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Review Article

Extracellular vesicles-mediated signaling in the osteosarcoma microenvironment: Roles and potential therapeutic targets

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ABSTRACT

Osteosarcoma (OS) is the most common non-hematologic malignant tumor of bone in children. It is usually characterized by a high risk of developing lung metastasis and poor prognosis. Extracellular vesicles (EVs) are cell-derived nanoparticles with a small size of 50–200 nm in diameter. As a communicator, the contents of the EVs secreted via either fusing with lysosomes for degradation and recycling or fusing with the cell plasma membrane into the extracellular environment, which play an important role in regulating the tumor microenvironment of OS and mediating the Wnt/β-catenin and TGF-β signalings. Increasing evidences suggest that EVs have significant role in OS growth, progression, metastasis and drug resistance. In this study, the roles of EVs in the physiology and pathogenesis of OS and the potential attractive therapeutic target for the treatment of OS were reviewed.

1. Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. Current treatment for newly diagnosed OS includes three aspects: preoperative chemotherapy, surgical resection and postoperative chemotherapy. These management strategies have improved the outcomes of patients with localized OS. However, patients with advanced, metastatic and recurrent OS continue to experience a quite poor prognosis. Although current multidisciplinary treatments have been used for OS, there is still no drastic change in the overall prognosis during the past two decades. The 5-year survival rate of OS patients with metastases is 20% compared with 65% of patients with localized disease [1].

Extracellular vesicles are lipid bilayer membrane vesicles with a small size of 50–200 nm in diameter. As a communicator in the cancer microenvironment, previous evidences revealed that extracellular vesicles can directly stimulate target cells with their membrane molecules or deliver their contents into multiple types of cells for direct influence [2]. Extracellular vesicles are released by all types of cells, including OS cells. Indeed, recent studies revealed that extracellular vesicles secreted by tumor cells played a critical role in cancer cell development, survival, metastasis and drug resistance [3–5]. However, the role of extracellular vesicles in the biological and pathophysiological processes of OS was still not clear. In this review, we provide an overview regard to the currently available data to illustrate the role of extracellular vesicles in OS.

2. Biogenesis and functions of extracellular vesicles

Extracellular vesicles are produced by all normal and pathological cells and secreted from the internal vesicles. The diameters of them are 50–200 nm. Extracellular vesicles are derived from cells via a multivesicular body endocytic process [6], and are found in nearly all extracellular space and body fluids, including blood plasma, cerebrospinal fluid, saliva, breast milk, urine and semen. Also, extracellular vesicles are observed abundantly in tumor microenvironment [7].

After extracellular vesicles are formed, a variety of molecules, such as multiple proteins, nucleic acids, enzymes and other soluble factors are contained in them. Extracellular vesicles may differ according to the tissue birthplace and specific cell type from which they originate, and may be subjected to the stimulation and physiological variation that the cells experience. The components of extracellular vesicles could partly reflect the contents of the original cells [8]. Study indicated that double-stranded genomic DNA contained in extracellular vesicles derived from cancer cells could partly reflect the mutational status of the originate cells [9]. Also, Ismail et al. [10] reported that RNAs contained in

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extracellular vesicles can exchange genetic information with target cells, and the expression of genes and intercellular communication in the target cells was influenced by extracellular vesicles. Notably, a significantly higher expression of extracellular vesicles was found in tumor cells than normal cells, which meant extracellular vesicles may play a special role in cancer development and drug resistance [11].

The contents of the extracellular vesicles secreted via either fusing with lysosomes for degradation and recycling or fusing with the cell plasma membrane into the extracellular environment. Notably, extracellular vesicles production and release are signal and stimuli dependent, and various proteins are associated with the process of extracellular vesicles secretion. Members of the Rab family are demonstrated to accurately regulate the secretion of extracellular vesicles, especially Rab27a and Rab27b affecting the size and localization of extracellular vesicles [12]. Also the factor p53 is shown to be involved in the extracellular vesicles release [13]. Previous studies revealed that elevated intracellular calcium concentration, acidosis, cAMP levels and P2 × 7 receptor activation modulated the pool of extracellular vesicles output [3]. After extracellular vesicles secreted into the extracellular space, they may be taken up by the target cells via direct fusion with the plasma membrane; receptor-ligand interaction; endocytosis by phagocytosis and degradation in the lysosome [14].

3. Extracellular vesicles in the microenvironment of OS

As a communicator, the main function of extracellular vesicles in intercellular communication is to exchange information with target cells. Increasing studies revealed that extracellular vesicles had significant roles in tumor development, progression, metastasis and chemo-resistance [3]. Detection of extracellular vesicles in osteoblastic and osteoclastic lesions provided a strong rationale to study the function of extracellular vesicles in messaging OS bone microenvironment [15]. Studies have reported the characterization of extracellular vesicles derived from OS cells and its potential implications on the bone marrow stroma. It clearly reported that abundant of the extracellular vesicles have diameters within 50 to 200 nm [16].

Biomechanical stress in the bone marrow stroma can elevated intracellular calcium concentration, which in turn accelerates the production of extracellular vesicles, and up-regulate the expression of extracellular remodeling enzymes, such as matrix metalloproteinases (MMPs). The significantly higher expression of MMPs and down-regulation of miRNA143 are correlated with the poor prognostic outcomes in patients with OS. Therefore, detection of MMPs in extracellular vesicles is a valuable finding for predicting OS prognosis [16,17]. Casimiro et al. [18] identified RANKL as the important regulatory factor for osteoclast differentiation due to it playing a special role in the activation of MMPs and stimulation of osteoclastogenesis. Lim et al. observed that the transfer of nucleic acid from bone microenvironment to breast cancer cells through extracellular vesicles may have a significant role in the quiescence of bone metastases [19].

CD-9 belongs to tetraspanin protein family and is found enriched in extracellular vesicles. It can regulate osteoclast differentiation and suppress the formation of mature polykaryons. In osteotropic cancers, CD-9 not only induces the homing of cancer cells in the bone microenvironment, but also enhances osteoclastic bone resorption via overexpression [20]. Herr et al. [21] reported that blocking of CD-9 by KMC8 would suppress multinucleated osteoclast formation and mediate osteoclast differentiation. Yi et al. [22] also indicated the regulatory function of CD-9 in the mediation of MMP-9 induced migration and invasion in cancer.

The expression of TGF-β is highly in the serum of patients with OS compared with those without OS. TGF-β can regulate the bone marrow stroma and stimulate migration of OS cells directly [23]. TGF-β contained in extracellular vesicles can increases the accumulation of immature myeloid cells, and the function of immature myeloid cells from the osteolytic bone marrow stroma in accelerating osteoclastic bone resorption was demonstrated [24].Thus, targeting the OS bone microenvironment and inhibiting extracellular vesicles secretion may prevent tumorigenesis.

4. Wnt signaling and extracellular vesicles in OS

The Wingless (Wnt) signaling pathway plays an important role in cell proliferation and tumorigenesis. Previous studies revealed that maintenance of cancer cells are regulated by the Wnt/β-catenin signaling pathway in several cancers. And aberrantly activated the Wnt/β-catenin pathway is correlated with the progression of OS [25]. Chen et al. established that activation of autocrine Wnt/β-catenin signaling in the tumor cell-derived extracellular vesicles would enhance the development and survival of OS cells [26].

The function of Wnt signaling in OS remains controversial. Some studies suggested an oncogenic role for this pathway, but other studies supported an anti-tumorigenic role for it. Goldstein et al. found that treatment with BHQ880 (an antibody against the Wnt signaling inhibitor) would induce increased nuclear localization of β-catenin, which resulted in elevated expression of a number of Wnt target genes released from extracellular vesicles and inhibited OS metastasis. These studies indicated that Wnt signaling pathway promoted bone differentiation in OS, and prevented tumor progression and metastasis [27].

Several valuable molecular strategies for therapeutic intervention by targeting Wnt signaling in OS have been reported. Two groups of secreted Wnt antagonists are characterized by their inhibition mechanisms. The first group of antagonists directly bind to Wnt ligands and promot inhibitory reaction, such as sFRP family and Wnt inhibitory factor-1. CD82 and CD9 were abundantly found in extracellular vesicles, which would suppress β-catenin-mediated Wnt signaling activity. It revealed that the function of CD82 and CD9 in mediating the down-regulation of Wnt signaling induced discharge of *β*-catenin [28].The second group of antagonists suppress the Wnt signaling pathway by binding to transmembrane receptors, such as the Dickkopf (Dkk) family and sclerostin. The Dkk family comprises four secretory proteins, which could mediate Wnt signaling pathway via binding to the transmembrane receptors LRP5/6 [29]. Dkk-3 can suppress the motility of *β*-catenin nuclear in OS cells, and the decreased expression of Dkk-3 was shown to prevent progression and migration of OS cells [30].

Recently, a number of microRNAs were found to be played important roles in the pathogenesis of OS. These microRNAs were detected in tumor-derived extracellular vesicles and acted as oncogenic [31, 32] or suppressive RNAs [33]. For example, miR-370 could suppress the invasion of OS cell by inhibiting the Wnt/β-catenin signaling pathway [34].

Based on the findings above, the Wnt signaling pathway plays a significant role in the progression and metastasis of OS. Thus, preventing autocrine activation of Wnt/β-catenin signaling by regulating tumor cells extracellular vesicles may be an effective therapeutic method for inhibiting OS development and metastasis.

5. TGF-β signaling and extracellular vesicles in OS

Transforming growth factor β (TGF-β) super family plays significance roles in the development of various diseases. It is one of the most abundant molecules in the tumor microenvironment. Study revealed that tumor cells derived from extracellular vesicles are able to enhance the proliferation and migration of tumor cells through activating an anti-apoptotic pathway regulated by exosome-associated TGF- β [35]. In particular, TGF-β is strongly associated with the development and progression of OS. Increased expression of TGF-β was found in the serum of patients with OS compared with those without OS [36]. And a significantly higher expression of TGF-β was found in OS patients with metastasis than those without metastasis [37]. Also the elevated serum TGF-β level was correlated with high-grade OS [38].

As one of the most widely studied pathways in OS, variations in the