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Disruption of Crosstalk between Mesenchymal Stromal and Tumor Cells in Bone Marrow as a Therapeutic Target to Prevent Metastatic Bone Disease

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Abstract

Skeletal metastasis is a serious complication of many primary cancers. A common feature of tumor cells that metastasize to the bone marrow microenvironment is that they initiate a cascade of events, recruiting and presumably/potentially altering the phenotype of bone marrow mesenchymal stromal cells (MSC) to produce an environment that allows for tumor growth and in some cases, drug-resistant dormancy of latent cancer cells. Consequently the MSC population can contribute to metastatic disease through several distinct mechanisms by differentiating into cancer-associated fibroblasts (CAFs). Understanding the expression and epigenetic changes that occur as normal MSCs become associated with metastatic tumors would reveal possible therapeutic targets for treating skeletal metastasis.

Keywords

Metastasis; Mesenchymal stromal cell; Cancer-associated fibroblast

Bone is Integrally Involved in Cancer Metastasis

Bone is a preferential target site for many types of cancer metastasis. Because of the frequency of osteotropic metastasis, an estimated 280,000 adults in the United States are living with metastatic bone disease (Li et al., 2012). Bone is a site of primary metastases most frequently occurring in patients with breast, prostate, and lung cancers although many other cancer types are also osteotropic. The presentation of skeletal metastases is often a pivotal and potentially catastrophic event for patients with breast cancer, as metastatic bone lesions can cause serious complications including compression of nerves and spinal cord, fractures, and severe pain requiring palliative care, all of which may negatively affect

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survival (Coleman, 2006; Selvaggi & Scagliotti, 2005). Metastatic bone disease is highly aggressive, destroying bone structure due to tumor cell growth and production of osteolytic factors (Weilbaecher, Guise, & McCauley, 2011). Our current understanding of why bone is a preferential site of metastasis is based on the acquired osteomimetic properties of primary tumor cells; that is, their ability to produce bone-related developmental factors that promote their vascular invasion, homing, and residence in the bone microenvironment where tumor cells survive. This knowledge suggests that bone directly contributes to the metastatic phenotype and may be a critical component in treating cancer progression.

Bone is a reservoir of extracellular cytokines and contains rich population of pluripotent cells in the marrow. Hematopoietic stem cells (HSCs) in the marrow cavity can form and regenerate components of the hematopoietic system and mesenchymal stromal cells (MSCs) can maintain and repair bone, muscle and adipose tissue. In instances of tissue damage, such as fracture repair, MSCs are rapidly recruited to sites of inflammation where they differentiate into cell types that will contribute to new tissue (Quante et al., 2011; Taichman et al., 2010). A negative consequence of this ability to respond to inflammation mediators is that MSCs may be recruited to sites of spurious inflammation or cytokine production associated with tumor growth. MSCs recruited to primary tumors facilitate tumor growth by differentiating into cancer-associated fibroblasts (CAFs) (Jung et al., 2013). Although cytokine expression patterns indicate that CAFs are important in establishing tumor progression and migration (Liu et al., 2011), how bone-derived MSCs are recruited into primary tumor sites and what regulates their conversion into CAFs is not entirely understood. These events could represent a critical step in initial tumor formation and progression.

Metastasis Requires a Suitable Host Microenvironment

Metastasis is an integral part of cancer progression, and as such, cancer treatment should include a focus on preventing or minimizing metastatic disease at a secondary organ (e.g., bone or the marrow stroma) as part of a comprehensive, multifaceted regime. Cancer metastasis has frequently been described as a process that requires “seed and soil”—an interaction between metastatic cells “seeds” and tissue microenvironments that can support their growth “soil” (Fidler, 2003). Several lines of evidence would suggest that effective therapy should address both the metastatic cell and the host environment to prevent metastasis. For example, metastasis of primary cancer cells to a new site is highly inefficient. Malignant primary tumors can release thousands of circulating tumor cells into the blood stream, but only a minute fraction of these cells are able to establish a viable colony in a distal organ (Gupta & Massagué, 2006; Kienast et al., 2010). This suggests that only a subset of tumor cells have the appropriate properties for attraction to bone and the properties of the host tissue are integrally involved in the establishment of metastatic tumors. Additionally, the selective tropism of different cancer cell types cannot be explained entirely by gene expression patterns from primary tumors (Weigelt et al., 2003). The microenvironment supporting secondary tumor growth would therefore dictate when and where metastatic cells of specific tissue origin can attach and survive. Potentially, the stroma of the bone marrow not only supports invasion, but differentiates *in situ* to surround metastatic “seed” cells, forming the “soil” that supports survival and growth at the

secondary site (Figure 1). These events, in establishing secondary tumor attachment and growth, parallel events that established the primary tumor: recruitment of MSCs and differentiation into CAFs.

MSCs of the bone marrow are involved in the formation and maintenance of the HSC niche. The HSC niche is a source of pluripotent, undifferentiated blood precursors that have the ability to regenerate circulating blood cells throughout the life of an individual. Signaling events within this niche regulate HSC self-renewal and differentiation while dysregulation of the niche can lead to hematopoietic abnormalities (Renström, Kröger, Peschel, & Oostendorp, 2010). In addition, because of the hospitable environment, the HSC niche is an ideal environment for the establishment of an attachment site for metastatic tumor cells that would allow for new growth or dormancy. Metastatic cancer cells can compete directly for occupancy of the HSC niche and have been postulated to evict HSCs into the peripheral blood or drive them into premature maturation so they vacate an available niche (Shiozawa, Havens, Pienta, & Taichman, 2008). The preservation of the HSC niche is an important consideration as it represents a vital, yet fragile structure that would require protection from metastatic cells in cancer patients. Due to the complex nature of the MSCs in the bone microenvironment, there is a necessity to understand the molecular mechanisms that define specific interactions dictated or regulated by subsets of MSCs. Such studies could lead to the identification of diagnostic markers and potential targets for reversion of the differentiated, tumor-associated phenotype.

CXC-Related Proteins as Biomarkers for MSC-to-CAF Differentiation

Recent studies have demonstrated that MSC-derived CAFs can be identified by distinct cell surface markers that differentiate these cancer-associated cells from normal MSCs and associated tumor cells. Compared with normal MSCs, CAFs have increased extracellular matrix production and secretion of cytokines that promote tumor growth (Polanska & Orimo, 2013). In addition, growing evidence suggests that specific members of the CXC family of G protein-coupled receptors (CXCRs) and associated ligands (CXCLs) are upregulated in MSCs that will become CAFs (Zhang et al., 2013). The differentiation of MSCs into CAFs is associated with an increase in CXCL12 production, which supports metastasis, growth and proliferation of tumor cells at secondary sites such as bone (Figure 1). The upregulation of CXC-related receptors and ligands in MSCs can be viewed as a defining characteristic in the differentiation of normal, pluripotent MSCs to a committed, tumor-associated phenotype. As such, these cell surface markers and expressed genes could be exploited as a tool for distinguishing normal MSCs from those that have begun to differentiate into CAFs.

In addition to the CXC family genes, several other genes have been shown to be upregulated in MSC-derived CAFs (compared to normal fibroblasts) (Nakayama et al., 2014); however, a full characterization of mRNA, microRNA, and long noncoding RNA expression in normal vs. differentiating MSCs in human samples has not been done. Identifying a global RNA expression signature associated with the onset of differentiation into CAFs could provide new biomarkers to identify CAFs and new insight into mechanisms of metastasis. It

would be informative to conduct such a study using MSCs isolated from biopsies or surgically resected tissues from patients with metastatic tumors.

Understanding Epigenetic Control of MSC Differentiation is Critical to Defining MSC Populations

Cell-fate decisions and differentiation events are regulated by the inheritance of epigenetic modifications as a cell progresses through the cell cycle, which in turn lead to changes in gene expression patterns in the progeny cell population. Many epigenetic patterns are inherited as post-translational modification of histone proteins associated with chromatin. Defining specific histone modifications in a cell line or tissue can identify regulatory regions that influence gene expression through activating or repressive mechanisms resulting in unique chromatin modification states. For example, H3K36me3 or H3K79me2 marks throughout gene coding regions signify that the genomic territory is a region of active gene elongation and can be correlated to gene expression (Rivera & Ren, 2013; Wagner & Carpenter, 2012). Poised or active enhancers can be identified by H3K4me1 and H3K27ac modifications, while repressed domains, enhancers and genes are usually marked by H3K27me3 (Creyghton et al., 2010; Zhu et al., 2013). Histone modification profiling has been useful in understanding the widespread changes in gene expression observed during epithelial-mesenchymal transitions (Tam & Weinberg, 2013) and loss of transcription plasticity in prostate carcinoma (Coolen et al., 2010), but the unique epigenetic signature for MSC-derived CAFs has yet to be defined.

Epigenetic Modifications as Therapeutic Targets

In addition to providing a molecular epigenetic signature of tumor-associated CAFs, analysis of global histone modification changes could also serve as a basis for therapeutic strategies to prevent metastasis. Since each type of histone modification is mediated by a specific chromatin-modifying enzyme or enzyme complex, global changes in particular histone marks can be attributed to the activity of the associated enzyme or enzyme complex. For example, H3K27me3 levels above or below normal values would suggest that enzymes associated with the polycomb protein complex PRC2 are involved. PRC2 maintains repression of a large number of genes through EZH2, a histone methyltransferase that specifically modifies H3K27 residues (Helin & Dhanak, 2013). To address this, highly potent and selective chemical inhibitors of EZH2 have been developed (e.g., GSK126, GSK343), at least one of which (EPZ-6438) has entered human clinical trials for B-cell lymphoma and solid tumors with elevated H3K27me3 profiles (Prinjha & Tarakhovsky, 2013). Similarly, enzyme complexes involved with facilitating other epigenetic modifications may also prove to be viable targets for therapeutic intervention. Defining an epigenetic signature of cancer-associated MSCs in the tumor bone environment would establish potential diagnostic targets and regulatory mechanisms that can be targeted for new therapies aimed at restoring or maintaining MSCs in their nascent, undifferentiated state.

Future Considerations on the Prevention of Skeletal Metastasis

Considering how drastically skeletal metastasis can reduce quality of life for cancer patients, the available treatment options for this step in the progression of many common cancers are inadequate. Previous research into the development of bone metastases indicates that the microenvironment of the bone marrow is extremely important in the formation of metastases and that MSCs, in particular, contribute to the “successful” attachment and growth of circulating tumor cells at secondary sites. Scientific understanding of the role of MSCs in promoting bone metastases has accumulated to a point at which research scientists, working with orthopedic surgeons, can develop methods to directly compare genetic expression and epigenetic signatures in normal MSCs and CAFs from the same patient. Markers identified in MSCs associated with metastatic tumors could conceivably be used to identify pre-metastatic or analogous mechanisms in MSC-derived stroma surrounding primary breast tumors. Such studies also have potential for identifying signature epigenetic and/or expression patterns related to osteotropism of specific tumor types (such as breast, prostate, and other cancers).

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Figure 1. Bone Marrow Cells Facilitate Cancer Metastasis and Tumor Growth

Bone marrow-derived MSCs differentiate to form CXCL12 expressing CAFs that facilitate cancer growth at the primary tumor site. Metastatic cancer cells invade the bone environment and newly formed tumors associate with MSCs that will become CAFs.