Novel molecular and metabolic aspects in osteosarcoma

Evangelos Tsiambas[1]*, Panagiotis P. Fotiades[2]*, Chrissa Sioka[3]*, Dimitrios Kotrotsios[3], Evangelia Gkika[3], Andreas Fotopoulos[3], Stylianos N. Mastronikolis[4], Ilianna E. Armata[5], Evangelos Giotakis[3], Vasileios Ragos[6]

[1]Department of Immunohistochemistry & Molecular Biology, 401 GAH, Athens, Greece; [2]Department of Surgery, Leicester General Hospital, Leicester, UK; [3]Department of Nuclear Medicine and Neurosurgical Research Institute, University of Ioannina, Ioannina, Greece; [4]Medical School, University of Crete, Heraklion, Crete, Greece; [5]Medical School, St George's, University of London Medical Programme, University of Nicosia, Nicosia, Cyprus; [6]Department of Maxillofacial Surgery, School of Medicine, University of Ioannina, Ioannina, Greece

* These authors contributed equally to this work

Summary

Osteosarcoma (OS) is the most frequent bone-forming malignancy in children and adolescents. Concerning its molecular landscape, there is no a direct relationship with a specific gene, but a combination of genetic events. A broad spectrum of activated oncogenes and downregulated suppressor genes has been already explored and considered crucial for its progressive pathogenesis. Mechanisms of gene deregulation include amplifications, point mutations, allelic losses and also epigenetic abnormalities such as aberrant promoter methylation. Although a significant progress in understanding the molecular nature of the OS has been achieved, its aggressive phenotype - characterized by high metastatic potential - remains unexplored. Novel targeted therapeutic strategies include monoclonal antibodies (mABs) and also tyrosine-kinase inhibitors (TKIs). Additionally, sophisticated and innovative diagnostic techniques, such as 18 fluorodeoxyglucose positron emission tomography plus CT (18F-FDG/PET/CT), provide critical data regarding its biological behavior. In the current paper, we present novel molecular and metabolic advances by analyzing OS genetic profile and biochemical microenvironment.

Key words: *gene, metabolic, osteosarcoma, tomography*

Introduction

OS is the most frequent bone-forming malignancy in young adults and adolescents demonstrating an aggressive phenotype characterized by high metastatic potential [1]. In fact, it represents the eighth most common tumor in childhood, occurring predominantly at the ages of 10 to 14 years in association with pubertal growth. The malignancy involves the long bones of the extremities adjacent to metaphyseal growth plates [2]. It is located predominantly in the femur (42%), followed by tibia (19%), humerus (10%), skull or jaw (8%) and pelvis (8%) [3]. Metastases from OS occur in approximately 11-16% of the cases during initial diagnosis, primarily involving the lungs or bones [4]. However, throughout the course of the disease, lung metastases arise in 95% of the patients, followed by metastases to the bones (33%), bone marrow and liver (10%), and the brain (5%) [5]. Due to its aggressive biological behaviour OS demonstrates moderate to high levels of resistance to

specific chemo-targeted therapeutic regimens [6]. In the current study, we present novel molecular and metabolic advances by analyzing OS genetic profile and biochemical microenvironment.

Molecular landscape of OS

Concerning the genetic substrate of OS, extensive molecular analyses have shown that there is a complicated gene signature including amplifications, point mutations, allelic losses and also epigenetic abnormalities such as aberrant promoter methylation [7]. Overactivation has been detected in a variety of oncogenes including growth factor receptors such as Epidermal Growth Factor Receptor type 2 (HER2/neu), Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor/Receptors (PDGF/PDGFR) and also Insulin-Like Growth Factor Receptor (IGF-R). Overexpression of these molecules - due to gene amplification predominantly - motivates a cataract of downstream tyrosine-kinase chain reactions leading to an aberrant signal transduction to the nucleus [8]. The HER2 and IGFR depended PI3K/PTEN/Akt/mTOR pathway is the most critical in this deregulated genetic process. Additionally, Notch, Wnt, NF-κB, p53, and MAPK pathways are also upregulated during OS progression. Furthremore, Sex determining region Y-box protein 5 (SOX5), a transcription factor, which plays important role in the regulation of embryonic development and in the determination of the cell fate is also deregulated in OS. According to a recently published study, SOX5 promotes epithelial-mesenchymal transition (EMT) in OS via regulation of Snail transcription factor [9]. Other proto-oncogenes such as Apurinic/Apyrimidinic exonuclease 1 (APEX1), Myc, bone morphogenetic protein typeII receptor (BMPR2) and high mobility group box1 (HMGB1) seem to play a significant role in its progressive pathogenesis. APEX overexpression is a negative predictive factor for OS patients correlated with increased metastatic activity, resistance to specific chemotherapy and also lesion recurrence [10]. In conjunction, Myc protein - acting as strong transcription factor - is also found to be amplified associated to resistance to specific chemotherapy [11]. Recently, a study group showed that the Highly Up-regulated in Liver Cancer (HULC) long non-coding RNA which is strongly over expressed in hepatocellular carcinoma demonstrated also the same aberrant upregulation in OS [12]. In contrast to these oncogenes, suppressor genes such as tumor protein p53 (TP53) and Retinoblastoma 1 (RB1) are downregulated by point somatic mutations and allelic losses. Both of them are correlat-

ed with increased proliferation, downregulation of apoptotic mechanism and high resistance to DNA damage [13].

Among the molecular mechanisms that are involved in normal gene expression, microRNAs (miRNAs) demonstrate an increasing interest for understanding their role in cancer and also in handling patients via targeted therapeutic agents. miRNAs are short, non-coding RNAs consisting of 20-25 nucleotides located at intra- or intergenic regions [14]. RNA polymerase II is responsible for their transcription. Initially, primary-miRNAs (pri-miRNAs) are reformed to pre-miRNAs followed by a maturation process. In the nucleus, the RNase III enzyme Drosha complex provides release of the pre-miRNAs to the cytoplasm where the final single-stranded mature miRNA is produced. Functional miRNAs mediate a positive regulation of posttranscriptional gene silencing. miRNA deregulation in cancer cells due to genetic (mutations, translocations), epigenetic (DNA hypermethylation of tumor suppressor genes, extensive genomic DNA hypomethylation, aberrant histone modification patterns) and transcriptional alterations leads to a loss of miRNA-mediated repression of target mRNA. Concerning the influence of specific mRNAs in OS, a broad spectrum of miRNAs (miR-21, -34a, -107, -143, -148a, -195a, -199a-3p, -382) regulate multiple target genes, pathways, and processes essential for its pathogenesis [15].

Metabolic abnormalities in OS: the role of 18F-FDG/PET/CT

Metabolic changes in the microenvironment of OS are correlated with a variety of biochemical markers. Furthermore, it is well established that 18F-FDG PET/CT appears to be increasingly important in the management of cancer patients, contributing to final diagnosis, staging, assessing therapeutic response and prognosis for several pediatric and adult tumors including bone and soft tissue sarcomas. Previous studies have showed that 18F-FDG PET/CT can predict tumor necrosis and response after chemotherapy, progression-free survival (PFS) and overall survival (OVS) [16]. Overall, 18F-FDG PET/CT can detect OS with a sensitivity, specificity and accuracy of 95%, 98% and 98% respectively, which can be increased up to 100% if the above test can be combined with bone scan. A study group analyzed patients treated with adjuvant chemotherapy, showed sensitivity, specificity and accuracy of 71 %, 85 % and 77 % respectively [17]. In contrast, another study based on OS patients who received neoadjuvant chemo-

therapy reported no significant predictive value of 18F-FDG PET/CT for evaluating subsequent response [18]. Besides, another study group that focused on OS patients who underwent the scan prior and post neoadjuvant chemotherapy, showed that 18F-FDG uptake had a prognostic value only after chemotherapy, but not before [19]. Specifically, 18F-FDG PET/CT scan was able to predict the metastatic potential of the residual tumor outside the area of the surgically excised tumor. Based on these analyses, high 18F-FDG uptake was found to be associated with worse PFS and poor OVS than low uptake.

Semi-quantitative dynamic-contrast MRI and diffusion weighted imaging (DWI) are additional imaging methods, useful for the evaluation of OS patients. According to some previous referred studies, 18F-FDG PET/CT is superior to MRI in distinguishing responders from non-responders in pediatric OS or that both PET/CT and DWI are equally useful imaging methods for predicting a histologic response, with comparable sensitivity, specificity and accuracy. It appears though, that the combination of 18F-FDG PET/CT along with MRI may be superior to each method alone for predicting a favorable histologic response to neoadjuvant chemotherapy. Furthermore, pulmonary metastatic disease may be confirmed when a pulmonary lesion is seen in both 18F-FDG PET and thoracic CT. On the other hand, a mismatch in these two imaging methods, with negative 18FFDG PET/CT scan in lesions seen in CT, cannot rule out metastatic disease. However, positive 18F-FDG PET/CT findings in the lungs indicate metastases since its specificity is high [20]. Thus, in the clinical setting, a pulmonary lesion detected by 18F-FDG PET/CT should be further analyzed by spiral CT. On the contrary, where a metastatic pulmonary lesion is depicted on a spiral CT no further imaging is required for this specific lesion.

Conclusions

OS is characterized by a complicated molecular signature based on a combination of gene imbalances. Many aberrant gene expression profiles are different targets for specific therapeutic strategies, but its high metastatic potential and recurrence ability - due to increased resistance to those regimens - remains a main issue for improving treating strategies with innovative novel agents. Concerning metabolic changes, high 18F-FDG tumor uptake indicates aggressive tumor and is associated with worse survival than low uptake. Metabolic tumor volume indicates large, aggressive tumor prone to metastasis and worse prognosis. Tumor to background uptake is an 18F-FDG/PET parameter that can differentiate responders from non-responders after chemotherapy, since if its value is low indicates tumor necrosis and good response to therapy, and if it is high indicates poor tumor response to chemotherapy. Total lesion glycolysis is another 18F-FDG/PET parameter that can differentiate non-responding patients to chemotherapy if it is high, from responders when it is low. In fact, 18F-FDG/PET and its specific parameters offer an invaluable assistance during evaluation and treatment of OS.

Conflict of interests

The authors declare no confict of interests.

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