Osteosarcoma Pathogenesis

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Osteosarcoma (OS) is thought to originate from mesenchymal stem cells and is the primary malignant bone tumor that most commonly affects children, adolescents, and young adults.

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1. Introduction

OS preferentially develops in rapidly growing bone, especially in the metaphysis of long bones, like the distal femur, proximal tibia, or humerus [1][2]. Disease etiology remains unclear and controversial, but multiple associations have been made between OS development and race, gender, age, genomic alterations, and certain exposures such as to ultraviolet and ionizing radiation or chemical agents like methylcholanthrene, asbestos, or chromium salts [1][3][4]. The current management strategy for newly diagnosed OS includes neoadjuvant chemotherapy (ChT) followed by surgical removal of the primary tumor and all clinically evident metastatic disease with adequate margins, plus the addition of adjuvant ChT after surgery [2]. ChT protocols have traditionally included doxorubicin, cisplatin, ifosfamide, and methotrexate, although data raised in nonrandomized studies have questioned the use of methotrexate in OS treatment [2][5][6]. After surgery, it is important to assesses necrosis in the resected tumor. Patients with at least 90% of necrosis in the primary tumor after ChT have a better prognosis than patients with less necrosis [7]. These data have been critical in the attempts to identify patients who may benefit from therapy modifications, but new data revealed that, despite increasing the number of good responders, neoadjuvant ChT intensification did not alter overall survival, diminishing the prognostication value based on histologic response [8]. Another topic of discussion was the survival effect of changing post-operative ChT based on histologic response. The EURAMOS-1 trial addressed this question by randomizing good and poor responders to standard ChT or intensification therapy with pegylated interferon (IFN) alfa-2b. No significant differences in overall survival were identified between treatment arms in this study, showing that the degree of tumoral necrosis should not be used to guide decisions about postoperative systemic treatment [9].

Prior to 1970, localized OS treatment primarily relied on surgical resection, with 5-year survival rates below 20% [10]. However, with the developments in neo-adjuvant ChT, these have increased to 66–82% over the past 40 years [10]. Despite all these developments, OS remains a poor-prognosis disease, with 5-year survival rates of only 20% in patients with metastases, and is also a high-burden disease, which significantly impacts the patients' quality of life and the community, as it affects patients in the prime of their lives, often with disabling surgery and long rehabilitation periods [11].

2. Osteosarcoma (OS) Pathogenesis

The difficulties in OS biology research are related to the complexity of the OS genome, low incidence of this tumor, and significant biologic differences between OS subtypes. Different OS neoplastic clones develop, during tumor growth, from normal cells that earn the first cancer-promoting mutations to start tumor formation [12]. Various cell types along the osteogenic lineage have been suggested as cell-of-origin. Not only the cell-of-origin, but also their derived cancer stem cell (CSC) subpopulations are strongly affected by both environmental and epigenetic elements and it is then simple to understand that molding and shaping the OS-CSC environment and niche is the strategy behind different recently postulated therapies [12].

The intricacy and complexity of karyotypes and the nature of changes in multiple genes and cell pathways characterize, specifically, OS among sarcomas. The resulting significant genetic instability of operating system cells leads to the development of several different cell types within the same tumor, with consequent changes in cellular behavior. These changes may be responsible for the aggressiveness of cancer cells and result in the emergence of resistance to ChT treatment [11]. Understanding the main mechanisms of OS molecular pathogenesis, discussed below in this article, can help to unravel novel therapeutic approaches.

Several chromosomal and genetic syndromes, like Li-Fraumeni or hereditary retinoblastoma, have been linked to OS as well as 6p21, 8q24, and 12q14 chromosome amplifications and loss of heterozygosity of 10q21.1, described as the most common genomic alteration in OS [13]. Mutations in both the p53 or Rb suppressor genes have also been implicated in OS pathogenesis, but without evidence that they impact tumor behavior [14].

Transcription factors such as the activator protein 1 complex, found to be significantly upregulated in high-grade OS and associated with propensity to metastatic development, may play a future role as potential therapeutic targets [15]. Amplification of Myc, a transcription factor that exerts its effects in the nucleus promoting cell growth and division, has been involved in OS pathogenesis and resistance to chemotherapeutics [16]. OS cells have the capacity to develop and secrete a range of growth factors that exert autocrine and paracrine effects. Abnormal production and expression of these factors can lead to accelerated cell proliferation. Transforming growth factor (TGF)-β influences a wide variety of cell processes such as differentiation, proliferation, apoptosis, and matrix production, and is found be significantly overexpressed in high-grade compared with low-grade OS [17]. IGF (insulin-like growth factor)-I and IGF-II are growth factors frequently overexpressed in OS. They bind to specific receptors such as the IGF-1 receptor (IGF-1R), activating the PI3K and MAPK transduction pathways [18]. Parathyroid hormone-related peptide (PTHrP) and its receptor have also been implicated in OS progression and metastasis development, with PTHrP conferring OS chemoresistance by blocking signaling via p53 [19].

Another relevant factor in OS molecular pathogenesis is the resistance of OS cells to anoikis. Anoikis consists of a type of apoptosis that specifically takes place when cells lose their attachment to a basement membrane or matrix. It is particularly important in OS given the propensity of this tumor's clones to detach from the matrix components and metastasize. The pathways involved in this process are intricate and comprise interactions between integrin signaling, Rho GTPases, PI3 kinase, and PKB/Akt activation, along with many key components of the intrinsic and extrinsic apoptosis pathway [20][21].

Tumor angiogenesis is essential for sustained OS growth and metastatic development. Vascular endothelial growth factor (VEGF) is a very well characterized pro-angiogenic factor, promoting endothelial cell proliferation, migration, and blood vessel maturation. These actions are potentiated via phospholipase Cγ, protein kinase C, and the c-Raf-MEK-MAPK cascades [22]. Readjustment of the actin cytoskeleton, crucial for endothelial cell migration, develops via phosphorylation of T cell-specific adapter and interaction with Src, a protein kinase [23]. VEGF also upregulates matrix metalloproteinase, responsible for breaking down extracellular matrix, inducing antiapoptotic factors, and releasing other pro-angiogenic factors such as platelet-derived growth factor (PDGF) or angiopoietin 1 [24][25].

As stated before, matrix metalloproteinases play an important role in extracellular matrix degradation, opening the possibility of the invasion of surrounding tissues. Another important mediator of this process is the urokinase plasminogen activator (uPA) system, which once activated cleaves plasminogen to plasmin. An inverse relationship between uPA levels and survival has been shown, and in vivo models have shown that downregulation of this system results in reduced primary tumor growth and fewer metastases [26].

Ultimately, bone invasion relies on interactions between osteoblasts and osteoclasts. Osteoclasts play a main role as bone-resorbing cells, and significant osteolysis exhibited in some OS cases is the direct consequence of the increased osteoclastic activity. Throughout the first stages of OS invasion, growth factors like TGF-β are liberated from the degraded bone matrix and have a direct action on OS cells, stimulating the release of PTHrP, interleukin(IL)-6, and IL-11 [27]. These cytokines stimulate osteoclasts, facilitating further invasion and release of pro-resorptive cytokines. Osteoclast pathways of differentiation, maturation, and activation constitute possible therapeutic targets, since the inhibition of bone resorption at the tumor–bone interface may conduct to reduced local OS invasion. The crucial role played by the receptor activator of nuclear factor kappa-B ligand (RANKL) in osteoclast function makes it a particularly compelling target. Osteoprotegerin, a soluble decoy receptor for RANKL, vigorously suppresses osteoclast differentiation, both in vitro and in vivo [28].

Several signaling pathways have been associated with tumorigenesis in OS such as the Wnt and Notch pathways. Lately, deregulation of microRNAs (miRNAs)—non-coding RNAs that participate in post-transcriptional regulation of gene and protein expression—have shown a role in carcinogenesis, as discussed further below.

OS immunogenicity is closely linked with the intrinsic immunogenic properties of cancer clones, while the activity patterns of different immune cells that are part of the OS microenvironment influence the nature of the elicited immune response [29].

OS is typically associated with high levels of chromosome structural variations. Among these are rearrangements resulting from chromothripsis (20–89%) and mutation clusters known as kataegis (50–85% of cases), which result in a significant degree of genomic instability, with a predicted elevated burden of antigens and neoantigens that may provide

immunogenic potential in OS [29][30]. Interestingly, the high levels of genomic rearrangements and moderate point mutation burden are associated with low levels of predicted neoantigen expression and are not associated with increased immune infiltrate levels [29]. Rather than evoking a vigorous immune response, this genomic complexity seems to contribute to multiple immune-suppressive mechanisms that may represent targets for novel therapeutic approaches [29].

Moreover, research has shown that poor tumor infiltration by immune cells, low activity from available T-cells, lack of immune-stimulating neoantigens, and multiple immune-suppressing pathways all combine to dampen response to immunotherapy in this tumor.

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