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REVIEW ARTICLE

Precision medicine for diagnosis and treatment of osteosarcoma

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Abstract

Osteosarcoma is one of the most common primary malignant bone tumors, most commonly affecting children and adolescents. With a low 5-year survival rate, osteosarcoma is among the most dangerous threats to the health and life of young people. In many cases, lung micro-metastases are detected at the time of osteosarcoma diagnosis, which makes it very difficult to save patients' lives even with very radical treatments such as surgical amputation to remove the primary lesion. Patients with osteosarcoma often die of lung metastatic disease. The diagnosis of osteosarcoma at an early stage is therefore very important for disease prognosis. Osteosarcoma shows a remarkable variation in its pathologic presentation between its different pathologic sub-types and from patient to patient. Prior to displaying any abnormalities in cellular morphology, molecular and biochemical metabolic changes may occur, leading to increases in abnormally functioning oncoproteins. New evidence from molecular biological and genomic studies provides critical information about the occurrence, development, metastasis, and prognosis of osteosarcoma. The precision medicine approach, which allows for individualized treatment, has improved the prognosis and treatment outcomes for osteosarcoma. This review aims to comprehensively summarize the recent key discoveries in osteosarcoma and to highlight optimal strategies for diagnosis and treatment.

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Osteosarcoma is a very complex disease that does not have a clear genetic background or pathogenesis [1]. Osteosarcoma is cellularly sparse, with tumor cells that are embedded into the hard extracellular matrix. Thus, the characterization of osteosarcoma tumor cells is more difficult than that of soft tissue sarcomas [2]. Because of the relatively low morbidity of osteosarcoma, large-scale prospective randomized controlled studies are impractical and attempts to improve the patients' survival rate with evidence-based medicine is unrealistic [3]. In recent decades, osteosarcoma has been treated by using chemotherapeutic adjuvant treatment [4]. However, the failure of chemotherapy and the presence of pulmonary metastasis often hinder the doctors' efforts due to the lack of effective alternative therapies [5]. The challenges faced in the treatment of osteosarcoma arise from our poor knowledge of the mechanism and pathways involved in the early stages of the disease. With the rapid development of genetic sequence-based biomedicine, especially in the field of oncology, some specific genes or molecular markers associated with cancer diagnosis and therapeutic effect have been revealed for many tumor types [6]. Recently, it was found that inducing IL-12 results in enhanced innate anti-tumor activity in a fibrosarcoma mouse model [7]. Precision medicine is a novel medical model that seeks to customize healthcare, with medical decisions, practices, and/or products being tailored to the individual patient [8]. By utilizing precision medicine for the treatment of osteosarcoma, we hope to maximize the effects of treatment, while minimizing potential side effects.

Diagnosis and classification of osteosarcoma

Osteosarcoma is a primary intramedullary high-grade malignant tumor that produces osteoid, even if only in small amounts [9]. Osteosarcoma is the most common pri-

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mary malignant bone tumor found in teenagers, at an annual incidence rate of 1–3 per 1-million people, with 70%–80% of 10- to 20-year-old patients [10]. Most lesions occur near the metaphysis of long bones such as the distal femur, proximal tibia, and proximal humerus, have a single nidus, and rarely occur in the spine, pelvis, or sacrum. There is no typical clinical symptom indicative of osteosarcoma and, at present, the main complaint from patients is local pain and swelling with occasional joint dysfunction. In younger patients, the symptoms of osteosarcoma can be easily confused with trauma and growing pain [11]. Very few patients are initially admitted to the hospital with a pathologic fracture. Imaging examinations such as computed tomography (CT) and magnetic resonance imaging (MRI) scans show bone destruction, irregular new bone formation, soft tissue masses, local pathological periosteal reaction, satellite lesions, leap metastases, or lung metastases [12]. Positron electron tomography (PET-CT) scans display an abnormally high signal around the osteosarcoma tumor [13]. However, changes noted on advanced imaging are poorly specific to osteosarcoma at an early stage [14]. Although the levels of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) can often be determined in laboratory examination, no specific difference in the levels of either of these factors has been detected; in fact, the levels are comparable to those detected in many other diseases or during childhood [15]. A tissue biopsy and pathological examination are necessary to make a definite diagnosis.

In addition to limitations in sample collection, the lack of tumor biomarkers with high specificity and sensitivity to osteosarcoma, makes early diagnosis challenging. The difference in the prognosis of each of the different pathologic sub-types of osteosarcoma is significant. However, the etiology and pathogenesis of osteosarcoma remain unclear. The polyomavirus was found to induce the formation of osteogenic sarcoma in animal models [16]. Previous studies also indicated that ionizing radiation exposure could promote osteosarcoma formation [17]. While many patients with osteosarcoma often present with a history of trauma [18], the relationship between traumatic events and osteosarcoma is inconclusive. Radiotherapy was also recognized as a risk factor for secondary osteosarcoma.

Precision medicine and osteosarcoma

In 2011, "Toward Precision Medicine" was proposed by the National Academy of Sciences, the American Academy of Engineering, and the United States National Institutes of Health (NIH). Through advances in research, technology, and policies that empower patients, the Precision Medicine Initiative (PMI) has enabled us to enter a new era of medicine in which researchers, providers, and patients work together to develop individualized care [19]. Five years ago, the National Research Council (US) Committee approved the framework to build a Knowledge Network for Biomedical Research and a New Taxonomy of Disease (http://www.ncbi.nlm.nih.gov/books/NBK91503/). According to the NIH, precision medicine is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person." This approach will allow doctors and researchers to predict the treatment and prevention strategies for a particular disease more accurately and on an individual scale. This is in contrast to a "one-size-fits-all" approach in which disease treatment and prevention strategies are developed for the average person, with less consideration for the differences between individuals. Although the term "precision medicine" is relatively new, the concept has been a part of healthcare for many years and has been suggested as a modality for the more accurate diagnosis and treatment of osteosarcoma.

Precision medicine in the diagnosis of osteosarcoma

Osteosarcoma has a complicated genetic background that includes an extensive and heterogeneous gene mutation spectrum that varies between its different pathologic types [20]. Several large-scale sequencing studies indicated that osteosarcoma was associated with some common oncogenic gene mutations such as in the p53 or the PI3K pathway [21–22]. However, there is no direct evidence that a single mutation is associated with osteosarcoma. In the last few years, the abnormal regulation of some genes, including Snail, MHC class I chain-related gene A (MICA), RASSF1A, HER-2, matrix metalloproteinase (MMP) -2/-9, c-kit2, nuclear factor-κB (NF-κB), microRNA (miRNA), circulating tumor cell (CTC), and circulating DNA (ctDNA), was found to be associated with osteosarcoma formation. However, further studies must be performed to evaluate the causality behind each gene pathway.

The Snail family of zinc-finger transcription factors consists of Snail-1 (Snail), Snail-2 (Slug), and Snail-3 (Smuc) [23]. Snail was the first identified and is likely to be the most important transcriptional repressor of E-cadherin. By interacting with smad-interacting protein-1 (SIP1), Snail functions as a suppressor of the transcription of shotgun (an E-cadherin homolog) to control embryogenesis in Drosophila [24]. Snail also plays a fundamental role in the epithelial-mesenchymal transition (EMT) by suppressing E-cadherin expression in mammalian cells. Snail is not expressed in most cell types, but is overexpressed in osteosarcoma cells [25]. Therefore, detecting the level of Snail in osteosarcoma tissue may help evaluating the degree and progression of the tumor. Treatment targeting Snail can also be a potential new strategy to inhibit

osteosarcoma metastasis and increase the sensitivity of the primary tumor to chemotherapy.

MHC class I chain-related gene A (MICA) is the most frequently expressed ligand for the natural-killer group 2 member D (NKG2D) receptor in osteosarcoma cells [26]. Tumor cells express NKG2D ligands on their cell surface that activate NKG2D receptor, which is expressed on the surface of cytotoxic immune cells such as NK cells [27]. There are two families of NKG2D ligands, the MHC class I-related chain molecules A and B (MICA and MICB) and the UL16-binding proteins (ULBP), in humans. The binding of NK cells to tumor cells through the interaction of NKG2D and its ligands induces the cytolysis of the tumor cells, which is important in immunologic surveillance. However, to prevent this cytolysis, tumor cells produce soluble forms of NKG2D ligands by means of proteolytic cleavage of their extracellular domains. These soluble forms of the NKG2D ligands interfere with the binding of the NKG2D ligands on the surface of tumor cells to the NKG2D receptors on the surface of the cytotoxic immune cells, thereby downregulating the number of NKG2D receptors on the surface of cytotoxic immune cells [28]. A decrease in the expression of NKG2D ligands on the surface of tumor cells and an increase in the secretion of soluble NKG2D ligands therefore attenuate the susceptibility of tumor cells to cytotoxic immune cells. Hypoxia downregulates the expression of cell surface MICA without increasing soluble MICA (sMICA) in osteosarcoma cells in a HIF-1 alpha-dependent manner [29]. sMICA is increased during disease development and tumor metastasis. MICA can therefore be regarded as an independent prognostic factor when evaluating the complete survival and progression-free survival of patients with osteosarcoma. Human epidermal growth factor receptor 2 (HER2) is a 185-kDa transmembrane receptor tyrosine kinase (RTK), belonging to the epidermal growth factor receptor (EGFR) family [30]. Increased expression of HER2 is detected in various types of tumors, including osteosarcoma [31]. HER2 activates the downstream PI3K/AKT, MAPK, and mTOR signaling pathways with subsequent transcriptional activation [32]. HER2 barely exists on the surface of normal cells and overexpression can be observed in osteosarcoma [33]. High levels of HER2 expression are associated with recurrence and death in many types of malignant tumors, but are not prognostic factors for osteosarcoma [34]. However, various studies revealed that targeting HER2 is an important therapeutic strategy for treating osteosarcoma [35].

Matrix metalloproteinases (MMPs) are a family of 26 calcium dependent, zinc-containing human endopeptidases that are responsible for tissue remodeling and the degradation of components of the extracellular matrix such as gelatin, elastins, collagens, matrix glycoproteins, and proteoglycans [36]. Tumor aggression and metastasis have been correlated with increased MMP expression [37]. Overexpression of MMPs, especially MMP-2 and -9, and low levels of TIMPs are associated with more aggressive cases of osteosarcoma. Increased expression of MMP-9 correlates with clinical osteosarcoma metastasis, and inhibitors of MMPs such as TIMP-1 inhibit the invasiveness of osteosarcoma tumor cells *in vitro* [38]. Intratumoral vessels and perivascular ECM are positive for MMP-9 and negative for TIMPS [39]. MMP regulation is therefore of utmost importance in the development of anti-primary and anti-metastatic therapies against osteosarcoma.

The proto-oncogene c-kit located on the long arm of chromosome 4 and encodes a trans-membrane tyrosine kinase receptor known as CD117 that binds to the ligand stem cell factor [40]. The c-kit signal appears to play a central role in the regulation of normal cell differentiation and proliferation. Mutation of the c-kit gene results in the constitutive activation of the c-kit protein, which is well documented in many tumors [41]. C-kit expression is observed in osteosarcoma, with a high percentage of positive staining. It is also a clinical prognostic factor [42]. However, because of the diversity in tumorigenesis, the instability of disease phenotypes, and the complexity of osteosarcoma prognosis, a clear relationship between the expression of c-kit and the behavior of osteosarcoma requires further investigation.

Extensive studies demonstrated that the rapid-acting primary transcription factor nuclear factor-κB (NF-κB) is constitutively active in osteosarcoma cell lines [43]. Furthermore, tumor angiogenesis is regulated by numerous NF-κB-regulated gene products, including vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF). This evidence indicates the importance of NF-κB in osteosarcoma and suggests that agents that block NF-κB activation could reduce osteosarcoma chemoresistance and angiogenesis may possibly be used as a novel therapeutic regimen for osteosarcoma [44].

MicroRNA molecules play a variety of roles in cellular development and proliferation, including normal osteogenesis [45]. These effects are exerted through the post-translational inhibition of target genes. Altered miRNA expression has been demonstrated in several cancers, both in the tumor tissue and in the peripheral circulation. This may influence carcinogenesis if specific miRNA targets are encoded by tumor suppressor genes or oncogenes [46]. To date, most studies investigating the role of microRNAs and primary bone tumors focused on osteosarcoma and Ewing's sarcoma [47]. Several microRNAs, including the miR-34 family, have been implicated in osteosarcoma tumorigenesis via their effects on the Notch signaling pathway [48]. The progression, invasion, and metastasis of osteosarcoma tumor cells are also influenced by microRNA expression [49]. MicroRNA expression may affect the osteosarcoma's response to chemotherapy, and thus hold

potential for future use as either a prognostic indicator or a therapeutic target [50]. MicroRNA expression profiling may have some potential in the prediction of osteosarcoma disease progression and survival.

The detection and surveillance of circulating tumor cells (CTCs) and circulating DNA (ctDNA) have significant clinical utility in the diagnosis and prognosis of epithelial malignancies such as carcinomas of the colon, breast, and prostate [51]. While osteosarcoma research is still in its relative infancy, the detection of CTCs in patients with osteosarcoma may help to diagnose and predict disease recurrence or metastasis and improve the overall management of these patients [52]. Especially for osteosarcoma, pulmonary metastasis is often the main cause of death. CTCs are most often detected via reverse transcription polymerase chain reaction (RT-PCR) or antibody-based detection of cell surface proteins, including flow cytometry. The DNA from tumors, known as ctDNA, can be detected as well. Samples may be obtained from either the peripheral blood or the bone marrow. The detection of translocations in CTCs of patients with osteosarcoma is perhaps the most promising as a recurrent abnormal gene fusion product can be detected in effected individuals, but not in a normal patient. Studies on Ewing's sarcoma, a peripheral neuroectodermal tumor, have confirmed the feasibility of this approach [53]. While it remains in its relative infancy, the use of CTCs in the detection and the management of osteosarcoma has shown promise.

Prospect of precision medicine in the treatment of osteosarcoma

Amputation has been the standard method for the treatment of osteosarcoma primary tumors. However, only about 20% of patients have a long-term (> 25 years) survival outcome and many have serious limb dysfunction [54]. With improvements in modern medical imaging and surgical techniques, especially the extensive use of individualized chemotherapy regimens, the results of the comprehensive treatment of osteosarcoma are better than ever. Limb salvage treatment has therefore become popular [55]. Unlike the treatment of other malignant tumors, the model of pre-operative chemotherapy, surgery, and post-operative chemotherapy is employed for osteosarcoma. Post-operative chemotherapy is adjusted according to the surgeon's assessment of the tumor burden during the operation [56]. In patients who display a good response to pre-operative chemotherapy, a high tumor necrosis rate can be detected in the pathologic sections. The protocol used for pre-operative chemotherapy would then be maintained as the post-operative regimen. For patients who responded poorly to pre-operative chemotherapy, the drug dosage and chemotherapy protocol should be altered [57]. Radiotherapy is only considered for patients with unresectable tumors even after pre-operative chemotherapy [58]. With improvements in chemotherapy, about 90% of patients have a chance for limb retention. However, a good pre-operative chemotherapy response would also be necessary and the operation should be individually designed for each patient due to the specificity of each osteosarcoma [59]. So far, some concepts behind individualized treatment have been applied in the management of osteosarcoma, although it cannot yet be described as precision medicine. There is no single therapeutic approach for the treatment of all the sub-types of osteosarcoma or even for different patients with the same type of tumor. Moreover, metastases, chemoresistance, and serious side effects remain major reasons for the failure of osteosarcoma treatment. Today, physicians must prescribe detoxification drugs to patients in order to fight the side effects of chemotherapy. Intra-arterial infusion chemotherapy has also been employed in order to reduce systemic side effects [60]. However, many patients still need to stop the treatment prior to the completion of chemotherapy because of severe side effects such as leukopenia [61].

New tumor targeting agents that can enhance the local drug concentration at the tumor site, while reducing undesirable side effects are used to improve the overall osteosarcoma survival rate [62]. Further studies must be conducted to find solutions against osteosarcoma metastases and chemoresistance. In osteosarcoma, precision medicine is a multidisciplinary science, requiring not only the collaboration of different departments, but also requiring tailored therapies based on the different biologic characteristics of the patients and different types of osteosarcomas [63]. The composition of osteosarcoma at the molecular level is very important in allowing the physician to identify the origin of the disease and to predict tumor development and metastasis. Treatment of osteosarcoma may lie in the critical understanding of the specific defect promoting the disease pathogenesis [64]. With the development of personalized genomics research, we hope to observe a tendency towards individual-based osteosarcoma treatment [65]. Genetic testing should not be limited to the detection of genes associated with osteosarcoma, but should also be applied to understand the wider meaning of the expression of these genes [66]. New genomic indices such as the whole genome DNA sequence, whole exon sequences, small RNA, epigenetic modifications, and metabolomics can reflect the essential osteosarcoma tumor characteristics and help us create a precise treatment for this disease [67]. With the development of a gene delivery system, gene and stem cell therapies for the treatment of osteosarcoma provide a promising future for the permanent cure of this deadly and debilitating disease [68].

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Conflicts of interest

The authors indicated no potential conflicts of interest.

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