulation. In aggressive cancer, many cancer cells are shed into the circulation every day, now referred to as circulating tumor cells (CTCs), but the efficacy of CTCs to successfully colonize distant tissues and develop into clinically overt metastases is relatively low. Once that these CTCs have colonized the metastatic site (e.g. the bone/bone marrow), they are referred to as disseminated tumor cells (DTCs). DTCs may remain dormant for years [5] and, due to largely unknown mechanisms, develop into macrometastases. The reciprocal interaction between cancer cells and the tissue-specific stroma is critical for primary and metastatic tumor growth progression. Prostate and mammary cancer cells metastasize to bone, where they induce either an osteoblastic or osteolytic response respectively. These opposite stromal responses suggest that different types of cancers adopt distinct strategies to

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hijack the bone marrow/bone stroma for their growth support ('metastatic niche'). However, the molecular signals underlying these divergent responses are largely elusive [6].

In the past decade, preclinical studies have addressed the putative role of non-coding RNAs in tumorigenesis, metastasis and therapy response in osteotropic cancers. Besides long non-coding RNAs, microRNAs (miRs) represent a class of small non-coding RNAs, (18–25 nucleotides long), that regulate protein abundance by promoting mRNA degradation or translational repression [7], thus acting as oncogenes (oncomiRs) or tumor suppressor miRs. Several miRs have been identified as key molecules in tumorigenesis, bone tropism and the development of metastatic bone disease [8].

In this review, we provide a concise description on the role of miRs in bone metastases. In particular, we will focus on the processing of the supportive microenvironment in bone marrow prior and after the bone colonization mediated by specific miRs. Finally, future therapeutic perspectives are also discussed.

2. Osteotropism and miRs

The dissemination of prostate and breast cancer metastatic cells specifically to the skeleton is defined as osteotropism and is determined by multiple factors expressed by the tumor cells and the bone microenvironment. The interactions between cancer cells and the endothelium of the bone marrow vasculature is one of the key processes which precedes extravasation from the blood vessels and suggested to underlie the bone-specific dissemination [9]. During this process, surface molecules expressed on cancer cells such as the chemokine (C-X-C motif) receptor (CXCR) 4 (CXCR4), αvβ3 integrin, CD44 and RANK are directly involved in homing to bone [10]. Briefly, the interaction between CXCR4 and its ligand stromal derived factor 1 (SFD1, also known as CXCL12) is critically important in the formation of prostate and breast cancer bone metastasis. CXCR4 is significantly elevated in breast carcinoma compared to normal tissue and miR-218 has been shown to up-regulate CXCR4 [11]. Moreover, CXCR4 signaling induces expression of matrix metallopeptidase (MMP) 9 and MMP13 in tumor cells. Interestingly, miR-218 has also been shown to reduce MMP9 expression [12] and appears to be a crucial regulator of osteomimicry in breast cancer, a process which regulate the expression of osteoblast specific genes by tumor cells, that may facilitate the growth of metastatic cells in the bone microenvironment [13]. MMP13 is, on the other hand, regulated by miR-126, which has been shown to reduce the formation of breast cancer bone metastases [14] and has also been associated with prostate cancer metastases [15]. Osteotropic breast cancer cells express vascular-endothelial molecule-1 (VCAM1) that is also targeted by miR-126 [16]. VCAM1 binds α4β7 and α4β1 integrins on osteoclast progenitors that, in turn, can induce excess osteoclastogenesis and subsequently lead to radiologically-evident osteolytic lesions. Furthermore other members of the integrin family, αv integrins, have been shown to be required for the maintenance of cancer stem cell properties and to be involved in bone colonization, angiogenesis by activated endothelial cells and osteoclastic bone resorption [17]. CXCL12 has been shown to induce the activation of integrin αvβ3, that mediates multiple cell-extracellular matrix interactions during tumor progression and skeletal metastasis, including stromal processes like osteoclastic bone resorption and angiogenesis. Recently, our group demonstrated that miR-25 is strongly decreased in the highly osteotropic cancer stem/progenitor subpopulation of human prostate cancer cells and directly regulates integrin-αv expression [18]. Overexpression of miR-25 reduces the metastatic dissemination, thus supporting the notion that miR-25 is a key regulator of cancer stemness and in the formation of distant bone metastases.

3. miRs and the 'pre-metastatic' bone niches

As described above, the reciprocal interaction between cancer cells and the tissue-specific stroma is critical for primary and metastatic tumor growth progression. In organ-confined primary tumors soluble factors and extracellular vesicles (e.g. exosomes) can be released in the circulation. These factors may contribute to the conditioning of the future, distant metastatic sites, the so-called 'pre-metastatic niche' [3]. During this process, hematopoietic progenitor cells (HPCs) expressing VEGF receptor 1 (VEGFR1) are recruited to metastatic target organs by specific factors released by the primary tumor. Among these factors, LOXL enzymes, VEGFA, VEGFC, TNFα and TGF-β produced by the primary tumor stimulate inflammation, attachment, differentiation and recruitment of, for example, immunosuppressive myeloid cells. Recently, miR-26a and miR-29 have been shown to decrease LOXL2 [19] which suggest a tumor suppressive role for these two miRs. Additionally, miR-29 has been proven to inhibit cell migration in prostate cancer [20]. Interestingly, it has been proposed that extracellular vesicles and exosomes released from the primary tumor represent a mechanism of communication between the primary cancer cells and the metastatic sites during the induction of the "pre-metastatic niche", recently reviewed in [21]. Additionally, extracellular vesicles released from bone marrow mesenchymal stem/stromal cells (MSCs) have been shown to transport tumor supportive miRs [22]. Together these observations reinforce the notion that bi-directional interactions tumor-bone stroma participate in the establishment of bone metastases.

The miR signature of exosomes isolated from bulk prostate cancer cells (Fig. 1**A**, blue cells) and from their cancer stem cell compartment (Fig. 1**A**, green cells), display high levels of miR-21, miR-30 and miR-218 [23] (Fig. 1**A**, blue arrow). miR-21 and miR-30 have been identified as key regulators of osteoblast differentiation and have been included in a panel of microRNA biomarkers designated as "OstemiR" [24]. Therefore, high levels of these miRs may lead to increased bone remodeling "at a distance" and may facilitate subsequent metastatic colonization and cancer cell growth in the bone marrow microenvironment. Moreover, miR-21 increased expression of MMP2, MMP9 and MMP13, inducing extracellular matrix (ECM) remodeling and facilitating EMT. In the same study, CSC-derived exosomes (Fig. 1**A**, green arrow), were found to contain high amounts of miR-183 (Fig. 1). Furthermore, miR-183 has been shown to increase osteoclastogenesis by repressing heme oxygenase-1 (HO-1) [25]. It appears, therefore, that exosomal miR-183 may represent a supportive factor in the conditioning of the bone microenvironment by highly metastatic cells and reinforce their role in the conditioning and formation of the 'receptive pre-metastatic niches'.

4. miRs and metastatic bone niches

In primary and metastatic cancers, tumor cells interact with different cell types that constitute the bone/bone marrow stroma such as osteoblast and osteoclasts, tumor-associated macrophages (TAMs), bone marrow stromal fibroblasts, endothelial cells, pericytes, MSCs and immune cells like myeloid-derived suppressor cells (MDSCs) [26]. Multiple miRs have been associated with the interaction between tumor cells and stromal cells, reviewed in [27]. miR-511-3p reduced the pro-tumoral activity of TAMs [28]; up-regulation of miR-31 and miR-214 while inhibition of miR-155, abrogated the CAF phenotype [29]. Tumor cells produce several factors that "activate" the surrounding stromal cells and induce remodeling of extracellular matrices. These factors include fibroblast growth factor 2 (FGF2), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth