



Biological evidence of cancer stem-like cells and recurrent disease in osteosarcoma

Camille Jubelin, Javier Munoz-Garcia, Denis Cochonneau, Emilie Moranton, Marie Françoise Heymann, Dominique Heymann

► To cite this version:

Camille Jubelin, Javier Munoz-Garcia, Denis Cochonneau, Emilie Moranton, Marie Françoise Heymann, et al.. Biological evidence of cancer stem-like cells and recurrent disease in osteosarcoma. Cancer Drug Resistance, 2022, 5, 10.20517/cdr.2021.130 . inserm-03550410

HAL Id: inserm-03550410

<https://inserm.hal.science/inserm-03550410>

Submitted on 1 Feb 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Review

Biological evidence of cancer stem-like cells and recurrent disease in osteosarcoma

Camille Jubelin^{1,2,3}, Javier Muñoz-García², Denis Cochonneau², Emilie Moranton², Marie-Françoise Heymann², Dominique Heymann^{1,2,4}

¹ Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France

² Institut de Cancérologie de l'Ouest, "Tumor Heterogeneity and Precision Medicine" Laboratory, Saint-Herblain, France

³ Atlantic Bone Screen, Saint-Herblain, France

⁴ University of Sheffield, Department of Oncology and Metabolism, Medical School, Sheffield, UK

Correspondence to: Prof. Dominique Heymann, Institut de Cancérologie de l'Ouest, Blvd Jacques Monod, 44805 Saint-Herblain, France; Email: dominique.heyman@univ-nantes.fr; ORCID: 0000-0001-7777-0669

Received: 01st December 2021

Abstract

Sarcomas are a large family of cancers originating in the mesenchyme. Composed of more than 100 histological subtypes, soft tissue and bone sarcomas remain clinically challenging, particularly in children and adolescents in whom sarcomas are the second most common malignant entities. Osteosarcoma is the main primary bone tumor in adolescents and young adults, and is characterized by a high propensity to induce distant metastatic foci and to become multi-drug resistant. The innate and acquired resistance of osteosarcoma can be explained by high histological heterogeneity and genetic/molecular diversity. In the last decade, the notion of cancer stem-like cells (CSCs) has emerged. This subset of cancer cells has been linked to drug resistance properties, recurrence of the disease, and finally therapeutic failure. Although CSCs remain controversial, many elements are in favor of them playing a role in the development of the drug resistance profile. The present review gives a brief overview of the most recent biological evidence of the presence of CSCs in osteosarcomas and their role in the drug resistance profile of these rare oncological entities. Their use as promising therapeutic targets will be discussed.

Keywords: Cancer stem cells, bone sarcoma, soft tissue sarcoma, drug resistance, tumor microenvironment, recurrent disease, residual disease

INTRODUCTION

Sarcomas are composed of highly heterogeneous soft tissue and bone oncological entities that are members of the mesenchymal tumor family^[1,2]. Osteosarcoma is the main bone sarcoma, with high prevalence in adolescents and young adults. Two peaks of incidence are described in the literature, a main peak around 18 years and a second, in the 6th decade, more frequently diagnosed in patients following Paget's disease or radiotherapy, and referred to as secondary osteosarcomas^[2-4]. The conventional



© The Author(s) 2021. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or

format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

therapeutic regimen for osteosarcoma is based on a sequential approach combining surgery and neoadjuvant and adjuvant polychemotherapies^[5]. Considered to be radioresistant, radiotherapy is nevertheless part of the therapeutic arsenal, proposed in osteosarcomas for which the surgical procedure is delicate, such as tumors in high-risk locations, and can be used for better local control of the tumor^[6]. Unfortunately, the therapeutic response in osteosarcoma patients has not improved in the last 4 decades, with an overall survival rate of around 70% after 5 years for localized disease. This rate drops dramatically to 30% when lung metastases can be detected^[7].

As described in other types of cancer, osteosarcoma evolves under the pressure of random mutational changes^[8,9], with preferential clonal proliferation and epigenetic modifications^[10-13] within the clonal population, leading to genetic instability, high genetic diversity, and high tumor heterogeneity^[13,14]. Therapeutic failure is frequently attributed to this intratumoral heterogeneity, and more specifically to the emergence of oligoclonal tumor cells capable of evading the therapeutic drugs. From this observation, has emerged the concept of CSCs, in reference to embryonic stem (ES) cells. CSCs express transcription factors (e.g. Nanog, Oct4, Sox2) initially detected in ES cells, and exhibit pluripotent differentiation properties into various functional cells able to reconstitute the complete tumor mass. The tumor-initiating cells, the CSCs, have been described as tumor cells capable of reproducing all features of the initial tumor mass, and have been associated with tumor recurrence, propagation, and drug resistance^[15-18]. Unfortunately, long-term relapse in patients considered clinically disease-free is observed in numerous cancers, including osteosarcoma^[19,20]. Minimal residual disease is defined as malignant cells that are resistant to treatment, and that remain in patients after remission, leading to relapse and metastasis. Minimal residual disease is composed of drug-resistant tumor cells and is presented dynamically as persister/dormant/quiescent/cancer cells in residual tumors, such as circulating tumor cells in peripheral blood, and disseminated tumor cells in bone marrow and other metastatic sites^[13-15, 21]. In this context, tumor recurrence may be related to the persistence of CSCs. More and more evidence highlights the existence of CSCs in osteosarcomas, although their real contribution to pathogenesis remains speculative. The present review aims to give a brief overview of the most recent knowledge available on CSCs in osteosarcoma and their potential clinical interest as new therapeutic targets.

PROPERTIES OF CANCER STEM-LIKE CELLS IN OSTEOSARCOMA AND BIOLOGICAL *IN VIVO* EVIDENCE

Around 5% of osteosarcoma patients develop local recurrence of their disease between 6 and 28 months after their first line of treatment and disease-free survival of up to 12 years is usually observed in 46% of patients^[22]. A large series confirmed a relatively low rate of local recurrence of high-grade osteosarcoma in contrast to the relapse disease associated with lung metastases^[23,24]. In 2010, Perrot *et al.* described local recurrence with metastatic foci in patients with telangiectatic osteosarcoma of the humerus after 13 years of complete remission^[20]. The local recurrence exhibited the same histological subtype as the initial tumor and was observed at the injection site of autologous fat grafts that had been performed 18 months before the recurrence for plastic surgery. More recently, Pennati *et al.* studied a series of autologous fat grafts in sarcomas and did not exclude an increased risk of local recurrence after the fat grafting procedure^[25]. These clinical cases raise the question of the persistence of cancer cells that remain quiescent at the primary tumor site during the remission phase and are reactivated by alteration to their local microenvironment. Interestingly, in 2018, Le Nail *et al.* identified osteosarcoma cells with CSC properties from high grade osteosarcoma samples^[26]. Of the isolated cells, two showed a high ability to

form spheroids and even though they were not tumorigenic, these cells supported tumor growth when they were co-inoculated with human osteosarcoma cell lines in immunodeficient mice.

Table 1: Biological characteristics and functional properties of CSCs identified in human osteosarcoma

Biomarkers studied	Biological properties	Models	Reference
CD24	- Sphere formation - Expression of stemness markers (Oct4, Nanog, Sox2, BMI1) - Properties of tumor-initiating cells - Drug resistance	- Human MNNG-HOS, U2OS, MG-63 and OSC228 cell lines - Primary cultures of human cancer cells	27
CD117, Stro-1	- Expression of stemness markers (CD133, CXCR4, Nanog, Otc4) - <i>In vivo</i> properties of tumor-initiating cells - Drug resistance (ABCG2): resistance to methotrexate, cis-platin	- Mouse K7M2 cell line - Mouse 318-1, P932 and K7M2 cell lines / Human KHOS and MNNG/HOS cell lines - Human U2OS cell line - Human MG63, MNN/HOS, 143B cell lines and patient-derived cells	28 29 30 31
CD133	- Sphere formation - Expression of stemness markers (Sox2, Oct3/4, Nanog) - Expression of ABCG2, MDR1 - Expression of ABCB1, ABCC2, and the metastasis-associated genes β4-integrin, ezrin, MMP-13, and CXCR4 - Concomitant CD133/CXCR4 expression significantly associated with lung metastasis - Expression of CD133, ALDH1 positively associated with lymph node metastasis, distant metastasis	- Human SaOS2, MG63 and U2OS cell lines - Primary cultures of human cancer cells and human MG63 cell line - FFPE samples and MG63 cell line - SaOS2 cell line - FPPE samples and human SaOS2, U2OS, MG63, HOS, MNNG/HOS, HuO9, 143B cell lines - FFPE samples	32 33 34 35,36 37 38, 39
CD271	- Sphere formation - Ability for self-renewal - Resistance to DDP therapy - Overexpression of Nanog, Oct3/4, STAT3, DNA-PKcs, Bcl-2 and ABCG2 - <i>In vivo</i> tumorigenicity	- FFPE samples and human U2OS, MNNG/HOS, SaOS2 cell lines	40
ALDH1	- Sphere formation - Ability for self-renewal - Expression of stemness markers	- FPPE samples - Human MG63 cell line	39 41

	(CD133, CXCR4, Nanog, Oct4, Sox2, KLF4) - Drug resistance - <i>In vivo</i> tumorigenicity	- Huma HuO9, OS99-1, MG63, SaOs2 cell lines - Human HOS, MG63, MHM, MNNG/HOS, OHS, U2OS cell lines	42 43
hTERT enrichment	- Expression of CD117, Stro-1 - Spheroid formation	Primary osteosarcoma cell lines (OS1-4) - MG63, MNNG/HOS, 143B cell lines	31
Metabolic properties	- Specific metabolic feature of osteosarcoma stem like cells: amino acid, fatty acid, energy, and nucleic acid. - Involvement of the Rap1 and Ras signaling pathways in methotrexate resistance - High aerobic glycolysis and oxidative phosphorylation: association to LINB28 expression - Downregulation of the citrate cycle and elevation of oxidized glutathione levels. Upregulation of most of the amino acid metabolisms - Uncoupling Warburg and stemness in CD133 ⁺ cells under hypoxia	- Human 143B and MG63 cell lines - Human OS13 cell line - Human HOS cell line - Human SaoS2 cell line	44 45 46 47
N-methyltransferase	- Sphere formation - Expression of CD133, CD44, Oct4, Sox2, Nanog, Nestin, ABCG2, and BMI-1	- Human MG-63 cell line	48
m ⁶ A methylome	- Multidrug resistance - Sphere formation - Overexpression of CD117, stro-1, CD113, stemness markers (SOX2, POU5F1, NANOG and KLF4) - Upregulation of METTL3 and ALKBH5 of and downregulation of METTL14 and FTO	- Human MG63 cell line	49

86

87 Asymmetric cell division has been described in stable cancer cell lines, leading to the development of
88 proliferating and quiescent cells that were functionally related to sensitive and drug resistant cells
89 respectively^[15]. The identification of CSCs in osteosarcoma has been extensively described in the
90 literature (Table 1). CSCs express CD24^[27], CD177^[28-31], Stro-1^[28-31], CD133^[32-39], ALDH1^[39,41-43] and

show specific metabolic properties^[44-47]. Telomerase (hTert) controls the lengthening of chromosome telomeres by catalyzing the addition of repetitive DNA sequence to their end. CD271, and Stro-1 were enriched in hTert and showed metabolic specificities such an uncoupling Warburg under hypoxia^[31, 47]. In addition, as expected, these cells which expressed stemness markers (e.g. *Nanog*, *OCT4*, *Sox2*), were able to form spheroids *in vitro*, and exhibited the properties of tumor-initiating cells in preclinical mouse models^[47]. Among the other metabolic particularities, CSCs exhibit high aerobic glycolysis and oxidative phosphorylation^[45], a downregulation of the citrate cycle and an increased oxidative glutathione levels^[46] and show more generally an upregulated of most of the amino acid metabolism^[44,46]. A drug resistant profile have been associated with the stemness properties of CSCs which can be modulated by epigenetic mechanisms such as DNA and mRNA methylation^[48,49] and with an increase in ALDH activity and ABC transporter expression^[50,51]. Interestingly, anti-cancer therapies based on cytotoxic agents result in enrichment of CSCs in cancer cells, highlighting the potentially harmful link between CSCs and the establishment of drug resistance^[52-54]. CSCs may be a specific subset of tumor cells with high potential for tumor-initiation and self-renewal as has been recently observed in all primary cultures from cases of patient-derived Ewing sarcoma^[55].

MOLECULAR REGULATION OF CANCER STEM-LIKE CELLS IN OSTEOSARCOMA

Osteosarcoma growth and the distant dissemination of cancer cells are controlled by a permanent dialog between cancer cells and their microenvironment^[2,56]. These soluble and membranous mediators trigger specific intracellular molecular cascades that lead to control of cellular processes, including cell death, epithelial-mesenchymal transition, or spreading, but also proliferation and quiescence. In this context, the behavior of CSCs is controlled by the tumor microenvironment. In recent decades, key signaling pathways regulating CSCs have been identified and become the source of therapeutic development (Figure 1).

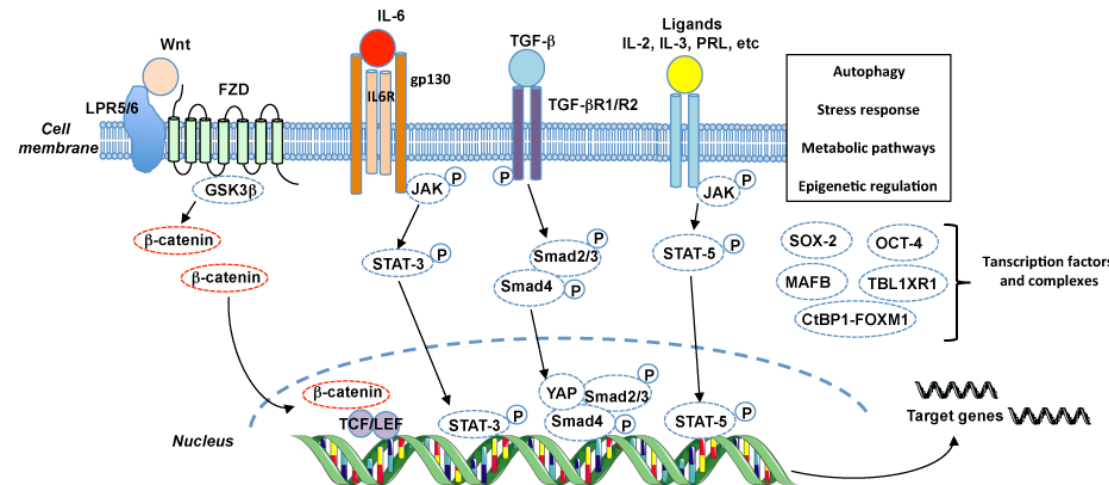


Figure 1: Main signaling pathways and mechanisms regulating the maintenance of cancer stem-like cells in osteosarcoma. LPR: Lipoprotein Receptor-related Protein FZD: Frizzled receptor; PRL: PRLactin receptor

The **Wnt/β-catenin** pathway contributes to the regulation of numerous cellular processes (e.g. proliferation, differentiation, polarization) and is thus strongly associated with embryonic development. Wnt glycoprotein family is composed by 19 secreted members that interact with cell membranes after

binding to on of the 10 Frizzled (FZD) receptors identified which are G protein-coupled receptors or to a co-receptor such as LRP-5 or -6 or tyrosine kinase receptor chains including retinoic acid-related orphan receptor (ROR) and RyK. In absence of Wnt ligand, β -catenin is degraded by the proteasome after sequestration associated GSK-3 β and the Wnt/ β -catenin pathway is considered as inactive. The Wnt/ β -catenin pathway is activated by the binding of one Wnt ligand to its receptor/co-receptor complex that leads to a series of phosphorylation cascade and recruitment of the receptor chains and then to the inactivation of the β -catenin degradation process. Consequently, β -catenin accumulates to the cytoplasm and is translocated into the nucleus before to interact with transcription factors members of the TCF/LEF family and to activate target genes (Figure 1). Any disturbances (e.g. mutations, activation) in this molecular pathway lead to pathological situations^[57]. Recently, Deng *et al.* studied the involvement of Indian Hedgehog (IHH) signaling in cartilage and bone tumors by deleting *Ptch1* encoding an inhibitor of IHH receptor^[58]. These authors demonstrated that deleting *Ptch1* in mice was associated with an increase in Wnt member expression and the development of skeletal diseases, including osteosarcoma. Interestingly, inhibiting the Wnt/ β -catenin pathway abolished the development of osteosarcoma, highlighting the key role played by this molecular pathway in the pathogenesis of bone sarcomas^[58]. The Wnt/ β -catenin pathway may be the link between tumor development, drug resistance and CSCs in osteosarcoma. Whether or not the Wnt/ β -catenin cascade was related to chemoresistance, it appeared to be a driver of cancer by acting directly on tumor cells, but also by modulating the immune microenvironment^[59]. This cancer driver is persistently activated in the CSCs of osteosarcoma, and the stemness properties induced by chemotherapies are related to activation of the Wnt/ β -catenin cascade^[60,61]. In this context, most molecular machineries that modulate the expression level of Wnt/ β -catenin may affect cancer cell behavior. Thus, epigenetic regulation of Wnt/ β -catenin using the LncRNA DLX6-AS1/miR-129-5p/DLK1 axis or histone methyltransferase SETD2 results in increased stemness properties for osteosarcoma cells, tumor growth, and drug resistance^[62,63]. The key contribution of Wnt/ β -catenin in the maintenance of CSCs may lead to the development of new targeted therapies in osteosarcoma as described below.

IL-6/STAT3 signaling has also been identified as a crucial regulator of bone remodeling and primary bone tumors^[64]. IL-6 family of cytokines composed by 10 members including e.g. IL-11, OSM and LIF induces redundant and pleiotropic activities such as embryogenesis, differentiation or inflammation. Most of the members of this family share the transducing receptor β -subunit gp130 as part of a multimeric receptor complex that includes a specific receptor α -subunit (e.g. IL-6R). The oligomerization of receptor subunits induced by each ligand results in various transduction of signaling pathways dominated by JAK/STAT3 activation and others such as MAPKs, p38 and JNK (Figure 1). In addition to its functions on the tumor microenvironment (e.g. bone and immune cells), the IL-6 signaling pathway controls the maintenance of CSCs in osteosarcoma^[65]. IL-6 released by activated mesenchymal stem cells (MSCs) in the tumor microenvironment promoted osteosarcoma stemness and the spreading properties of cancer cells^[65]. In addition, MSCs supported drug resistance through STAT-3 signaling in cancer cells activated by IL-6^[66]. MSCs and osteosarcoma cells then established a reciprocal dialog initiated by TGF- β containing extracellular vesicles secreted by cancer cells that induced the production of IL-6 by MCSs, which in turn supported stemness, drug resistance, and tumor progression^[67]. The use of active drugs confirmed the contribution of the IL-6/STAT3 axis in osteosarcoma stemness^[68,69].

The **TGF- β /Smad** axis regulates the self-renewal of osteosarcoma cells. TGF- β $\square\square\square\square\square\square$ belong to a large family of at least 30 secreted proteins sharing structural similarities. TGF- β growth factors are secreted as latent precursors which can bind to specific receptor chains after activation in mature form. TGF- β induces the assembly of type I and type II TGF β -receptors leading to the formation of

heteromeric receptors and the initiation of the signal transduction. TGF- β type I receptor shows intrinsic tyrosine kinase activity, phosphorylates the type II chain and initiates the downstream signaling which include Smads phosphorylation. Phospho-Smads complexes are translocated into the nucleus where they cooperate with YAP/TAZ transcription regulators and modulates the transcription of target genes (Figure 1). Zhang *et al.* studied the functional impact of TGF- β 1 on osteosarcoma stemness in a hypoxic environment^[70]. They demonstrated the crucial role played by TGF- β 1 on the proliferative state of cancer cells which acquired the stem cell phenotype for self-renewal, drug resistance, neoangiogenesis, and tumorigenicity, and on the contrary, blocking the TGF- β 1 signaling pathway reduced the dedifferentiation program of osteosarcoma cells. Similarly, by using gamabufotalin, a bufadienolide extracted from toad venom, it has recently been demonstrated that blockading the TGF- β /periostin/PI3K/AKT axis resulted in suppression of CSCs in osteosarcoma.^[71] CSCs associated with TGF- β activity were also linked to drug resistance, as shown for EGFR inhibitors highlighting once again the role played by CSCs in the drug resistance process.^[72]

Recently, a series of **transcription factors** were identified as regulators of cancer stemness in osteosarcoma. The transcription factor Sox determining the region Y-box 2 (Sox2) plays a key role in developing and controlling the embryonic stem cell state and was identified as a biomarker for CSCs in osteosarcoma (Table 1). In addition, the proliferation of osteosarcoma cells and tumor development requires Sox2^[73]. These authors compared tumor growth in Cre-bearing mice with identical Rb and p53 genotypes in a background of Sox2 deficient- or wild-type mice. Tumor development was significantly slowed down in the Sox2 deficient mice compared to the other groups, and the survival rate was also higher in the Sox2 knockout mice. Sox2 appeared essential for the survival and proliferation of all osteosarcoma cells, including CSCs. The Hippo pathway, which is under the transcriptional control of Sox2, was directly related to the same activities, and deactivating Sox2 effectors (e.g. YAP) resulted similarly in a reduction in tumor growth^[73]. Chen *et al.* demonstrated that musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) is highly expressed in osteosarcoma and more specifically in CSCs and this transcription factor, like Sox2, is required for the proliferation and tumorigenicity of osteosarcoma cells^[74]. Interestingly, they observed that maintaining the self-renewal potential of CSCs was under the transcriptional control of Sox-9, a stem cell regulator^[74]. More recently, STAT-5 associated signaling was identified as a key regulator^[75]. The knockdown of STAT-5 (A and B isoforms) using an siRNA approach reduced pimezone-induced tumor growth in mice in addition to suppressing *in vitro* sphere formation. Inhibiting STAT-5 signaling thus impaired osteosarcoma self-renewal and development^[75]. JAK/STAT-5 activation belongs to the downstream signaling associated to various cytokine/hormone induced signaling pathways including prolactin, IL-2, IL-3, etc. Oct4 promoted osteosarcoma development by supporting the maintenance of CSCs through the increase in AK055347, a long-noncoding (lnc) RNA. Oct4 knockdown with siRNA induced a significant decrease in cell proliferation, invasion and apoptosis^[76]. TBL1XR1 is a transcriptional co-factor which is overexpressed in osteosarcoma patients^[77]. Its overexpression in MG63 and U2-OS cell lines induced a CSC phenotype in contrast to its repression. TBL1XR1 thus provides osteosarcoma cells with tumorigenic properties and promotes the recurrence of osteosarcoma in a STAT-3 signaling dependent manner^[77]. Transcriptional complexes can also modulate osteosarcoma drug resistance. Thus, CtBP1-FOXM1 transcriptional complex increased *MDR1* expression in osteosarcoma CSCs, which is associated with drug resistance^[78]. Interestingly, small molecules targeting this complex reversed the *MDR1*-mediated resistance both *in vitro* and in murine preclinical models.

Regulating osteosarcoma growth through the oct4-lncRNA axis highlights the epigenetic regulation of osteosarcoma CSCs^[79]. This observation is supported by the rich literature emerging in the last 10 years^[76]

(Table1). In this context, chromodomain helicase DNA binding protein 1-like (CHD1L) significantly reduced osteosarcoma proliferation and drug resistance through its binding to DNA. It also controls chromosomal integrity maintenance, DNA repair, and transcriptional regulation^[79]. Ubiquitin-specific peptidase 39 (USP39) is a crucial factor for assembling mature spliceosome complex and its knockdown leads to the inhibition of osteosarcoma cell proliferation combined with an increase in apoptosis^[80]. Human antigen R (HuR) is involved in stabilizing mRNA, and its repression in osteosarcoma cells reduced their stemness properties and increased the drug response^[81]. These activities were related to YAP activation. Several recent publications showed the role played by specific miRNA in controlling stemness in osteosarcoma and the list includes miR29b and its target Spin1^[82], miR34a^[84] and the DNMT1/miR34a/Bcl2 axis^[84,85], TNF- α -miR155 signaling^[87], miR335 and its target POU5^[88], miR429 and its target Sox2^[89], and the TGF β -miR-499a-SHKBP1 89 axis^[90]. Very recently, leukemia inhibitory factor (LIF) was shown to belong to the IL-6 family of cytokines, similarly activating STAT-3, and was recently revealed as a super-enhancer-controlled regulator of CSC properties, confirming the role of STAT-3 transcription factor in the functional regulation of CSCs in osteosarcoma^[91]. TSSC3 tumor-suppressing STF cDNA 3 (TSSC3), the first apoptosis-related gene reported to be imprinted, repressed the self-renewal of osteosarcoma CSCs^[92]. Finally, lncRNAs also play a part in the biological regulation of CSCs in osteosarcoma^[76,93].

Autophagy^[94,95], **stress response**^[96,98] as well as **numerous enzymatic pathways**^[99-104] complete the landscape of osteosarcoma CSC-regulation mode. Autophagy was shown as a critical biological process for maintaining CSCs in OS^[94] and defective autophagy was directly associated with the decrease in CSCs^[95]. Similarly, the knockdown of stress-induced phosphoprotein 1 (STIP1) resulted in the inhibition of CSC invasiveness and migration^[96]. STIP-1 is a co-chaperone that binds to HSP70 and 90, and consequently inhibits Hsp90 by 17-AAG reduced stem cell-like properties and decreased drug resistance in OS^[97].

THERAPEUTIC TARGETING OF CANCER STEM-LIKE CELLS IN OSTEOSARCOMA

The recent evidence of CSCs in osteosarcoma, and better understanding of the molecular pathways required for their maintenance, led to the identification of new therapeutic targets summarized in Table 2. Repressing the signaling pathways related to the maintenance of CSCs (see Table 1) resulted in the slowdown of tumor growth and inhibition of the metastatic process^[105-116]. As previously mentioned, Wnt/ β -catenin appeared crucial for the maintenance of CSCs and its attenuation by using tankyrase inhibitor or tegavivint was associated with a decrease in both CSC numbers and tumor progression^[105,106]. GSK3 appeared highly expressed in osteosarcoma and targeting Akt/GSK3/ β -catenin or Akt/GSK3-/Notch-1 respectively with $\square$ dioscein or tideglusib repressed CSC and tumor growth^[107,108]. Gamabufotalin induced similar activities by targeting TGF- β /periostin/PI3K/Akt signaling^[109]. Similar results were obtained by targeting BMP2R^[110]. Drugs targeting transcription factors (e.g. STAT-3, STAT5) controlling the development of CSCs may be also used to improve the therapeutic approaches to osteosarcoma^[75,111,112]. Activation of hormone signaling can reduce stemness in osteosarcoma, as shown by the activation of estrogen receptor alpha by decitabine^[113]. Most of the cytokine induced signaling pathways results in the translocation of transcription factors which modulate the transcription of target genes. Targeting of such transcription factors (e.g. KLF4, Sox9) may be used for reducing CSCs in osteosarcoma^[114-116]. Similarly, ROCK inhibition by fasudil suppressed *in vitro* cell proliferation and reduced their tumorigenicity *in vivo*^[100]. Cell metabolism is significantly modulated in CSCs (e.g.

autophagy, cell cycle) and these specificities can be used for targeting CSCs in osteosarcoma. For instance, thioridazin and metformin target autophagy and metformin and wogomin modulated ROS-mediated apoptosis in CSCs and resensitize CSCs to cell death^[114-116]. Similarly, regulation of cell cycle by DMAMCL or inhibition of γ -secretase by DAPT will affect the behavior of CSCs and their function in tumor growth^[122-123].

Table 2: Potential therapeutic approach to CSCs in osteosarcoma

Drug	Molecular pathway involved or therapeutic approaches	Reference
Wnt/β-catenin targeting		
Tankyrase inhibitor (IWR-1)	Attenuation of Wnt/ β -catenin signaling	105
Tegavivint	β -catenin/transducin β -like protein 1 (TBL1) inhibition	106
Dioscein	Akt/GSK3/ β -catenin	107
Tideglusib	GSK-3 β /NOTCH1	108
TGF-β/BMP2 targeting		
Gamabufotalin	TGF- β /periostin/PI3K/AKT	109
BMP2	BMP2 receptor signaling	110
Other receptor signaling targeting (STAT-3, STAT-5, ER-α, TRAF-2, etc) and transcription factors		
Bruceine D	STAT-3 inhibition	111
Pimozide	STAT-5 signaling	75,112
Decitabine	Activation of estrogen receptor alpha (ER- α)	113
NCB-0846	TRAF2- and NCK-interacting protein kinase	114
Melatonin	Suppression of SOX9 mediated signaling	115
Statins	KLF4	116
Targeting of kinase activities		
Fasudil	Rho-associated coiled-coil containing kinase (ROCK) inhibition	100
Autophagy and metabolic targeting		
Thioridazine	Autophagy	94
Metformin	- Inhibition of mitochondrial functions (decrease in oxygen assumption, decreased mitochondrial membrane potential, decreased ATP production)	117
	- Pyruvate kinase isoenzyme M2 (PKM2)	118
	- ROS-mediated apoptosis and autophagy	119
	- Activation and phosphorylation of the energetic sensor AMPK	120
Wogonin	ROS regulation	121
DMAMCL	Cell cycle	122
DAPT	γ -secretase inhibition	123
Combinations with chemotherapy and sensitization to chemotherapy		
Ascorbate	Sensitization to cisplatin	124
Ouabain	Sensitization to cisplatin: Na ⁺ /K ⁺ ATPase inhibition	125
Tangeretin-assisted platinum	Combination with doxorubicin	126

nanoparticles		
Senolytic drug (Fisetin)	Combination with etoposide	127
Immunotherapy		
Immunotherapy based on cytokine induced killer cells	CSCs spared after chemotherapy or other targeted therapies	129
Modulation of epigenetic events		
Epigenetic targeting	- USP39 silencing	80
	- HuR knockdown	81
	- Disruption of the DNMT1/miR34a/Bcl-2 axis by Isovitexin	85
	- LncRNA HOXD-AS1 knockdown	
	- RAB39A silencing	92
	- Targeting of LncRNA SOX2OT variant 7 by EGCG (polyphenol isolated from green tea)	99
		130
Photo Therapy		
Graphene Oxide Nanoparticle loaded Ginsenoside Rg3	Photodynamic therapy	131
CD271 antibody-functionalized HGNs	Photothermal therapy	132
Drug delivery systems		
Salinomycin-loaded PLA nanoparticles	Delivery of solinomycin	133
Lipid-polymer nanoparticles with CD133 aptamers	Delivery of all-trans retinoic acid	134
Lipid-polymer nanoparticles with EGFR and CD133 aptamers	Delivery of salinomycin	135

Drugs/effective agents can be used as sensitization agents to chemotherapy^[124,125] or in combination with chemotherapeutic drugs^[126,127]. Numerous cytokines are involved in the control of local immunity of cancer cells^[128] and immunotherapies have been proposed for targeting CTCs^[129]. Specific silencing of the epigenetic partners of CSCs can induce similar regression in tumor growth and metastatic development by altering CSC maintenance^[80,81,95,92,99,129]. Nanoparticles can be used for developing phototherapies and drug delivery systems. In this context, nanoparticles have been functionalized and adapted for phototherapy with a specific aim to improve the targeting of CSCs using^[131,132]. Finally, drug delivery systems has also been proposed^[133-135]. All these therapeutic approaches, the question of the general toxicity in healthy tissue stem cells and the specificity of the targeting remains unanswered.

CONCLUSION

Long considered as controversial, today CSCs are a realistic therapeutic target in osteosarcoma^[1,2]. Osteosarcoma remains a highly heterogeneous oncological entity in perpetual evolution due to a strong clonal dynamic^[136] leading to very efficient adaptation to drugs and the establishment of drug resistance^[15]. The dynamic properties of tumor evolution have led to numerous questions about CSCs and their functional impact: i) Can we detect CSCs in the bloodstream and can we use circulating tumor cells to follow the minimal residual disease and identify personalized therapeutic options^[137]? ii) Are CSCs capable of migrating to distant organs to establish metastatic foci? iii) Is the dynamic evolution of

osteosarcoma similar in the primary site and in the metastatic foci? iv) What is the functional regulation of CSCs and are they under the control of proliferating osteosarcoma cells? v) Are CSCs regulated by the tumor microenvironment, and by which molecular pathways? vi) Can we use immune therapies in combination with other drugs (e.g. chemotherapy) to target CSCs and improve overall survival in osteosarcoma? vii) How can we specifically control CSC metabolism and consequently can we set up specific therapeutic options to control CSC wake-up? viii) As osteosarcoma is a form of cancer that originates in the mesenchyme, can we use the fibrogenic reprogramming of CSCs as a therapeutic option^[138]? Even whether sarcomas are considered as an immune desert explaining the current poor clinical efficacy of immune therapies^[1,128]. Macrophage and stromal cells contribute to the establishment of drug resistance and could be envisaged as therapeutic target in osteosarcoma^[139]. For instance, M2 macrophage may be associated with tumor angiogenesis. Tumor cells released high amount of protons that induced local acidosis, favored the release of inflammatory mediators by local stromal cells which in turn facilitated tumor invasiveness and metastasis in osteosarcoma^[140]. Overall, it has been demonstrated that stromal cells significantly contributed to increase the stemness properties of osteosarcoma cells by inducing metabolic reprogramming of cancer cells^[141,142]. Consequently, stromal cells constitute an interesting reservoir of stemness targeting to reduce osteosarcoma progression as it as been shown recently^[1413]. A better understanding of the role of stromal cells in the control of stemness would help to identify new mediators associated with stemness, drug resistance and tumor progression. Overall, CSCs are promising targets in osteosarcoma as demonstrated by the most recent data described in this review, paving the way for a new therapeutic era focused on better-controlled residual disease in osteosarcoma through targeting CSCs.

DECLARATIONS

Authors' contributions

DH supervised the work proposed and took the lead in writing the manuscript. CJ, JMG, DC, MFH and contributed to the preparation of the manuscript. All authors approved the version submitted.

Financial support and sponsorship

This work was supported by an internal ICO grant 2018 (ref# "DorSarc-2018-ICO-DH").

Conflicts of interest

Camille Jubelin is an employee of Atlantic Bone Screen and prepared her PhD at the Université de Nantes (FR). Dominique Heymann is a member of the Editorial board of Cancer Drug Resistance.

Copyright

© The Author(s) 2021.

REFERENCES

- [1] Grünewald TGP, Alonso M, Avnet S, et al. Sarcoma treatment in the era of molecular medicine. *EMBO Mol Med* 2020;12:e11131. [PMID: 33047515 DOI: 10.15252/emmm.201911131]
- [2] Brown HK, Schiavone K, Gouin F, Heymann MF, D Heymann. Biology of bone sarcomas and new therapeutic developments. *Calcif Tissue Int* 2018;102(2):174-95. [PMID: 29238848 DOI: 10.1007/s00223-017-0372-2]

- [3] Baumhoer D, Böhling TO, Cates JMM, et al. Osteosarcoma. In: WHO Classification of Tumours Editorial board. Soft tissue and bone tumours. Lyon (France): International Agency for Research on Cancer; 2020. pp 403-9.
- [4] Flanagan AM, Bridge JA, O'Donnel PG. Secondary osteosarcoma. In: WHO Classification of Tumours Editorial board. Soft tissue and bone tumours. Lyon (France): International Agency for Research on Cancer; 2020. pp 419-21.
- [5] Gill J, Gorlick R. Advancing therapy for osteosarcoma. *Nat Rev Clin Oncol* 2021;18(10):609-24. [PMID: 34131316 DOI: 10.1038/s41571-021-00519-8]
- [6] Strauss SJ, Whelan SJ. Current questions in bone sarcomas. *Curr Opin Oncol* 2018; 30(4):252-59. [PMID: 29782347 DOI:10.1097/CCO.0000000000000456]
- [7] The ESMO/European Sarcoma Network Working Group. Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012;23: vii100-vii109. [doi:10.1093/annonc/mds254]
- [8] Kovac M, Blattmann C, Ribi S, et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat Commun* 2015 Dec 3;6:8940. [PMID: 26632267 DOI: 10.1038/ncomms9940]
- [9] Behjati S, Tarpey PS, Haase K, et al. Recurrent mutation of IGF signalling genes and distinct patterns of genomic rearrangement in osteosarcoma. *Nat Commun* 2017;8:15936. [PMID: 28643781 DOI: 10.1038/ncomms15936]
- [10] Wu SP, Cooper BT, Bu F, et al. DNA Methylation-Based Classifier for Accurate Molecular Diagnosis of Bone Sarcomas. *JCO Precis Oncol* 2017;2017:PO.17.00031. [PMID: 29354796 DOI: 10.1200/PO.17.00031]
- [11] Morrow JJ, Bayles I, Funnell APW, et al. Positively selected enhancer elements endow osteosarcoma cells with metastatic competence. *Nat Med* 2018;24(2):176-185. [PMID:29334376 DOI: 10.1038/nm.4475]
- [12] Asano N, Takeshima H, Yamashita S, et al. Epigenetic reprogramming underlies efficacy of DNA demethylation therapy in osteosarcomas. *Sci Rep* 2019;9(1):20360. [PMID: 31889115 DOI: 10.1038/s41598-019-56883].
- [13] Brown HK, Tellez-Gabriel M, Cartron PF, Vallette FM, Heymann MF, Heymann D. Characterization of circulating tumor cells as a reflection of the tumor heterogeneity: myth or reality? *Drug Discov Today* 2019;24(3):763-72. [PMID: 30496853 DOI: 10.1016/j.drudis.2018.11.017]
- [14] Tellez Gabriel M, Heymann MF, Heymann D. Circulating tumor cells as a tool for assessing tumor heterogeneity. *Theranostic* 2019;9(16):4580-94. [PMID: 31367241 DOI: 10.7150/thno.34337].
- [15] Vallette FM, Olivier C, Lézot F et al. Dormant, quiescent, tolerant and persister cells: four synonyms for the same target in cancer. *Biochem Pharmacol* 2019;162:169-76. [PMID: 30414937 DOI: 10.1016/j.bcp.2018.11.004].
- [16] Nassar D, Blanpain C. Cancer stem cells: basic concepts and therapeutic implications. *Annu Rev Pathol* 2016;11:47-76. [PMID: 27193450 DOI: 10.1146/annurev-pathol-012615-044438]
- [17] Walcher L, Kistenmacher AK, Suo H, et al. Cancer stem cells-orgins and biomarkers: perspectives for targeted personalized therapies. *Front Immunol* 2020;11:1280. [PMID: 32849491 DOI: 10.3389/fimmu.2020.01280]
- [18] Clarke MF. Clinical and therapeutic implications of cancer stem cells. *N Engl J Med* 2019;380(23):2237-245. [PMID: 31167052 DOI: 10.1056/NEJMra1804280]
- [19] Halldorsson A, Brooks S, Montgomery S, Graham S. Lung metastasis 21 years after initial diagnosis

- of osteosarcoma: a case report. *J Med Case Rep* 2009;3:9298. [PMID: 20062787 DOI: 10.1186/1752-1947-3-9298]
- [20] Perrot P, Rousseau J, Bouffaut AL, et al. Safety control between autologous fat graft, mesenchymal stem cells and osteosarcoma recurrence. *PLoS One* 2010;5(6):e10999. [PMID: 20544017 DOI: 10.1371/journal.pone.0010999]
- [21] Pantel K, Alix-Panabières C. Tumour microenvironment: informing on minimal residual disease in solid tumours. *Nat Rev Clin Oncol* 2017;14(6):325-26. [PMID: 28397823 DOI: 10.1038/nrclinonc.2017.53]
- [22] Ferrari S, Bacci G, Picci P, et al. Long-term follow-up and post-relapse survival in patients with non-metastatic osteosarcoma of the extremity treated with neoadjuvant chemotherapy. *Ann Oncol* 1997;8(8):765-71. [PMID: 9332684 doi: 10.1023/a:1008221713505]
- [23] Bielack SS, Kempf-Bielack B, Delling G, et al. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 2002;20(3):776-90. [PMID: 11821461 DOI: 10.1200/JCO.2002.20.3.776]
- [24] Smeland S, Bielack SS, Whelan J, et al. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (European and American Osteosarcoma Study) cohort. *Eur J Cancer* 2019;109:36-50. [PMID: 30685685 DOI: 10.1016/j.ejca.2018.11.027]
- [25] Pennati A, Riggion E, Marano G, Biganzoli E. Autologous fat grafting after sarcoma surgery: Evaluation of oncological safety. *J Plast Reconstr Aesthet Surg* 2018;71(12):1723-29. [PMID: 30224167 DOI: 10.1016/j.bjps.2018.07.028].
- [26] Le Nail LR, Brennan M, Rosset P, et al. Comparison of tumor- and bone marrow-derived mesenchymal stromal/stem cells from patients with high-grade osteosarcoma. *Int J Mol Sci* 2018;19(3):707. [PMID: 29494553 DOI: 10.3390/ijms19030707].
- [27] Zhou Z, Li Y, Kuang M, et al. The CD24⁺ cell subset promotes invasion and metastasis in human osteosarcoma. *EBioMed* 2020;51:102598. [PMID: 31901872 DOI: 10.1016/j.ebiom.2019.102598]
- [28] Shao XJ, Xiang SF, Chen YQ, et al. Inhibition of M2-like macrophages by all-trans retinoic acid prevents cancer initiation and stemness in osteosarcoma cells. *Acta Pharmacol Sin* 2019;40(10):1343-350. [PMID: 31296953 DOI: 10.1038/s41401-019-0262-4]
- [29] Adhikari AS, Agarwal N, Wood BM, et al. CD117 and Stro-1 identify osteosarcoma tumor-initiating cells associated with metastasis and drug resistance. *Cancer Res* 2010;70(11):4602-12. [PMID: 20460510 DOI: 10.1158/0008-5472]
- [30] Tang QL, Liang Y, Xie XB, et al. Enrichment of osteosarcoma stem cells by chemotherapy. *Chinese J Cancer* 2011;30(6):426-32. [PMID 21627865 DOI: 10.5732/cjc.011.10127]
- [31] Yu L, Liu S, Zhang C, et al. Enrichment of human osteosarcoma stem cells based on hTERT transcriptional activity. *Oncotarget* 2013;4(12):2326-38. [PMID: 24334332 DOI: 10.18632/oncotarget.1554]
- [32] Tirino V, Desiderio V, d'Aquino R, et al. Detection and characterization of CD133⁺ cancer stem cells in human solid tumours. *PloS one* 2008;3(10):e3469. [PMID: 18941626 DOI: 10.1371/journal.pone.0003469]
- [33] Tirino V, Desiderio V, Paino F, De Rosa A, et al. Human primary bone sarcomas contain CD133⁺ cancer stem cells displaying high tumorigenicity in vivo. *FASEB J* 2011;25(6):2022-30. [PMID: 21385990 DOI: 10.1096/fj.10-179036]
- [34] He A, Qi W, Huang Y, Feng T, et al. CD133 expression predicts lung metastasis and poor prognosis

- in osteosarcoma patients: A clinical and experimental study. *Exp Ther Med* 2012;4(3):435-41. [PMID: 23181114 DOI: 10.3892/etm.2012.603]
- [35] Li J, Zhong XY, Li ZY, Cai JF, Zou L, Li JM, et al. CD133 expression in osteosarcoma and derivation of CD133(+) cells. *Mol Med Rep* 2013;7(2):577-84. [PMID: 23242469 DOI: 10.3892/mmr.2012.1231]
- [36] Ozturk S, Gorgun C, Gokalp S, Vatansever S, Sendemir A. Development and characterization of cancer stem cell-based tumoroids as an osteosarcoma model. *Biotechnol Bioeng* 2020;117(8):2527-2539. [PMID: 32391924 DOI: 10.1002/bit.27381]
- [37] Fujiwara T, Katsuda T, Hagiwara K, et al. Clinical relevance and therapeutic significance of microRNA-133a expression profiles and functions in malignant osteosarcoma-initiating cells. *Stem Cells* 2014;32(4):959-73. [PMID 24715690 DOI: 10.1002/stem.1618]
- [38] Mardani A, Gheythanvhi E, Mousavie SH, Jabari JM, Shooshtarizadeh T. Clinical Significance of Cancer Stem Cell Markers CD133 and CXCR4 in Osteosarcomas. *Asian Pac J Cancer Prev* 2020;21(1):67-73. [PMID: 31983166 DOI: 10.31557/APJCP.2020.21.1.67]
- [39] Bao Z, Cheng Z, Chai D. The expressions of CD133, ALDH1, and vasculogenic mimicry in osteosarcoma and their clinical significance. *Int J Clin Exp Pathol* 2018;11(7):3656-63. [PMID: 31949746]
- [40] Tian J, Li X, Si M, Liu T, Li J. CD271+ osteosarcoma cells display stem-like properties. *PLoS One* 2014;9(6):e98549. [PMID: 24893164 DOI: 10.1371/journal.pone.0098549]
- [41] Honoki K, Fujii H, Kubo A, et al. Possible involvement of stem-like populations with elevated ALDH1 in sarcomas for chemotherapeutic drug resistance. *Oncol Rep* 2010;24(2):501-5. [PMID:20596639 DOI: 10.3892/or_00000885]
- [42] Wang L, Park P, Zhang H, La Marca F, Lin CY. Prospective identification of tumorigenic osteosarcoma cancer stem cells in OS99-1 cells based on high aldehyde dehydrogenase activity. *Int J Cancer* 2011;128(2):294-303. [PMID:20309879 DOI: 10.1002/ijc.25331]
- [43] Martins-Neves SR, Paiva-Oliveira DI, Wijers-Koster PM, Abrunhosa AJ, Fontes-Ribeiro C, Bovee JV, et al. Chemotherapy induces stemness in osteosarcoma cells through activation of Wnt/beta-catenin signaling. *Cancer Lett* 2016;370(2):286-95. [PMID: 26577806 DOI: 10.1016/j.canlet.2015.11.013].
- [44] Wang F, Zhang Z, Li Q, Yu T, Ma C. Untargeted LC-MS/MS analysis reveals metabolomics feature of osteosarcoma stem cell response to methotrexate. *Cancer Cell Int* 2020;20:269. [PMID: 32587477 DOI: 10.1186/s12935-020-01356-y].
- [45] Mizushima E, Tsukahara T, Emori M, et al. Osteosarcoma-initiating cells show high aerobic glycolysis and attenuation of oxidative phosphorylation mediated by LIN28B. *Cancer Sci* 2020 ;111(1):36-46. [PMID: 31705593 DOI: 10.1111/cas.14229].
- [46] Zhong Z, Mao S, Lin H, Li H, Lin J, Lin JM. Alteration of intracellular metabolome in osteosarcoma stem cells revealed by liquid chromatography-tandem mass spectrometry. *Talanta* 2019;204:6-12. [PMID: 31357340 DOI: 10.1016/j.talanta.2019.05.088].
- [47] Koka P, Mundre RS, Rangarajan R, Chandramohan Y, Subramanian RK, Dhanasekaran A. Uncoupling Warburg effect and stemness in CD133⁺ cancer stem cells from Saos-2 (osteosarcoma) cell line under hypoxia. *Mol Biol Rep* 2018;45(6):1653-1662. [PMID: 30128626 DOI: 10.1007/s11033-018-4309-2].
- [48] Pozzi V, Salvolini E, Lucarini G, et al. Cancer stem cell enrichment is associated with enhancement of nicotinamide N-methyltransferase expression. *IUBMB Life* 2020;72(7):1415-1425. [PMID: 32150326]

- DOI: 10.1002/iub.2265]
- [49] Wang Y, Zeng L, Liang C, et al. Integrated analysis of transcriptome-wide m⁶A methylome of osteosarcoma stem cells enriched by chemotherapy. *Epigenomics* 2019; 11(15):1693-1715. [PMID: 31650864 DOI:10.2217/epi-2019-0262]
- [50] Menéndez ST, Gallego B, Murillo D, Rodríguez A, Rodríguez R. Cancer stem cells as a source of drug resistance in bone sarcomas. *J Clin Med* 2021;10(12):2621. [PMID: 34198693 DOI: 10.3390/jcm10122621]
- [51] Belayneh R, Weiss K. The Role of ALDH in the metastatic potential of osteosarcoma cells and potential ALDH targets. *Adv Exp Med Biol* 2020;1258:157-166. [PMID: 32767240 DOI: 10.1007/978-3-030-43085-6_10]
- [52] Tsuchida R, Das B, Yeager H, Koren G, Shibuya M, Thorner PS, et al. Cisplatin treatment increases survival and expansion of a highly tumorigenic side-population fraction by upregulating VEGF/Flt1 autocrine signaling. *Oncogene* 2008;27(28):3923-34. [PMID: 18332870 DOI: 10.1038/onc.2008.38]
- [53] Sun DX, Liao GL, Liu KG, Jian H. Endosialin-expressing bone sarcoma stem-like cells are highly tumor-initiating and invasive. *Mol Med Rep.* 2015;12(4):5665-70 [PMID: 26300407 DOI: 10.3892/mmr.2015.4218]
- [54] Di Fiore R, Santulli A, Ferrante RD, et al. Identification and expansion of human osteosarcoma-cancer-stem cells by long-term 3-aminobenzamide treatment. *J Cell Physiol* 2009;219(2):301-13. [PMID 19160414 DOI: 10.1002/jcp.21667]
- [55] Roundhill EA, Chicon-Bosch M, Jeys L, Parry M, Rankin KS, Droop A, Burchill SA. RNA sequencing and functional studies of patient-derived cells reveal that neurexin-1 and regulators of this pathway are associated with poor outcomes in Ewing sarcoma. *Cell Oncol (Dordr)* 2021;44(5):1065-1085. [PMID: 34403115 DOI: 10.1007/s13402-021-00619-8]
- [56] Alfranca A, Martinez-Cruzado L, Tornin J, et al. Bone microenvironment signals in osteosarcoma development. *Cell Mol Life Sci* 2015; 72(16):3097-113. [PMID: 25935149 DOI: 10.1007/s00018-015-1918-y]
- [57] Danieau G, Morice S, Rédini F, et al. New insights about Wnt/beta-catenin signaling pathway in primary bone tumors and their microenvironment : a promising target to develop therapeutic strategies ? *Int J Mol Sci* 2019;20(15):3751. [PMID: 31370265 DOI: 10.3390/ijms2015375152]
- [58] Deng Q, Li P, Che M, et al. Activation of hedgehog signaling in mesenchymal stem cells induces cartilage and bone tumor formation via Wnt/ β -Catenin. *Elife* 2019;8:e50208. [PMID: 31482846 DOI: 10.7554/eLife.50208]
- [59] Parsons MJ, Tammela T, Dow LE. Wnt as a driver and dependency in cancer. *Cancer Discov* 2021;11(10):2413-2429. [PMID: 34518209 DOI: 10.1158/2159-8290.CD-21-0190]
- [60] Martins-Neves SR, Corver WE, Paiva-Oliveira DI, et al. Osteosarcoma stem cells have active Wnt/ β -catenin and overexpress SOX2 and KLF4. *J Cell Physiol* 2016;231(4):876-86. [PMID: 26332365 DOI: 10.1002/jcp.25179]
- [61] Martins-Neves SR, Paiva-Oliveira DI, Wijers-Koster PM, et al. Chemotherapy induces stemness in osteosarcoma cells through activation of Wnt/ β -catenin signaling. *Cancer Lett* 2016;370(2):286-95. [PMID: 26577806 DOI: 10.1016/j.canlet.2015.11.013]
- [62] Zhang RM, Tang T, Yu HM, et al. LncRNA DLX6-AS1/miR-129-5p/DLK1 axis aggravates stemness of osteosarcoma through Wnt signaling. *Biochem Biophys Res Commun* 2018;507(1-4):260-266. [PMID 30442366 DOI 10.1016/j.bbrc.2018.11.019]
- [63] Jiang C, He C, Wu Z, et al. Histone methyltransferase SETD2 regulates osteosarcoma cell growth and

- chemosensitivity by suppressing Wnt/ β -catenin signaling. *Biochem Biophys Res Commun* 2018;502(3):382-388. [PMID 29842882 DOI: 10.1016/j.bbrc.2018.05.176]
- [64] Blanchard F, Duplomb L, Baud'huin M, et al. The dual role of IL-6 type cytokines on bone remodeling and bone tumors. *Cytokine Growth Factor Rev* 2009;20(1):19-28. [PMID: 19038573 DOI: 10.1016/j.cytogfr.2008.11.004]
- [65] Cortini M, Massa A, Avnet S, et al. Tumor-activated mesenchymal stromal cells promote osteosarcoma stemness and migratory potential via IL-6 secretion. *PLoS One* 2016;11(11):e0166500. [PMID: 27851822 DOI: 10.1371/journal.pone.0166500]
- [66] Tu B, Zhu J, Liu S, Wang L, Fan Q, Hao Y, Fan C, Tang TT. Mesenchymal stem cells promote osteosarcoma cell survival and drug resistance through activation of STAT3. *Oncotarget* 2016;7(30):48296-48308. [PMID: 27340780 DOI: 10.18632/oncotarget.10219]
- [67] Baglio SR, Lagerweij T, Pérez-Lanzón M, et al. Blocking tumor-educated MSC paracrine activity halts osteosarcoma progression. *Clin Cancer Res* 2017;23(14):3721-3733. [PMID: 28053020 DOI: 10.1158/1078-0432.CCR-16-2726]
- [68] Zhang C, Ma K, Li W. Cinobufagin suppresses the characteristics of osteosarcoma cancer cells by inhibiting the IL-6-OPN-STAT3 pathway. *Drug Des Devel Ther* 2019;13:4075-4090. [PMID: 31824138 DOI: 10.2147/DDDT.S224312]
- [69] Wang S, Hu H, Zhong B, et al. Bruceine D inhibits tumor growth and stem cell-like traits of osteosarcoma through inhibition of STAT3 signaling pathway. *Cancer Med* 2019 ; 8(17):7345-7358. [PMID: 31631559 DOI: 10.1002/cam4.2612]
- [70] Zhang H, Wu H, Zheng J et al. Transforming growth factor β 1 signal is crucial for dedifferentiation of cancer cells to cancer stem cells in osteosarcoma. *Stem Cells* 2013;31(3):433-46. PMID: 23225703 DOI: 10.1002/stem.1298.
- [71] Ma K, Zhang C, Li W. Gamabufotalin suppressed osteosarcoma stem cells through the TFG-beta/periostin/PI3K/AKT pathway. *Chem Biol Interact* 2020;331:109275. [PMID: 33010222 DOI: 10.1016/j.cbi.2020.109275].
- [72] Wang T, Wang D, Zhang L, Yang P, Wang J, Liu Q, Yan F, Lin F. The TGFbeta-miR-499a-SHKBP1 pathway induces resistance to EGFR inhibitors in osteosarcoma cancer stem cell-like cells. *J Exp Clin Cancer Res* 2019;38(1):226. [PMID: 31138318 DOI: 10.1186/s13046-019-1195-y]
- [73] Maurizi G, Verma N, Gadi A, et al. Sox2 is required for tumor development and cancer cell proliferation in osteosarcoma. *Oncogene* 2018;37(33):4626-4632. [PMID 2974593 DOI: 10.1038/s41388-018-0292-2]
- [74] Chen Y, Wang T, Huang M, et al. MAFB promotes cancer stemness and tumorigenesis in osteosarcoma through a Sox9-mediated positive feedback loop. *Cancer Res* 2020;80(12):2472-2483. [PMID: 32234710 DOI: 10.1158/0008-5472.CAN-19-1764]
- [75] Subramaniam D, Angulo P, Ponnurangam, et al. Suppressing STAT5 signaling affects osteosarcoma growth and stemness. *Cell Death Dis* 2020;11(2):149. [PMID: 32094348 DOI: 10.1038/s41419-020-2335-1]
- [76] Fan H, Liu G, Zhao C, et al. Transcription factor Oct4 promotes osteosarcoma by regulating lncRNA AK055347. *Oncol Lett* 2017;13(1):396-402. [PMID: 28123573 DOI: 10.3892/ol.2016.5400]
- [77] Xi X, Wu Q, Bao Y, et al. Overexpression of TBL1XR1 confers tumorigenic capability and promotes recurrence of osteosarcoma. *Eur J Pharmacol* 2019;844:259-267. [PMID 30529193 DOI: 10.1016/j.ejphar.2018.12.013]
- [78] Chen X, Zhang Q, Dang X et al. Targeting the CtBP1-FOXM1 transcriptional complex with small

- molecules to overcome MDR1-mediated chemoresistance in osteosarcoma cancer stem cells. *J Cancer* 2021;12(2):482-497. [PMID: 33391445 DOI: 10.7150/jca.50255]
- [79] Fan GT, Ling ZH, He ZW, Wu SJ, Zhou GX. Suppressing CHD1L reduces the proliferation and chemoresistance in osteosarcoma. *Biochem Biophys Res Commun* 2021 May 21;554:214-221. [PMID: 33813077 DOI: 10.1016/j.bbrc.2020.12.109]
- [80] Gan Z, Han K, Lin S, et al. Knockdown of ubiquitin-specific peptidase 39 inhibited the growth of osteosarcoma cells and induced apoptosis in vitro. *Biol Res* 2017;50(1):15. [PMID 28403900 DOI: 10.1186/s40659-017-0121-z]
- [81] Xu W, Chen C, Xu R, et al. Knockdown of HuR represses osteosarcoma cells migration, invasion and stemness through inhibition of YAP activation and increases susceptibility to chemotherapeutic agents. *Biomed Pharmacother* 2018;102:587-593. [PMID 29597092 DOI: 10.1016/j.biopha.2018.03.098]
- [82] Li S, Bai H, Chen X et al. Soft substrate promotes osteosarcoma cell self-renewal, differentiation, and drug resistance through miR-29b and its target protein Spin 1. *ACS Biomater Sci Eng* 2020;6(10):5588-5598. [PMID: 33320589 DOI: 10.1021/acsbiomaterials.0c00816]
- [83] Zou Y, Huang Y, Yang J, et al. miR-34a is downregulated in human osteosarcoma stem-like cells and promotes invasion, tumorigenic ability and self-renewal capacity. *Mol Med Rep* 2017 ;15(4):1631-1637. [PMID: 28260055 DOI: 10.3892/mmr.2017.6187]
- [84] Liang X, Xu C, Wang W, et al. The DNMT1/miR-34a axis Is Involved in the stemness of human osteosarcoma cells and derived stem-like cells. *Stem Cells Int* 2019;2019:7028901. [PMID: 31781245 DOI: 10.1155/2019/7028901]
- [85] Liang X, Xu C, Cao X, et al. Isovitexin suppresses cancer stemness property and induces apoptosis of osteosarcoma cells by disruption of the DNMT1/miR-34a/Bcl-2 axis. *Cancer Manag Res* 2019;11:8923-8936. [PMID: 31686915 DOI : 10.2147/CMAR.S222708]
- [86] Yao J, Lin J, He L, et al. TNF- α /miR-155 axis induces the transformation of osteosarcoma cancer stem cells independent of TP53INP1. *Gene* 2020;726:144224. [PMID: 31669646 DOI: 10.1016/j.gene.2019.144224]
- [87] Guo X, Yu L, Zhang Z, et al. miR-335 negatively regulates osteosarcoma stem cell-like properties by targeting POU5F1. *Cancer Cell Int* 2017;17:29. [PMID: 28239298 DOI: 10.1186/s12935-017-0398-6]
- [88] Zhang L, Yang P, Liu Q, et al. KLF8 promotes cancer stem cell-like phenotypes in osteosarcoma through miR-429-SOX2 signaling. *Neoplasia* 2020;67(3):519-527. [PMID: 32122144 DOI: 10.4149/neo_2020_190711N624]
- [89] Wang T, Wang D, Zhang L, et al. The TGF β -miR-499a-SHKBP1 pathway induces resistance to EGFR inhibitors in osteosarcoma cancer stem cell-like cells. *J Exp Clin Cancer Res* 2019;38(1):226. [PMID: 31138318 DOI: 10.1186/s13046-019-1195-y]
- [90] Qu Y, Zheng S, Kang M, et al. Knockdown of long non-coding RNA HOXD-AS1 inhibits the progression of osteosarcoma. *Biomed Pharmacother* 2018;98:899-906. [PMID 29571260 DOI: 10.1016/j.biopha.2018.01.024]
- [91] Lu B, He Y, He J, et al. Epigenetic profiling identifies LIF as a super-enhancer-controlled regulator of stem cell-like properties in osteosarcoma. *Mol Cancer Res* 2020;18(1):57-67. [PMID: 31615908 DOI: 10.1158/1541-7786.MCR-19-0470]
- [92] Qu Y, Zheng S, Kang M, et al. Knockdown of long non-coding RNA HOXD-AS1 inhibits the progression of osteosarcoma. *Biomed Pharmacother* 2018;98:899-906. [PMID: 29571260 DOI: 10.1016/j.biopha.2018.01.024]
- [93] Yan GN, Tang XF, Zhang XC, et al. TSSC3 represses self-renewal of osteosarcoma stem cells and

- Nanog expression by inhibiting the Src/Akt pathway. *Oncotarget* 2017;8(49):85628-85641. [PMID 29156746 DOI: 10.18632/oncotarget.20429]
- [94] Camuzard O, Trojani MC, Santucci-Darmanin S, Pagnotta S, Breuil V, Carle GF, Pierrefite-Carle V. Autophagy in osteosarcoma cancer stem cells is critical process which can be targeted by the antipsychotic drug thioridazine. *Cancers (Basel)* 2020;12(12):3675. [PMID: 33297525 DOI: 10.3390/cancers12123675]
- [95] Zhang D, Zhao Q, Sun H, et al. Defective autophagy leads to the suppression of stem-like features of CD271⁺ osteosarcoma cells. *J Biomed Sci.* 2016;23(1):82. [PMID: 27863492 DOI: 10.1186/s12929-016-0297-5].
- [96] Wang JH, Gong C, Guo FJ, et al. Knockdown of STIP1 inhibits the invasion of CD133-positive cancer stem-like cells of the osteosarcoma MG63 cell line via the PI3K/Akt and ERK1/2 pathways. *Int J Mol Med* 2020;46(6):2251-2259. [PMID: 33125116 DOI: 10.3892/ijmm.2020.4764]
- [97] Shu X, Liu H, Zhen R, et al. Hsp90 inhibitor 17-AAG inhibits stem cell-like properties and chemoresistance in osteosarcoma cells via the Hedgehog signaling pathway. *Oncol Rep* 2020;44(1):313-324. [PMID: 32377704 DOI: 10.3892/or.2020.7597]
- [98] Zhao T, Meng Y, Wnag Y, Wang W. NDRG1 regulates osteosarcoma cells via mediating the mitochondrial function and CSCs differentiation. *J Orthop Surg Res* 2021 ;16(1):364. [PMID: 34099022 DOI: 10.1186/s13018-021-02503-5]
- [99] Prominent role of RAB39A-RXR β axis in cancer development and stemness. *Oncotarget* 2018;9(11):9852-9866. [PMID 29515775 DOI: 10.18632/oncotarget.23955]
- [100] Takahashi N, Nobusue H, Shmizu T, et al. ROCK inhibition induces terminal adipocyte differentiation and suppresses tumorigenesis in chemoresistant osteosarcoma cells. *Cancer Res* 2019;79(12):3088-3099. [PMID: 30992323 DOI: 10.1158/0008-5472.CAN-18-2693]
- [101] Liu F, Li L, Li Y, et al. Overexpression of SENP1 reduces the stemness capacity of osteosarcoma stem cells and increases their sensitivity to HSVtk/GCV. *Int J Oncol* 2018;53(5):2010-2020. [PMID 30226577 DOI: 10.3892/ijo.2018.4537]
- [102] Tang, ML, Bai XJ, Li Y, et al. MMP-1 over-expression promotes malignancy and stem-like properties of human osteosarcoma MG-63 cells in vitro. *Curr Med Sci* 2018;38(5):809-817. [PMID 30594980 DOI: 10.1007/s11596-018-1947-5]
- [103] Zhao T, Meng Y, Wang et al. NDRG1 regulates osteosarcoma cells via mediating the mitochondrial function and CSCs differentiation. *J Orthop Surg Res* 2021;16(1):364. [PMID 34099022 DOI: 10.1186/s13018-021-02503-5]
- [104] Feng J, Lan R, Cai G, et al. TREX1 suppression imparts cancer-stem-cell-like characteristics to CD133⁺ osteosarcoma cells through the activation of E2F4 signaling. *Int J Clin Exp Pathol* 2019;12(4):1134-1153. [PMID: 31933929]
- [105] Martin-Neves SR, Paiva-Oliveira DI, Fontes-Ribeiro C, et al. IWR-1, a tankyrase inhibitor, attenuates Wnt/ β -catenin signaling in cancer stem-like cells and inhibits in vivo the growth of a subcutaneous human osteosarcoma xenograft. *Cancer Lett* 2018;414:1-15. [PMID: 29126913 DOI: 10.1016/j.canlet.2017.11.004]
- [106] Nomura M, Rainusso N, Lee YC, et al. Tegavivint and the β -Catenin/ALDH axis. *J Natl Cancer Inst* 2019 ;111(11):1216-1227. [PMID: 30793158 DOI: 10.1093/jnci/djz026]
- [107] Liu W, Zhao Z, Wang Y, et al. Dioscin inhibits stem-cell-like properties and tumor growth of osteosarcoma through Akt/GSK3/ β -catenin signaling pathway. *Cell Death Dis* 2018;9(3):343. [PMID: 29497056 DOI: 10.1038/s41419-018-0363-x]

- [108] Wei D, Zhu X, Li S, et al. Tideglusib suppresses stem-cell-like features and progression of osteosarcoma by inhibiting GSK-3 β /NOTCH1 signaling. *Biochem Biophys Res Commun* 2021; 554 :206-213. [PMID: 33813076 DOI: 10.1016/j.bbrc.2020.12.055]
- [109] Ma K, Zhang C, Li W. Gamabufotalin suppressed osteosarcoma stem cells through the TGF- β /periostin/PI3K/AKT pathway *Chem Biol Interact* 2020;331:109275. [PMID: 33010222 DOI: 10.1016/j.cbi.2020.109275]
- [110] Xiong Q, Wang X, Wang L, et al. BMP-2 inhibits lung metastasis of osteosarcoma: an early investigation using an orthotopic model. *Onco Targets Ther* 2018 ;11:7543-7553. [PMID: 30464502 DOI: 10.2147/OTT.S176724]
- [111] Wang S, Hu H, Zhong B, et al. Bruceine D inhibits tumor growth and stem cell-like traits of osteosarcoma through inhibition of STAT3 signaling pathway. *Cancer Med* 2019;8(17):7345-7358. [PMID: 31631559 DOI: 10.1002/cam4.2612]
- [112] Gonçalves JM, Barcellos S CA, Correa Rivero ER, et al. Inhibition of cancer stem cells promoted by Pimozide. *Clin Exp Pharmacol Physiol* 2019;46(2):116-125. [PMID: 30383889 DOI: 10.1111/1440-1681.13049]
- [113] Lillo Osuna MA, Garcia-lopez J, El Ayachi I, et al. Activation of estrogen receptor alpha by decitabine inhibits osteosarcoma growth and metastasis. *Cancer Res* 2019;79(6):1054-1068. [PMID: 30593524 DOI: 10.1158/0008-5472.CAN-18-1255]
- [114] Hirozane T, Masuda M, Sugano T, et al. Direct conversion of osteosarcoma to adipocytes by targeting TNIK. *JCI Insight* 2021;6(3):e137245. [PMID: 33400690 DOI: 10.1172/jci.insight.137245.
- [115] Qu H, Xue Y, Lian W, et al. Melatonin inhibits osteosarcoma stem cells by suppressing SOX9-mediated signaling. *Life Sci* 2018;207:253-264 [PMID: 29689273 DOI: 10.1016/j.lfs.2018.04.030]
- [116] Li Y, Xian M, Yang B, et al. Inhibition of KLF4 by statins reverses adriamycin-induced metastasis and cancer stemness in osteosarcoma cells. *Stem Cell Rep* 2017;8(6):1617-1629. [PMID: 28552603 DOI: 10.1016/j.stemcr.2017.04.025]
- [117] Deguchi T, Hosoya K, Kim S, et al. Metformin preferentially enhances the radio-sensitivity of cancer stem-like cells with highly mitochondrial respiration ability in HMPOS. *Mol Ther Oncolytics* 2021;22:143-151. [PMID: 34514095 DOI: 10.1016/j.omto.2021.08.007]
- [118] Shang D, Wu J, Guo L, et al. Metformin increases sensitivity of osteosarcoma stem cells to cisplatin by inhibiting expression of PKM2. *Int J Oncol* 2017;50(5):1848-1856. [PMID: 28393220 DOI: 10.3892/ijo.2017.3950]
- [119] Zhao B, Luo J, Wang Y, et al. Metformin suppresses self-renewal ability and tumorigenicity of osteosarcoma stem cells via reactive oxygen species-mediated apoptosis and autophagy. *Oxid Med Cell Longev* 2019;2019:9290728. [PMID: 31827709 DOI: 10.1155/2019/9290728]
- [120] Paiva-Oliveira DI, Martin-Neves SR, Abrunhosa AJ, et al. Therapeutic potential of the metabolic modulator Metformin on osteosarcoma cancer stem-like cells. *Cancer Chemother Pharmacol* 2018;81(1):49-63. [PMID: 29086064 DOI: 10.1007/s00280-017-3467-6]
- [121] Koh H, Sun HN, Xing Z, et al. Wogonin influences osteosarcoma stem cell stemness through ROS-dependent signaling. *In Vivo* 2020;34(3):1077-1084. [PMID: 32354895 DOI: 10.21873/invivo.11878]
- [122] Ba G, Hua Z, Xu N, et al. Novel agent DMAMCL suppresses osteosarcoma growth and decreases the stemness of osteosarcoma stem cell. *Cell Cycle* 2020;19(12):1530-1544. [PMID: 32401122 DOI: 10.1080/15384101.2020.1762041]

- [123] Dai G, Deng S, Guo W, et al. Notch pathway inhibition using DAPT, a γ -secretase inhibitor (GSI), enhances the antitumor effect of cisplatin in resistant osteosarcoma. *Mol Carcinog* 2019;58(1):3-18. [PMID: 29964327 DOI: 10.1002/mc.22873]
- [124] Oka N, Kumuro A, Amano H, et al. Ascorbate sensitizes human osteosarcoma cells to the cytostatic effects of cisplatin. *Pharmacol Res Perspect* 2020;8(4):e00632. [PMID: 32725721 DOI: 10.1002/prp2.632]
- [125] Guo W, Wei B, Cheng T, et al. The Na⁺/K⁺ ATPase inhibitor ouabain attenuates stemness and chemoresistance of osteosarcoma cells. *Med Sci Monit* 2019;25:9426-9434. [PMID: 31822650 DOI: 10.12659/MSM.919266]
- [126] Gurunathan S, Jeyarai M, Kang MH, et al. Tangeretin-assisted platinum nanoparticles enhance the apoptotic properties of doxorubicin: combination therapy for osteosarcoma treatment. *Nanomaterials* (Basel) 2019;9(8):1089. [PMID: 31362420 DOI: 10.3390/nano9081089]
- [127] Ferreira de Oliveira JMP, Pacheco AR, Coutinho L, et al. Combination of etoposide and fisetin results in anti-cancer efficiency against osteosarcoma cell models. *Arch Toxicol* 2018;92(3):1205-1214. [PMID: 29270805 DOI: 10.1007/s00204-017-2146-z]
- [128] Heymann MF, Schiavone K, Heymann D. Bone sarcomas in the immunotherapy era. *Br J Pharmacol*. 2021;178(9):1955-1972. [PMID: 31975481 doi: 10.1111/bph.14999]
- [129] Mesiano G, Grignani G, Fiorini E, et al. Cytokine Induced Killer cells are effective against sarcoma cancer stem cells spared by chemotherapy and target therapy. *Oncoimmunol* 2018;7(11):e1465161. [PMID: 30393581 DOI: 10.1080/2162402X.2018.1465161]
- [130] Wang W, Chen D, Zhu K. SOX2OT variant 7 contributes to the synergistic interaction between EGCG and Doxorubicin to kill osteosarcoma via autophagy and stemness inhibition. *J Exp Clin Cancer Res* 2018;37(1):37. [PMID: 29475441 DOI: 10.1186/s13046-018-0689-3]
- [131] Lu SL, Wang YH, Liu GF, et al. Graphene Oxide Nanoparticle-Loaded Ginsenoside Rg3 improves photodynamic therapy in inhibiting malignant progression and stemness of osteosarcoma. *Front Mol Biosci* 2021;8:663089. [PMID: 33968991 DOI: 10.3389/fmolb.2021.663089]
- [132] Tian J, Gu Y, Li Y, et al. CD271 antibody-functionalized HGNs for targeted photothermal therapy of osteosarcoma stem cells. *Nanotechnology* 2020;31(30):305707. [PMID: 32235073 DOI: 10.1088/1361-6528/ab8593]
- [133] Mineo P, Foti C, Vento F, et al. Salinomycin-loaded PLA nanoparticles: drug quantification by GPC and wave voltammetry and biological studies on osteosarcoma cancer stem cells. *Anal Bioanal Chem* 2020;412(19):4681-4690. [PMID: 32451642 DOI: 10.1007/s00216-020-02721-6]
- [134] Gui K, Zhang X, Chen F, et al. Lipid-polymer nanoparticles with CD133 aptamers for targeted delivery of all-trans retinoic acid to osteosarcoma initiating cells. *Biomed Pharmacother* 2019;111:751-764. [PMID: 30612000 DOI: 10.1016/j.biopha.2018.11.118]
- [135] Chen F, Zeng Y, Qi X, et al. Targeted salinomycin delivery with EGFR and CD133 aptamers based dual-ligand lipid-polymer nanoparticles to both osteosarcoma cells and cancer stem cells. *Nanomedicine* 2018;14(7):2115-2127. [PMID: 29898423 DOI: 10.1016/j.nano.2018.05.015]
- [136] Gambera S, Abarrategi A, González-Camacho F, Morales-Molina A, et al. Clonal dynamics in osteosarcoma defined by RGB marking. *Nat Commun* 2018;9(1):3994. [PMID: 30266933 DOI: 10.1038/s41467-018-06401-z]
- [137] Tellez-Gabriel M, Cochonneau D, Cadé M, et al. Circulating tumor cell-derived pre-clinical models for personalized medicine. *Cancers* (Basel) 2018;11(1):19. [PMID: 30586936 DOI: 10.3390/cancers11010019]

- [138] Zhang W, Zhao JM, Lin J, et al. Adaptive fibrogenic reprogramming of osteosarcoma stem cells promotes metastatic growth. *Cell Rep* 2018;24(5):1266-1277.e5. [PMID: 30067981 DOI: 10.1016/j.celrep.2018.06.103]
- [139] Heymann MF, Lézot F, Heymann D. The contribution of immune infiltrates and the local microenvironment in the pathogenesis of osteosarcoma. *Cell Immunol.* 2019;343:103711. [PMID: 29117898 DOI: 10.1016/j.cellimm.2017.10.011. 28].
- [140] Avnet S, Lemma S, Cortini M, et al. The release of inflammatory mediators from acidosis-stimulated stromal cells favours tumours invasiveness and metastasis in osteosarcoma. *Cancers (Basel)* 2021 22;13(22):5855. [PMID: 34831016 DOI: 10.3390/cancers13225855]
- [141] Avnet S, Lemma S, Cortini M, Di Pompo G, Perut F, Baldini N. Pre-clinical models for studying the interaction between mesenchymal stromal cells and cancer cells and the induction of stemness. *Front Oncol.* 2019;9:305. [PMID: 31114753 DOI: 10.3389/fonc.2019.00305].
- [142] Bonuccelli G, Avnet S, Grisendi G, et al. Role of mesenchmal stem cells in osteosarcoma and metabolic reprogramming of tumor cells. [*Oncotarget.* 2014;5(17):7575-88. [PMID: 25277190 DOI: 10.18632/oncotarget.2243]
- [143] Baglio SR, Lagerweij T, Pérez-Lanzón M, et al. Blocking tumour-educated MSC paracrine activity halts osteosarcoma progression. *Clin Cancer Res.* 2017;23(14):3721-3733. [PMID: 28053020 doi: 10.1158/1078-0432].