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Mini-review

Advances in osteosarcoma stem cell research and opportunities for novel therapeutic targets

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ABSTRACT

Osteosarcoma is the most common type of bone cancer, especially in children and young adults. The primary treatment for osteosarcoma is a combination of surgery and chemotherapy, however prognoses remain poor due to chemoresistance and early metastases. Osteosarcoma stem cells appear to play central roles in tumor recurrence, metastases and chemoresistance *via* self-renewal and differentiation. Targeting these cells may provide a novel strategy in the treatment of osteosarcoma. This review summarizes current knowledge of this rare phenotype and recent advances in understanding the functions OSCs (osteosarcoma stem cells) in osteosarcoma, with the aim of improving therapies in the future.

Introduction

Osteosarcoma is the most common type of bone cancer and the second leading cause of cancer-related deaths in children and young adults [1]. The majority of osteosarcomas originate in the long bones, such as the distal femur and proximal tibia. It is highly aggressive with a metastatic rate of ~20%, with the most common targets being the lung and other bones [2]. Although the origins of osteosarcoma remain unclear, the most likely candidates are considered to be mesenchymal stem cells (MSCs) or osteoprogenitor cells [3,4]. Currently, the primary treatment for osteosarcoma is a combination of surgery and chemotherapy. However, osteosarcoma frequently develops resistance to conventional chemotherapies resulting in tumor recurrence. Amputation of the affected limbs is often the only option but even this usually fails to save a patient's life due to early metastases [5]. A better understanding of tumor pathology in osteosarcoma and the mechanisms of initiation and recurrence are urgently needed to improve patient prognosis.

Discovery of osteosarcoma cancer stem cells

Emerging evidence has indicated that malignant tumors contain a hierarchy of cells responsible for tumor initiation, propagation, recurrence and resistance to therapy [6]. These include a rare phenotype termed cancer stem cells (CSC), that have the ability to retain their stem cell-like properties through self-renewal and differentiation [7]. CSCs are generally more malignant than differentiated cancer cells and may present a precise therapeutic target that circumvents conventional treatments to the tumor bulk. Elucidating the role of CSCs in osteosarcoma may improve prognosis in the treatment of osteosarcoma [8,9].

The existence of OSCs (Osteosarcoma stem cells) was first demonstrated by Gibbs et al. who identified a subpopulation of cells in human osteosarcoma tissue samples and cell lines that were capable of growing sarcospheres, or osteospheres, in serum-free conditions [10,11]. Martins-Neves et al. identified a subpopulation of cells in MNNG/HOS osteosarcoma cell lines that showed resistance to chemotherapeutic agents and irradiation and exhibited stem-like properties [12]. Other *in vitro* studies supporting the existence of OSCs included the formation of sarcospheres in MG63 cells under anchorage-independent conditions [13] and isolation and characterization of OSCs in human and canine osteosarcoma [14].

Side population (SP) cells have also been identified in osteosarcoma. These exhibit increased sphere-forming and colony formation capabilities and higher tumorigenicity compared to non-SP cells [15]. This was confirmed by Yang et al. who demonstrated that only the SP fraction had the capacity to self-renew [16].

Research now focuses on the regulatory mechanisms that underlie OSC initiation and activities and their potential as therapeutic targets in the treatment of osteosarcoma [1,8,11].

Techniques to isolate osteosarcoma stem cells

Surface markers, such as CD133, are commonly used to identify CSCs; however their biological function in osteosarcoma is unclear and suitable markers for separating OSCs from differentiated cells

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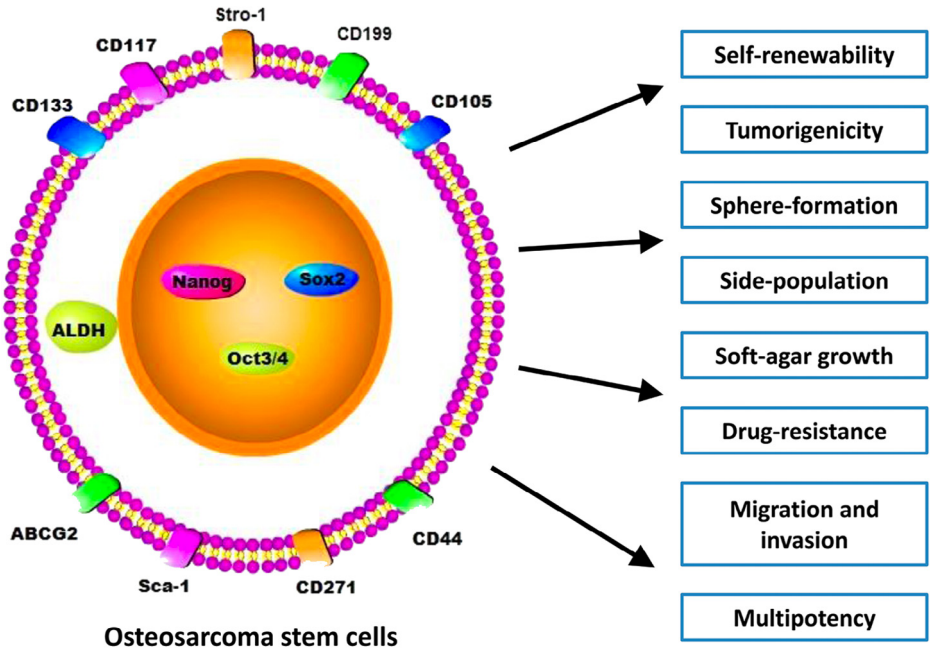

Fig. 1. Molecular markers used to isolate OSCs in tumors. The schematic diagram illustrates the functional roles of three key pluripotent molecules Oct3/4, Sox2 and Nanog and associated markers commonly used to identify OSCs.

remain to be found [17]. Gemei et al. detected 50 out of 245 membrane proteins that were differentially expressed between OSCs and differentiated cells through gene expression profiling. Their results have provided valuable data towards defining a cell-surface protein signature for OSC [18].

Fig. 1 provides a schematic illustration of these markers in OSCs; Table 1 summarizes their functions.

CD133

CD133 (AC133), a member of prominin family, is a glycoprotein with 3 isoforms that has been wildly used for isolating CSCs. Several studies have linked CD133-positive cells with a stem-cell phenotype. These include CD133-positive subpopulations exhibiting stem-like properties in SAOS2, MG63 and U2OS osteosarcoma cell lines [19–21]; elevated mRNA expression levels in stemness genes Oct4, Nanog and CXCR4 [22,23]; higher migration and invasive capabilities, particularly in lung metastasis [24]; and increased drug resistance [22,23]. In addition, cell populations with elevated levels of CD133 and CD199, along with low levels of CD44, exhibited higher levels of stem cell markers [25]; and CD49f-negative/CD133-positive cells possessed strong tumorigenicity and self-renewal capacities and were able to differentiate to CD49f-positive cells with more limited tumorigenicity [26].

CD117 and Stro-1

CD117 and Stro-1 are MSC markers. They have been found to be preferentially expressed in spheres and doxorubicin-resistant osteosarcoma cells and CD117-Stro-1 double-positive OSCs have been identified in both human and murine osteosarcoma cells and were found to exhibit high degrees of multipotency, invasiveness, drug resistance and elevated levels of self-renewal and metastatic potential [27]. Furthermore, they were enriched in metastasis-associated marker CXCR4 and drug-resistance marker ABCG2 [27]. Adhikari et al. also showed them to be highly effective in forming transplantable tumors. In contrast, CD117-Stro-1 double-negative cells lacked this ability [27]. Several studies have confirmed these findings [35,36,54], demonstrating the importance of CD117 and stro-1 as molecular markers for identifying and isolating OSCs in osteosarcoma. CD105 and CD44 have also been identified as effective MSC markers in osteosarcoma along with CD117 and stro-1 [10].

Other molecular markers

Other molecular markers that are associated with the OSC phenotype include the following: enhanced aldehyde dehydrogenase (ALDH) activity has been linked to increased levels of tumorigenicity, self-renewal and differentiation potential in osteosarcoma cell lines [28]; increased expression of ATP-binding cassette subfamily G member 2 (ABCG2) and tumor metastasis-associated marker CXCR4 have both been reported in sphere cells [16,24,27]; CD271, a marker of MSCs and human melanoma cancer stem cells, was able to distinguish a subpopulation of osteosarcoma cells that displayed stem-like features such as self-renewal, drug resistance and tumorigenicity [31]; and hematopoietic and MSC antigen Sca-1 was identified as an effective OSC marker [29,30]. Lower expression levels of CD326, CD24, CD44 and CBX3 have been identified in sarcospheres associated with the OSC phenotype, suggesting that ABCA5 may be a putative biomarker for OSCs [55].

Side population cells

Side population (SP) cells exhibit high levels of multidrug resistance and share similarities with CSCs. For example, SP cells have been linked to ABC protein transporters with high expressions of stemness genes Oct4 and Nanog [16]. The long-term label retention dye pKh26 can differentiate between rapidly dividing cells and quiescent cells, and studies using pKh26hi have identified SP cells as a subpopulation capable of self-renewal and tumor generation [32]. Hoechst dye exclusion assays are a common way to isolate drug-resistant cells and thereby establish SP cells [17].