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Mini-review

Mesenchymal stroma: Role in osteosarcoma progression

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ABSTRACT

The initiation and progression of malignant tumors are supported by their microenvironment: cancer cells *per se* cannot explain growth and formation of the primary or metastasis, and a combination of proliferating tumor cells, cancer stem cells, immune cells mesenchymal stromal cells and/or cancer-associated fibroblasts all contribute to the tumor bulk. The interaction between these multiple players, under different microenvironmental conditions of biochemical and physical stimuli (i.e. oxygen tension, pH, matrix mechanics), regulates the production and biological activity of several soluble factors, extracellular matrix components, and extracellular vesicles that are needed for growth, maintenance, chemoresistance and metastatization of cancer. In osteosarcoma, a very aggressive cancer of young adults characterized by the extensive need for more effective therapies, this aspect has been only recently explored. In this view, we will discuss the role of stroma, with a particular focus on the mesenchymal stroma, contributing to osteosarcoma progression through inherent features for homing, neo-vascularization, paracrine cross-feeding, microvesicle secretion, and immune modulation, and also by responding to the changes of the microenvironment that are induced by tumor cells. The most recent advances in the molecular cues triggered by cytokines, soluble factors, and metabolites that are partially beginning to unravel the axis between stromal elements of mesenchymal origin and osteosarcoma cells, will be reviewed providing insights likely to be used for novel therapeutic approaches against sarcomas.

Introduction

Tumors irrespective of their origin are heterogenous cellular entities whose growth and progression greatly depend on reciprocal interactions between genetically altered neoplastic cells and their non-neoplastic microenvironment. Cancer cells *per se* cannot explain growth of primary tumor and metastasis, but rather the combination and interaction between differentiated tumor cells, cancer stem cells (CSC), and normal cells reacting to the tumor, i.e. cells of mesenchymal origin and immune cells, combine to form the tumor bulk; the interplay between them regulates the production and biological activity of many soluble factors and extracellular matrix components that allow the growth and maintenance of solid tumors [1]. In this view, the reactive stroma is considered a foe in the development and progression of cancer. Only recently has this concept been suggested also in osteosarcoma (OS) [2–4]. In this malignancy, tumor cells and the mesenchymal stroma are indistinguishable from a morphologic point of view and share similar antigens (see Table 1); these features make the two populations very difficult to be distinguished in tumor biopsies and studied separately. OS originates and develops in bone where a high concentration of mesenchymal progenitors are present. Notably, in the context of OS, MSC are a cellular component of the tumor-associated stroma and the cancer-initiating cell with an ontogenic role. In line with this concept, we demonstrated the transformation of normal mesenchymal cells in OS, by lentiviral transduction of the MET oncogene, suggesting that MET overexpression is essential for the development of the cancer phenotype [5].

OS is the most common primary malignant bone tumor, arising in children and adolescents and occurring commonly in the metaphyseal regions of long bones; it develops during the puberal growth spurt at sites of rapid bone growth [4]. At the time of diagnosis, lung metastases are present in 20% of patients but it has been estimated that undetectable micrometastasis are present in 80% of cases [6]. Despite clinical application of

Abbreviations: mesenchymal stromal cells (MSC), osteosarcoma (OS); cancer-associated fibroblasts (CAF), interleukin-6 (IL-6); cancer stem cells (CSC), exosomes (EXO).

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Table 1
Cell surface antigens and mesenchymal markers expressed by MSC, OS or CSC.

	Normal MSC or MSC isolated from OS biopsies	OS or CSC
CD13	+ [25]	+ [129]
CD14	– [26,130]	nd
CD19	– [26,130]	nd
CD29	+ [25]	+ [131]
CD31	– [26]	nd
CD34	– [25,26,130]	nd
CD44	+ [25,26]	+ [129,132]
CD45	– [25,26,130]	– [133]
CD54	+ [25]	+ [129]
CD73	+ [25,26,130]	+ [134]
CD90	+ [25,26]	nd
CD105	+ [25,26,130]	+ [134]
CD106	+ [134]	+ [134]
CD117	+ [135]	+ [136]
CD133	+ [137]	+ [132,138]
CD146	+ [135]	+ [139]
CD166	+ [25,26]	nd
CD184	– [26]	nd
CD271	– [26,135]	+ [140]
c-Met	+ [141]	+ [72,142,143]
HER-2	+ [144]	+ [142]
IGF1R	+ [145]	+ [12,142]
VEGFR-3	+ [146]	+ [142]
IR	+ [147]	+ [12,142]
STRO-1	+ [135]	± [148] [136]
vimentin	+ [3,26]	+ [134]
alpha-smooth muscle actin	+ [3,26]	+ [149]

*nd, not determined.

standard treatment strategies (i.e. the combination of surgery resection and multi-chemotherapy) significantly improving patients' survival, outcomes have reached a plateau over the last decades [7]. Yet, the most aggressive treatment regimen is still not sufficient to eradicate chemotherapy-resistant subpopulations, and most patients die of widespread metastasis and tumor relapse, dropping overall 5-years survival to less than 70% [8–10]. Cytogenetic analyses of OS cells have shown highly aberrant karyotypes with numerous gene dysregulation and variations of DNA copy number and methylation [11]. Compared to other malignancies characterized by well-described single mutations, OS treatment cannot therefore take advantage of mutation-specific drugs such as mAbs. Despite the promising results obtained in preclinical models by using tyrosine kinase inhibitors, like anti-IGF1R and anti-IR strategies [12], molecular targeting has shown a lack of both significant and clear therapeutic benefit in patients [13]. Finally, since the discovery of the major role played by P-glycoprotein expression in conferring chemoresistance [14], the number of recurrence has not improved. In this context, the study of the interactions between tumor and stromal cells gains even more importance, as it might unveil novel therapeutic approaches.

Here, we will review the most recent advances in the cross-talk between OS stromal elements and sarcomas and its role in tumor progression and chemoresistance.

The osteosarcoma-associated mesenchymal stroma

Tumor-associated stroma mainly consists of two macro-categories. The first includes the extracellular matrix, formed of structural proteins, such as collagen and elastin, specialized proteins, such as fibronectin, and proteoglycans, such as hyaluronan [15]; the second is formed by the cellular elements: all cells surrounding the tumor tissue take part to the stromal response i.e. bone cells, vasculature and endothelial cells, pericytes, immune cells, such as macrophages and lymphocytes, and MSC. In addition, fibroblasts that differentiate from MSC and that usually switch to a tumor-promoting cells, the CAF [16], are also present in the tumor microenvironment.

A number of studies have suggested that stromal cells play a key role in OS development and progression [17]. Among these, also osteoclasts, the cells that are responsible for bone resorption both under physiological and pathological conditions, have a major role in OS, like in osteolytic bone metastases. Indeed, in the cancer environment, a number of cytokines, including the most important pro-osteoclastogenic factor, i.e. receptor activator of nuclear factor-κB ligand (RANKL), increase osteoclast resorption function, resulting in further bone destruction. In the primary OS site, osteoclasts reciprocally modulate the osteoblastic behavior of OS cells [18] and, as we previously demonstrated, increase OS aggressiveness; consequently, the presence of a high number of osteoclasts at the primary site is considered a bad prognostic factor. Given the role of osteoclasts in tumor formation, a number of studies have tested the effect of drugs affecting bone resorption, such as bisphosphonates. Indeed, zoledronic acid in combination with standard chemotherapy decreased OS growth by inhibiting OS-mediated osteolysis *in vivo* and *in vitro* [19,20]. Unfortunately, when tested in a randomized trial, zoledronate did not show any significant improvement in OS treatment [21].

Given the difficulties in targeting osteoclasts, the focus of the attention in OS therapy moved to MSC. Here, we are going to focus on the mesenchymal stroma, with particular emphasis on MSC and their role in OS progression.

The mesenchymal stroma

MSC are non-hematopoietic precursors residing in the bone marrow and contributing to the maintenance and regeneration of a variety of tissues of mesodermal lineage, including bone [22] (for an extensive review see [23]). MSC differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* when supplemented by the addition of several factors in the culture medium. As with other stem cell types, MSC possess an enormous capacity for self-renewal while maintaining pluripotency. In addition to being multi-potent, MSC possess immunomodulatory functions and can interact with cells of the innate and adaptive immune system, either through cell-cell contact or through their secretome [24]. MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules [25] (Table 1). Interestingly, as MSC acquire a transformed aggressive phenotype, as in the case of OS cells, the expression of surface receptors increases and, to a certain extent, changes. For example, CD271 positive OS cells acquire all the characteristics of aggressive CSC [26], whereas consensus sourcing indicates that MSC have to be CD127 negative [26].

In vivo, MSC home to the area of insult and inflammation, and it is generally assumed that these stem cells follow the same steps that were described for leukocyte homing [27,28]. Like a double-edged sword, stem cell and tumor biology both focus on similar cell phenotypes and functions, but the characteristics that make MSC beneficial for tissue regeneration are the same that make them harmful in the context of sarcoma tumorigenesis.

Mesenchymal stromal cells:anti-cancer vs pro-tumorigenic effects

Tumors are increasingly described as “wounds that never heal” because of their continuous production of inflammatory cytokines that maintain the tumor microenvironment and assist progression [29]. Moreover, this concept implies that cells that are involved in angiogenesis and response to injury, such as MSC, have a prominent