Bone Sarcoma and IGF/IGF-IR -signaling

Subjects: Oncology

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Bone sarcomas, mesenchymal origin tumors, represent a substantial group of varying neoplasms of a distinct entity. Bone sarcoma patients show a limited response or do not respond to chemotherapy. Notably, developing efficient chemotherapy approaches, dealing with chemoresistance, and preventing metastasis pose unmet challenges in sarcoma therapy.

Keywords: bone sarcoma ; IGF signaling ; IGF-1R ; extracellular matrix ; tumor microenvironment ; cancer therapy ; proteoglycans

1. Introduction

Sarcomas, mesenchymal origin tumors, represent a discrete group of varying neoplasms. Sarcomas develop from transformed mesenchymal cells of various connective tissues, like bone, cartilage, blood, or fibrous and adipose tissues, and are broadly defined as bone and soft tissue tumors [1][2], the former being the focus of this review.

Even though bone sarcomas occur in adults, the prevalence of some subtypes is distinctive for the pediatric population [3]. Thus, osteosarcoma and Ewing's sarcoma (EWS) predominantly present in children and adolescents, whereas chondrosarcoma can present at any age but mainly affects individuals in the 30 to 70 years group [4][5]. These malignancies exhibit heterogeneity at the intertumoral and intratumoral levels partly correlated with their stem cell origin [6]. Indeed, recent studies have provided evidence that osteosarcoma exhibits stem cell-like properties with subpopulations of CD133+ cells, indicating traits of self-renewal, high growth rates, and the formation of spherical colonies [7]. Based on respective tumor molecular bases, histology, or clinical characteristics, bone sarcomas are classified into different subtypes [8][9]. Primary bone tumors are rare malignancies as they account for less than 0.2% of all cancers registered in the EUROCARE (European Cancer Registry-based study on survival and care of cancer patients) database [10]. Bone sarcoma patients show a varying response to chemotherapy [9].

Osteosarcoma is the most common primary bone tumor, with the highest incidence in children and young adults [11][12]. Importantly, conventional osteosarcoma is a high-grade tumor [13]. Even though chemotherapy had initially significantly improved osteosarcoma patients' prognoses, as chemotherapy treatment of high-grade localized osteosarcoma increases disease-free survival probability from 10–20% to more than 60% [14][15], its effects on survival have plateaued over the last 30 years [16][17]. Notably, improving chemotherapy approaches, dealing with chemoresistance, and preventing metastasis are still major challenges in osteosarcoma therapy [18].

Chondrosarcomas, the second most common bone malignancy, representing 10–20% of all bone malignancies, is the most frequent bone sarcoma of adulthood [19]. Chondrosarcomas are mostly low-grade, locally aggressive, non-metastasizing tumors (grade I-atypical cartilaginous tumors), rather than high-grade (grades II-III), and after wide local excision [19] or after intralesional procedures with curettage and adjuvant treatments usually have a good prognosis [20]. These tumors, however, are resistant to chemotherapy [21]. Likewise, conventional chemotherapy has very limited efficacy in patients with high-grade, advanced chondrosarcoma [22][23], with the highest benefit being noted in mesenchymal and dedifferentiated chondrosarcoma [23]. Likewise, chondrosarcomas are primarily resistant to radiotherapy, except for highly selected cases or palliation [24][25]. Some factors that seem to impair this resistance are the chondrosarcoma extracellular matrix (ECM), the low percentage of dividing cells, and poor vascularity of tumors [26].

The Ewing sarcoma (EWS), an aggressive, primarily pediatric tumor, may develop as a bone sarcoma or a soft-tissue sarcoma [27]. The 2013 WHO classification of sarcomas [28] defines tumors carrying the pathognomonic FET–ETS gene fusions, in which a member of the FET gene family is fused with an ETS transcription factor, with the most common fusion being EWSR1–FLI1, as 'Ewing sarcoma' [29]. Notably, the majority of childhood sarcomas, including EWS, exhibit low recurrent genetic alteration except for pathognomonic and uniformly expressed driver mutations [27][30][31]. However, it has recently been suggested that the cooperation of tumorigenic driver-mutations with discrete regulatory germline variants

could account for the inter-individual variability of cancer clinical outcomes [32]. EWSR1-FLI1 fusion reprograms the epigenome by introducing de novo enhancers at GGAA microsatellites and modifying the gene regulatory element's state [33].

Before the development of chemotherapy, just 10% of EWS patients survived, whereas the application of chemotherapy increased survival to 75% in patients with localized tumors. Notably, only 25% of patients with metastatic/recurrent EWS achieve disease regression under current multifunctional treatment options consisting of local control either through surgery or radiation combined with systemic chemotherapy [34][35]. Metastatic patients thus still have a dismal prognosis [35]. Given the limitations of current medical therapies, novel treatment strategies are urgently needed.

2. IGF-IR/IGF-I Signaling in Sarcoma Pathogenesis

Aberrant expression of IGF pathway members has been determined in various sarcoma types [36]. Even early studies determined an elevated IGF-IR/IGF-I expression in osteosarcoma [37]. Indeed, it has been determined in other cancer models that the overexpression of IGF-IR/IGF-I may be initiated by depressing specific tumor suppressor genes, including BRCA1 and p53 [38][39].

Moreover, IGF-IR was the critical determinant of malignant transformation in EWS required for the EWS/FLI-1 transformation of fibroblasts [40]. Notably, the EWS/FLI-1 fusion gene downregulates the expression of the IGFBP-3 by binding the IGFBP-3 promoter and suppresses its activity. Since IGFBP-3 is a major regulator of IGF-1-dependent proliferation and survival signaling, Prieur et al. showed that the repression of IGFBP-3 is a crucial event in the development of Ewing's sarcoma [41].

As with other malignancies, sarcoma patients exhibit modified IGFBP circulatory concentrations when compared to healthy subjects. IGF-1, IGF-2, and insulin bind to both types of IGF-IR and IR. Although each receptor has its affinity for these ligands, there are overlapping profiles of action in the target cells, an issue that complicates the mechanisms of their activity [42].

Notably, a generalized IGF-IR signaling input in sarcoma progression was demonstrated by a recent meta-analysis correlating IGF-1R expression with poor outcomes regarding overall survival in sarcoma patients [43]. Likewise, a poor prognosis of patients expressing IGF-I was determined by implementing tissue microarray analysis [44][45].

In the following sections, we will briefly discuss IGF-signaling involvement in the pathogenesis of some bone sarcoma types.

2.1. Osteosarcoma

IGF-1 and IGF-1R push osteosarcoma progression through subsequent malignant transformation, proliferation, attenuated susceptibility to apoptosis, and the differentiation of a prone to metastasis phenotype [45][46][47]. Notably, the IGF signaling mediators have now been recognized as biomarkers for primary osteosarcoma detection [48]. A distinct correlation between IGF-IR downstream pathways and osteosarcoma disease progression seems to have been identified. The downstream PI3K/AKT pathway was over-activated during primary osteosarcoma development and pulmonary metastasis, whereas the RAS/MAPK pathway seems to contribute to later stages of pulmonary dissemination [45]. Furthermore, it has been shown that IGFBP5, the most profuse bone IGFBP stored in bone, attenuates tumor growth and human osteosarcoma metastasis [49]. IGF-IR expression has been highly correlated with ABC subfamily G member2 (ABCG2) expression in a cohort of osteosarcoma patients under 10 [50]. As ABCG2 bestows resistance to anticancer drugs [51], these data suggest that the two proteins in combination can be utilized as prognostic factors/therapy determinants.

IGF-1 gene polymorphisms were investigated for the association of risks and outcomes of osteosarcomas. Five single nucleotide polymorphisms (SNPs) of IGF-1 (e.g., SNPs like rs6214, rs6218, rs35767, rs5742612, and rs5742714) were genotyped. Out of all tested SNPs, rs6218 proved to have a predictive role for osteosarcoma's susceptibility and progression. Moreover, this SNP was associated with a later stage and elevated risk of osteosarcoma metastasis [52]. Furthermore, an exclusive nuclear localization of IGF-1R was associated with progression-free survival and overall survival in osteosarcoma patients treated with IGF-1R Ab therapy [53].

Notably, osteosarcoma, in contrast with other pediatric tumors [30], exhibits a high degree of mutational diversity and copy number variability [48][54]. Indeed, 7–14% of osteosarcoma cases exhibit mutations in IGF signaling genes (IGF1R, IGF1, IGF2R, and IGFBP5). Thus, even taking into account intratumor heterogeneity, these data indicate that taking advantage of anomalies in the osteosarcoma genome could offer novel therapeutic strategies [55].

2.2. Chondrosarcoma

IGF-signaling facilitates chondrosarcoma pathogenesis. Thus, treatment of human chondrosarcoma cells with IGFBP3 or IGF inhibitors enhanced their apoptosis rate, whereas mice expressing Gli2 presented fewer tumors upon IGF-2 downregulation. Therefore, Ho et al. suggest that IGF signaling-dependent apoptosis mediates chondrocytes' malignant transformation [56].

The genetic polymorphisms in IGF-1 pathway members have also been correlated with elevated risk and poor prognosis of conventional chondrosarcoma patients in Chinese populations. Thus, IGF-IR rs2016347 polymorphisms were associated with the risk of lung metastasis of CHS [57].

2.3. Ewing's Sarcoma

Notably, since EWS tumor cells express both IGF-IR and IGF-1, an autocrine loop enhances EWS progression [58]. Moreover, the inhibition of EWS-FLI1 fusion protein decreased IGF-1 and impaired the IGF-1/IGF-1R signaling correlated with increased EWS cell apoptosis, reduced migration, and repressed tumor xenograft growth in a mouse model [59]. Another study focused on EWS cell lines' high expression of focal adhesion kinase (FAK) transcript and potential interaction with IGF-IR. A dual inhibitor of FAK and IGF-IR, TAE226, was tested along with PF-562,271 as a combination inhibitor of FAK and proline-rich tyrosine kinase 2. TAE226 inhibited the cell growth of various EWS cell lines. The creation of FAK- and IGF-IR- deficient EWS cells induced dysregulation of different signaling pathways. Indeed, TAE226 induced cell cycle arrest, apoptosis, AKT dephosphorylation, and inhibition of invasion. In EWS mouse models, TAE226 was demonstrated to inhibit the local growth of primary tumors and hinder metastasis. Furthermore, the combination of TAE226 and chemotherapy agents showed that TAE226 could exhibit a synergistic effect with conventional chemotherapy and be possibly beneficial for EWS relapse and metastatic patients [60]. Moreover, a recent study demonstrated that CIC-DUX4 Ewing's sarcoma, an aggressive and often fatal high-grade childhood sarcoma, metastasizes to the lung, utilizing an autocrine IGF-IR/AKT signaling axis [61].

Thus, a deeper understanding of the IGF-signaling molecular facets is obligatory for developing new therapies involving these molecules.

3. The Sarcoma Tumor Microenvironment (TME)

The TME consists of tumor cells, non-malignant cells, stromal cells, infiltrating immune cells, and blood vessels embedded in the ECM [62][63]. The tumor cells have evolved mechanisms of interaction with the non-malignant components of the TME, which alter this compartment to facilitate tumor progression [64]. Notably, prominent differences in the immune constituents of the sarcoma TME, e.g., neutrophils, tumor-associated macrophages (TAMs), natural killer (NK) cells, dendritic cells (DCs), and B and T lymphocytes, have been determined and correlated with primary tumor location, sarcoma subtype, genetic or mutational burden and previous therapy exposure [65]. The potentials of the TME have been understudied in sarcoma therapy.

The TME of bone sarcomas is intrinsically different compared to epithelial-derived tumors. Thus, stromal cells are less likely to create distinctive compartments, as usually occurs in epithelial tumors. Indeed, they intermix with tumor cells, immune cells, and other cell types in a tumor-surrounding pseudocapsule. Furthermore, the function of non-malignant cells in sarcoma stroma is well less characterized [66]. Moreover, pediatric and adult sarcomas exhibit distinct characteristics regarding tumor tissue structure [66]. Notably, both tumor and stromal cells produce ECM components that offer structural support and modulate tumor cells' interaction with the TME.

The Non-Cellular TME Compartment in Sarcomas

The ECM is a network mainly consisting of collagens, proteoglycans (PGs), glycoproteins, and glycosaminoglycans such as hyaluronic acid (HA). It has the role of a plastic scaffold that bestows physical support to cells within the tissue and regulates the bioactivities of growth factors and cytokines in a time- and location-dependent manner [67]. Aberrant ECM contributes to the stromal cells' reprogramming and facilitates tumor cell' growth and dissemination [62][64][68].

In the last couple of decades, the crucial role of the ECM, the non-cellular section of the TME, has been acknowledged in cancer pathogenesis. Previous efforts in classifying the disease and therapy development had focused on the cellular compartment [69]. However, more recent developments have demonstrated the urgent need to understand the ECM component for tumor characterization and efficient therapy development [62][70].

Bone sarcoma extracellular matrices exhibit striking characteristics. Thus, osteosarcoma osteoid is an organic partly mineralized network that mainly consists of type I collagen, glycoproteins, and PGs [71]. The osteoid's structural components participate in signaling pathways correlated with specific pathogenic phenotypes of bone [72][73]. Indeed, it has been shown that the small leucine-rich proteoglycans (SLRPs), functionally involved in normal bone development and homeostasis [74], mediate various osteosarcoma cell functions [75][76]. Notably, transcriptional analysis of paired normal bone and osteosarcoma samples demonstrated significant alternations regarding mediators of extracellular matrix degradation and collagen biosynthesis [77]. As recently discussed by Cui et al., an increase in the expression of major ECM components, including collagens (I, III, IV, and V), fibronectin, laminin, and the PGs (biglycan and lumican), has been determined in osteosarcoma compared to normal bone samples [72]. The HA-binding PG, versican, is likewise overexpressed in osteosarcoma tissues relative to healthy bone tissue and facilitates osteosarcoma cell migration [78]. Considering the ECM as a crucial regulator of tumor progression [73] has allowed the identification of specific molecules of the tumor osteoid as putative therapeutic targets [72].

Chondrosarcoma cells are characterized by intense production of cartilage-like ECM, rich in collagen type II and proteoglycans [26][79][80], with different expression patterns compared to normal tissue [81][82]. Notably, somatic changes of the collagen 2A1 gene were identified in 19.3% of chondrosarcoma and 31.7% of enchondroma tumor cohort cases [83]. Interestingly, a fusion between activin receptor 2A and fibronectin 1 was detected in 57% of synovial chondromatosis cases and in 75% of chondrosarcoma secondary to synovial chondromatosis, showing that fibronectin1 and/or AVCR2A gene rearrangements are present in both benign and malignant synovial chondromatosis, with a higher incidence in malignant disease [84].

Normal chondrocytes predominantly synthesize collagen types II, IX, X, and XI and characteristic proteoglycans, depending on their differentiation state [85]. The fact that the cartilaginous-like matrix production by chondrosarcoma cells is so intensive may indicate they originate from multipotent mesenchymal stem cells, which differentiate along the chondrocytic lineage. Interestingly, despite the malignant transformation, chondrosarcoma cells continue to express some molecules that characterize normal tissue [85][86][87].

Regarding radiotherapy and conventional chemotherapy, chondrosarcoma is characterized as a resistant lesion [88] due to the tumor's specific hallmarks. Chondrosarcoma tumor tissue, like hyaline, is characterized by a dense ECM with poor blood and lymph vascularity, on which a low percentage of dividing cells is embedded. Thus, the ECM forms a physical semi-permeable barrier, inhibiting cytotoxic agents reaching their target, i.e., chondrosarcoma cells, while reduced blood circulation creates severe chronic hypoxia [89]. Moreover, the Schwan chondrosarcoma ECM disturbance by modifying the synthesis of ECM components, mainly PGs, attenuates this tumor growth [90]. The participation of non-cellular TEM components required during sarcoma progression and their interaction with IGF-effectors has not been systematically investigated.

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