REVIEW

Review of the Molecular Pathogenesis of Osteosarcoma

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Abstract

Treating the osteosarcoma (OSA) remains a challenge. Current strategies focus on the primary tumor and have limited efficacy for metastatic OSA. A better understanding of the OSA pathogenesis may provide a rational basis for innovative treatment strategies especially for metastases. The aim of this review is to give an overview of the molecular mechanisms of OSA tumorigenesis, OSA cell proliferation, apoptosis, migration, and chemotherapy resistance, and how improved understanding might contribute to designing a better treatment target for OSA.

Keywords: Osteosarcoma - pathogenesis - genetic factors - chemotherapy resistance

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Introduction

The most commonly diagnosed primary malignant tumor of the bone In humans is OSA. It is the third most frequent cause of cancer in adolescents and represents over 56% of all bone tumors. The estimated incidence rate worldwide is 4 cases per million people per year, with a bimodal age distribution with peaks at 15 to 19 years and 70 years (Mirabello et al., 2009); approximately 60% of tumors occur in patients under 20 years of age, patients older than 60 years constitutes approximately 10% of the patient population. OSA has a high tendency to metastatic spread, eighty percent of all metastases arise in the lungs, especially the periphery of the lungs. Metastatic OSA exhibits resistance to conventional chemotherapy (Bacci et al., 2008; Bielack et al., 2008; Harting et al., 2006; Hughes, 2009; Messerschmitt et al., 2009; Kager et al., 2003), more than 30% of them do not respond to chemotherapy (Mankin et al., 2004). Prognosis of non-metastatic OSA has improved dramatically since the invention of preoperative and postoperative chemotherapy in the 1970s. Currently the five-year overall survival rate is 75% to 77% for patients diagnosed with primary nonmetastatic extremity OSA (Smeland et al., 2003; Ferrari et al., 2005). However, the prognosis for patients with metastatic OSA is poor with 5-year event-free survival (EFS) of no more than 20% (Mialou et al., 2004).

In the pursuit of better treatment, recent years have witnessed exciting progress in the revelation of the nature and pathogenesis of OSA cells. The following discussion will focus on these important findings.

Progenitors of OSA Cells

Where does the OSA come from? The presence of osteoid has led to the traditional viewpoint that the tumor is derived from osteoblasts. OSA has traditionally been believed to arise from an osteoblast, but the data supporting that assertion is rather limited (Dorfman et al., 1995). Several lines of evidence suggest that OSA has a more pluripotent potential and may, in fact, arise from a more primitive precursor. Several studies have identified genetic and epigenetic changes that prevent normal osteoblastic differentiation from mesenchymal progenitor cells (Ritter et al., 2010; Hengartner, 2000; Barker et al., 2006; Lin et al., 2006) as a major factor leading to the development of OSA (Weeraratna et al., 2002). It is also known that these tumors are capable of differentiating toward fibrous tissue, cartilage, or bone and can have chondroblastic, fibroblastic and osteoblastic components, suggesting that the cell of origin may be more pluripotent than an osteoblast (Dorfman et al., 1995).

Regulation of Signal Pathways in the OSA

Preoperative (neoadjuvant) plus postoperative (adjuvant) polychemotherapy should be preferred, because it allows preparation for safe surgery and preparation of the appropriate prosthesis for the individual patient (Ritter et al., 2010). Adjuvant therapies almost benefit the patients through affecting the apoptosis of OSA cells. Apoptosis is a programmed cell death event which occurs during embryogenesis, metamorphosis, endocrine-dependent tissue atrophy and normal tissue turnover. In multicellular organisms, it is responsible for development, tissue homeostasis, and the immune response via different signal pathways (Hengartner, 2000).

The Wnt/catenin pathway is very important in OSA

Aberrant activation of Wnt signaling has been reported

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in a variety of bone and soft-tissue sarcomas (Barker et al., 2006; Lin et al., 2006; Weeraratna et al., 2002; Wissmann et al., 2003). Haydon et al., (Haydon et al., 2002) demonstrated that OSA harbors an accumulation of beta-catenin either in the cytoplasm or in the nucleus, a hallmark of Wnt signaling activation. The Wnt signaling pathway is initiated by a combination from 19 secreted Wnt ligands, 10 Frizzled receptors, and the co-receptor Lipoprotein Receptor-Related Protein 5/6 (LRP5/6). These ligand-receptor interactions then lead to activation of multiple intermediate Wnt effectors including beta-catenin, JNK and calcium-channel regulators. The accumulation of beta-catenin in the cytoplasm and its translocation to the nucleus represent the hallmark of the canonical Wnt pathway activation. In the nucleus, beta-catenin forms a complex with lymphocyte enhancer factor/T cell factor family of transcription factors (LEF/TCF) to activate many oncogenes, such as c-Myc, cyclin D1, metalloproteinases, c-Met, etc. Wnt/beta-catenin/TCF activation are responsible for the FoxO3a mediated repression of syndecan-2, a key modulator of apoptosis and chemosensitivity in OSA cells (Dieudonne et al., 2012), suggesting a role of Wnt signaling in chemotherapy resistance through FoxO3a expression to modulate the activity of syndecan-2 and Wnt/beta-catenin/TCF together.

Secreted Wnt antagonists are divided into two classes according to their mechanisms of action. One class directly binds to Wnt ligands to cause inhibition and includes the sFRP family, Wnt inhibitory factor-1 (WIF-1) and Cerberus (Kawano et al., 2003). The second class including the Dkk family exerts inhibition by endocytosis of co-receptors LRP5/6 (Kawano et al., 2003). Several Wnt antagonists, including Frzb/sFRP3 and Dkk-3, function as tumor suppressors (Zi et al., 2005; Guo et al., 2008; Abarzua et al., 2005; Rubin et al., 2010; Gurney et al., 2012). WIF-1 is a unique Wnt antagonist, structurally distinct from sFRP and Dkk families, which contains a WIF domain for Wnt binding activity and epidermal growth factor (EGF) repeats (Hsieh et al., 1999). The WIF domain has also been found in the Ryk orphan tyrosine kinase receptor (Lu et al., 2004). WIF-1 is down-regulated in a majority of OSA cell lines and tumor tissues through methylation of WIF-1 promoter and that WIF-1 re-expression markedly reduced both tumor growth rate and lung metastasis in mouse models of OSA by reducing MMP-9 and MMP-14 protein expression (Rubin et al., 2010).

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that can degrade the ECM and facilitate cellular invasion and migration (Baldini et al., 1995). High MMP-9 expression was observed in pre-treatment OSA tumor samples and in a majority of metastatic lesions, leading to speculation that MMP-9 expression is associated with the micrometastatic behavior of OSA (Himelstein et al., 1998). Membrane-type metalloproteinase (MT1-MMP), also known as MMP-14, has been shown to also play a critical role in metastasis (Itoh, 2006). MMP-9 and 14 are transcriptional targets of Wnt signaling (Wu et al., 2007) and have been correlated with poor disease-free survival in OSA (Foukas et al., 2002; Kido et al., 1999; Heikkila et al., 2003; Uchibori et al., 2006).

The role of GSK-3β/NF-kB activity in the differentiation, proliferation, and apoptosis of the OSA

Glycogen synthase kinase-3 plays a central role in at least four of these signaling pathways-the Wnt, Notch, Hedgehog, and nuclear factor-κB (NF-κB) pathways-with important roles in at least six more-the ras/mitogen-associated protein kinase (RAS/MAPK), cyclic-AMP, transforming growth factor-β/activin (TGF-β), phosphatidylinositol-3-kinase (PI3K), jun kinase/stress-activated protein kinase (JNK/SAPK), and janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways (James, 2012; McNeill et al., 2010).

Glycogen synthase kinase-3β (GSK-3β), a serine/threonine protein kinase, may function as a tumor suppressor or an oncogene, depending on the tumor type. Expression of active GSK-3β had an oncogenic effect on OSA cells. Silencing or pharmacological inhibition of GSK-3β resulted in apoptosis of OSA cells. Inhibition of GSK-3β resulted in inhibition of the NF-κB pathway and reduction of NF-κB-mediated transcription (Hoeflich et al., 2000). Similar results with other studies, combination treatments with GSK-3β inhibitors, NF-κB inhibitors, and chemotherapy drugs increased the effectiveness of chemotherapy drugs in vitro and in vivo (Tang et al., 2012). Patients whose OSA specimens had hyperactive GSK-3β, and nuclear NF-κB had a shorter median overall survival time (49.2 months) compared with patients whose tumors had inactive GSK-3β and NF-κB (109.2 months) (Tang et al., 2012). GSK-3β activity may promote OSA tumor growth, and therapeutic targeting of the GSK-3β and/or NF-κB pathways may be an effective way to enhance the therapeutic activity of anticancer drugs against OSA. However, NF-kB inhibitors could suppress the growth of OSA in U2OS cell lines but not in MG 63 cell lines, and that GSK-3β inhibition could enhance this effect both in vitro and in vivo (Rengan et al., 2012). Suggesting that we must pay more attention to the characteristics of the subtypes of OSA.

In addition to regulated by GSK-3beta, mammalian Rap1 forms a complex with IKKs (IκB kinases), and is crucial for the ability of IKKs to be recruited to, and phosphorylate, the p65 subunit of NF-κB to make it transcriptionally competent. Rap1-mutant mice display defective NF-κB activation and are resistant to endotoxic shock. Furthermore, levels of Rap1 are positively regulated by NF-κB, and human breast cancers with NF-κB hyperactivity show elevated levels of cytoplasmic Rap1 (Hsiangling et al., 2010).

Direct inhibition of NF-κB by expression of a dominant negative IκB mutant or siRNA to the p65 subunit of NF-κB suppressed tumor cell growth, whereas silencing of IκB expression partially reversed the pro-apoptotic effects of lithium treatment (James, 2012). The nuclear factor-κB (NF-κB) transcription factor family has been considered the central mediator of the inflammatory process and a key participant in innate and adaptive immune responses. Coincident with the molecular cloning of NF-κB/RelA and identification of its kinship to the v-Rel oncogene, it was anticipated that NF-κB itself would be involved in cancer development. Oncogenic activating mutations in NF-κB genes are rare and have been identified only in some

lymphoid malignancies, while most NF-κB activating mutations in lymphoid malignancies occur in upstream signaling components that feed into NF-κB. NF-κB activation is also prevalent in carcinomas, in which NF-κB activation is mainly driven by inflammatory cytokines within the tumor microenvironment. Importantly, however, in all malignancies, NF-κB acts in a cell type-specific manner: activating survival genes within cancer cells and inflammation-promoting genes in components of the tumor microenvironment. Yet, the complex biological functions of NF-κB have made it an important therapeutic target.

CDKs play an import role in the pathogenesis of the OSA

Cyclin-dependent kinases (CDKs) are essential for cell cycle regulation and cell division. In the human OSA cell line U-2OS, combined depletion of CDK1 and CDK2 but not anyone alone by RNA silencing has been proved to arrest cells in G2-M phase and to induce apoptosis (Hu et al., 2001; Cai et al., 2006; Wei et al., 2011).

In the past years, a number of CDKs inhibitors have become available for clinical use (Senderowicz, 2003). One of the most studied and promising of these inhibitors is roscovitine. Roscovitine is a purine analogue which binds to the catalytic subunit of kinase proteins that mainly targets CDK1 and CDK2 together with other CDKs (Senderowicz, 2003). Preclinical studies have shown a relevant efficacy of roscovitine in a broad range of human tumor cell lines (Senderowicz, 2003) and have also demonstrated positive interactions with conventional chemotherapeutic agents (Crescenzi et al., 2005; Lambert et al., 2008). Therefore, roscovitine appears to act both as an inhibitor of tumor cell growth and as an enhancer of conventional drug activity, with a consequent decrease of their toxicity to normal tissues and an increase of their therapeutic index. Toward the goal of developing new treatment options for OSA, the cyclin-dependent kinase (CDK) inhibitor SCH 727965 (SCH) was used to induce the apoptosis of several OSA cell lines including those resistant to doxorubicin and dasatinib.

Gene transcription requires the activity of CDK7 and CDK9. These CDKs phosphorylate the large subunit of RNA polymerase II (RNAP II) at distinct sites in its C terminus to facilitate promoter clearance (CDK7-cyclin H) and elongation of nascent transcripts (CDK9-cyclin T) (Wei et al., 2011; Dongpo et al., 2006). Cell-cycle progression requires the activity of CDK4 and CDK6 (collectively referred to as CDK4/6), CDK2, and CDK1. CDK4/6 (with cyclin D1, D2 or D3) and CDK2 (with cyclin E) promote S-phase entry by phosphorylating and inactivating the retinoblastoma (Rb) protein; CDK2 (with cyclin A) and CDK1 (with cyclin A or cyclin B) propel cells through S phase and into mitosis, respectively (Chen et al., 1999; Santamarina et al., 2008; Scrace et al., 2008; Kim et al., 2011). SCH initiates the apoptosis of OSA by inactivating CDK1 and CDK2. The apoptotic proteins Bax and Bim accumulated in mitochondria-enriched fractions of SCH-treated cells, whereas amounts of the anti-apoptotic proteins Bcl-xL and Mcl-1 decreased (Wei et al., 2011). Suggesting that CDK inhibitors promote apoptosis by Bcl2-regulated mitochondrion cytochrome C releasing. Apoptosis induced by codepletion of CDK1 and CDK2 was less than that induced by SCH (Wei et al., 2011). This may reflect inactivation of additional targets by SCH or the presence of residual amounts of CDK1 and CDK2 in depleted cells. CDK1 and CDK2 can substitute for each other; thus, the need to suppress both to elicit apoptosis is not surprising. Suggesting that CDKs play an important role in the development of OSA by affecting many pathways, including cell cycle controlling, translation of Bcl-x, Mcl-1 and mitochondrion cytochrome C releasing.

R*egulation downstream of the p53 gene also take part in the development of the OSA*

Most chemotherapeutic agents induce apoptosis via the mitochondrial pathway (Strasser et al., 2000). The importance of p53 in the pathogenesis of the OSA was described previously. Regulators of this pathway include the Bcl-2 proteins and p53. There are 3 types of Bcl-2 proteins: anti-apoptotic (e.g., Bcl-xL and Mcl-1), single-domain apoptotic (termed as BH3-only) and multidomain apoptotic (e.g., Bax). When oligomerized, Bax perforates the outer mitochondrial membrane to release Cyt C. Anti-apoptotic proteins block Bax oligomerization; BH3-only proteins such as Bim facilitate Bax oligomerization. p53 accumulates in cells exposed to chemotoxic drugs and promotes apoptosis by 2 mechanisms. It transactivates genes that encoded apoptotic proteins, and it translocates to mitochondria where it interacts with Bcl-2 proteins (Moll et al., 2001). Many OSAs exhibit p53 abnormalities, and mice expressing p53-null osteoblast progenitor cells will develop OSAs (Lengner et al., 2006; Kansara et al., 2007). Suggesting that Bcl-2 proteins are important for the regulation of the p53 in the OSA.

Down regulation of PI3K/AKT pathway induce anoikis of OSA cells

The phosphatidylinositol-3-kinase (PI3K)/AKT pathway plays an important role in various cellular processes including cell growth, survival and motility (Vivanco et al., 2002; He et al., 2013; Gong et al., 2012). Recently, accumulating evidence indicated that PI3K/Akt pathway plays a crucial role in tumorigenesis and tumor progression by promoting cell proliferation and inhibiting apoptosis. AKT prevents apoptosis by activating anti-apoptotic signals through phosphorylating glycogen synthase kinase 3 (GSK3), Bad and caspase-9 and through activating transcriptional factors, such as forkhead (FOXO-1) and NF-kappa B (Yu et al., 2006; Cardone et al., 1998; Brunet et al., 1999; Romashkova et al., 1999). In addition, abnormal function of the PI3K/AKT pathway has been reported in many human tumors (Roy et al., 2002), including the OSA developed by PI3KCA gene mutation (Choy et al., 2012) and this signal pathway has been suggested to be a potential target for cancer chemotherapy. Suppressing the phosphorylation of Akt and its substrates FOXO transcription factor and GSK3 in OSA cells cause the suppression of proliferation and induction of mitochondria- and caspase-dependent apoptosis, induced the release of cytochrome c accompanied by activation of caspase-9, caspase-3 and cleavage of poly (ADP-ribose)

polymerase (PARP) (Jin et al., 2007).

Therefore, inhibition of PI3K/AKT pathway may be a novel target for treating OSA, and many studies in vitro ware done to discover potential medicines, for example, cyclooxygenase-2 inhibitor, induces apoptosis in human OSA cells via down-regulation of PI3K/Akt (Liu et al., 2008). BMI-1 is a member of the polycomb family of transcriptional regulators that was originally identified as an oncogenic partner of c-Myc in murine lymphomagenesis (van Lohuizen et al., 1991). BMI-1 was highly expressed in malignant OSA, and it is essential for cancer cell proliferation, migration and in vivo tumorigenicity (Wu et al., 2011). Inhibition of the PI3K/AKT pathway after BMI-1 knockdown was found to play a role in the sensitivity of SAOS-2 cells to cispatin treatment (Wu et al., 2011). BMI-1 could regulate the ratio of BCL-2 to Bid in SAOS-2 cells, which turn on to affect the apoptosis of OSA cells (Wu et al., 2011). Suggesting that PI3K/AKT pathway inhibition will induce apoptosis of OSA cells in many mechanisms.

GLI2 transcription factor accelerated the progression of OSA through Hedgehog pathway

The Hedgehog (Hh) pathway is a major regulator of many fundamental processes in vertebrate embryonic development including stem cell maintenance, cell differentiation, tissue polarity and cell proliferation. Paracrine Hh signaling from the tumor to the surrounding stroma was recently shown to promote tumorigenesis. This pathway has also been shown to regulate proliferation of cancer stem cells and to increase tumor invasiveness.

Binding of Hh to PTCH results in the loss of PTCH activity and the consequent activation of Smoothened (SMO) transmembrane receptor protein, which transduces the Hh signal to the cytoplasm (Taipale et al., 2002; Chen et al., 2002; Cooper et al., 1998). The Hh signal is transmitted via an alteration of the balance between the activator and repressor forms of the Ci (cubitus interruptus)/GLI family of zinc-finger transcription factors. In mammals, the Hh signaling takes place in the nonmotile cilia to which the SMO and other downstream pathway components must need to transit to activate the Ci ortholog in mammals, the GLI transcription factors (Huangfu et al., 2003; Corbit et al., 2005; Huangfu et al., 2005; Rubin et al., 2006). The GLI transcription factors exist as three separate zinc-finger proteins, GLI 1 and GLI 2 functioning as transcriptional activators and GLI 3 as a transcriptional repressor (Ruiz, 1997). GLI2 was aberrantly over-expressed in human OSA biopsy specimens (Hirotsu et al., 2010; Nagao et al., 2011). GLI2 knockdown by RNA interferences prevented OSA growth and anchorage-independent growth (Hirotsu et al., 2010; Nagao et al., 2011). Knockdown of GLI2 promoted the arrest of OSA cells in G (1) phase and was accompanied by reduced protein expression of the cell cycle accelerators cyclin D1, SKP2 and phosphorylated Rb (Nagao et al., 2011). On the other hand, knockdown of GLI2 increased the expression of p21 (cip1) (Hirotsu et al., 2010). In addition, over-expression of GLI2 promoted mesenchymal stem cell proliferation and accelerated their cell cycle progression. GLI2 knockdown inhibited the growth of OSA in nude mice (Nagao et al., 2011). Suggesting that inhibition of GLI2 represent an effective therapeutic approach for patients with OSA. Although all mechanisms of the Hh signaling pathway are not completely understood, it is clear that aberrant Hh signaling causes tumor growth and proliferation, increases tumor aggressiveness and raises the frequency of metastasis.

T*he role of SPHK1/ASK1/JNK/CHK1, 2 in the differentiation, proliferation and apoptosis of the OSA*

The lipid kinase sphingosine kinase 1 (SphK1) catalyzes the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P) (Shida et al., 2008; Vadas et al., 2008). In vivo and in vitro studies have proven that SphK1 is associated with cancer cell survival, proliferation, transformation, and prevention of apoptosis, the chemotherapy resistance and angiogenesis (Shida et al., 2008; Vadas et al., 2008). Evidence from clinical samples demonstrates that SphK1 is over-expressed in many tumor types and that inhibitors of SphK1 may sensitize tumors to chemotherapeutic agents (Shida et al., 2008; Vadas et al., 2008). SphK1 is over-expressed in multiple clinical OSA tissues. Over-expression of SphK1 in OSA cell line U2OS promoted its growth and endorsed its resistance against doxorubicin, while knocking-down of SphK1 by shRNA inhibited U2OS cell growth and increased its sensitivity to doxorubicin. Co-administration phenoxodiol with doxorubicin synergistically inhibited SphK1 activity to trigger cellular ceramide accumulation, and achieved synergistic anti-OSA growth effect, accompanied with a significant increased of apoptosis and cytotoxicity. Increased cellular level of ceramide by the co-administration induced the association between Akt and Protein Phosphatase 1 (PP1) to dephosphorylate Akt, and to introduce a constitutively active Akt (CA-Akt) restored Akt activation and diminished cell growth inhibition. Further, phenoxodiol and doxorubicin synergistically activated apoptosis signal-regulating kinase 1 (ASK1)/c-jun-NH2-kinase (JNK) signaling, which also contributed to cell growth inhibition. Significantly, the role of SphK1 in OSA cell growth and the synergistic anti-OSA effect of phenoxodiol and doxorubicin were also seen in a mice OSA xenograft model (Yao et al., 2012). Therefore, SphK1 might be a critical oncogene of OSA as we discussed previously and co-administration phenoxodiol with doxorubicin synergistically inhibited the activity of SphK1 to suppress OSA cell growth both in vivo and in vitro.

Therefore, sphk1 may affect the cell cycle via the regulation of the cell cycle checkpoint kinase, and then determine the fate of the cells. So sphk1 may be a good target for treating OSA.

The role of PEDF in the prevention of the OSA

PEDF expression changes in the course of progression of different tumor types (Halin et al., 2004). Researchers did a number of studies and showed that there is opposite relation between PEDF levels, grade and metastatic potential of prostate tumors (Halin et al., 2004), pancreatic adenocarcinoma (Uehara et al., 2004), prostate, melanoma, ovarian, OSA, glioma (Murray et al., 2010), hepatocellular carcinoma (Matsumoto et al., 2004) and

Wilm's tumors (Abramson et al., 2003). PEDF not only reduces angiogenesis, but also can increase tumor cell apoptosis and differentiation (Dawson et al., 1999; Maik-Rachline et al., 2005; Broadhead et al., 2009).

Pigmented epithelium-derived factor (PEDF) is a 50kDa glycoprotein, which is a member of the serine protease inhibitor family, and it has multifunctional properties (Murray et al., 2010) and is a potent inhibitor of angiogenesis, via its ability to decrease proliferation and migration of endothelial cells. It is found to be a potent inhibitor of angiogenesis, proliferation and migration of endothelial cells, retinal vascular permeability, and tumor activity (Broadhead et al., 2009). These significant antiangiogenic properties led the scientists to shift focus on to studies examining the potential antitumor activities of PEDF. Angiogenesis underlies the processes of bone growth, repair, and remodelling and may account at least in part for the aggressive nature of OSA. In vitro and in vivo studies have revealed that in the case of OSA, PEDF can induce both indirect and direct suppression of tumor growth and progression by potent antiangiogenic capability of PEDF targeting tumor vasculature and induction of OSA cell apoptosis, differentiation, and inhibition of cell cycling, respectively (Ek et al., 2006). And the resistance of epiphyseal cartilage to OSA invasion is likely to be due to the differential expression of PEDF and VEGF in the zones of the epiphysis (Ek et al., 2007).

In addition to the pathways discussed before, many studies have also reported a lot of novel pathway in the pathogenesis of OSA. For example, U0126 blocks MAPK/ERK signaling and decreases cell proliferation in OS (Sasaki et al., 2011; Yu et al., 2011). Even though ERK5 silencing did not suppress the proliferation of OS cells. However, ERK5 silencing significantly reduced the number of invading cells in invasion assay. The expression of MMP-9 was specifically reduced after silencing ERK5. The zymography showed that the enzyme activity of MMP-9 was also reduced after ERK5 suppression. The expression of ERK5 regulates the invasion of OS cells by inducing MMP-9 expression (Kim et al., 2012; Jin et al., 2013). Moreover, Mitochondria-specific ERK activation might provide a key advantage to tumor cells during the oncogenic process, by placing the death/survival mitochondrial rheostat in an anti-apoptotic mode. Mitochondrial ERK inhibition cause ATP depletion and apoptosis. Inhibition of ERK prompted block of ATP synthase and mitochondrial depolarization (Rasola et al., 2010).

Cell growth and differentiation are usually antagonistic. Proteins of the basic helix-loop-helix (bHLH) family bind DNA and play important roles in the differentiation of specific cell types. Id proteins heterodimerize with bHLH transcription factors, blocking their activation of lineage-specific gene expression and thereby inhibiting cellular differentiation. Id-2 expression was able to reverse the inhibition of cellular proliferation and the block in cell cycle progression mediated by the product of the retinoblastoma tumor suppressor gene pRB (Iavarone et al., 1994). Id proteins inhibit differentiation by HLH-mediated heterodimerization with basic HLH transcription factors (Florio et al., 1998). Enforced expression of Id3 but not Id2 caused the MG-63 sarcoma cells to be more sensitive to CDDP-induced growth inhibition, through generation of ROS and caspase-3 activation (Koyama et al., 2004). The down regulation of poly (ADP-ribosyl) ation of nuclear proteins (PARP) results in an increase in both the hypophosphorylated active form of Rb and pRb/E2F complexes (De Blasio et al., 2005). These effects are accompanied by G1 arrest, downregulation of gene products required for proliferation (cyclin D1, beta-catenin, c-Jun, c-Myc and Id2) and upregulation of those implicated in the osteoblastic differentiation (p21/Waf1, osteopontin, osteocalcin, type I collagen, N-cadherins and alkaline phosphatase). PTH induction of c-fos proto-oncogene transcription also appears to occur principally through activation of PKA that then targets CREB and the c-fos calcium/cAMP response element (Evans et al., 1996).

Mechanisms of OSA Resistance to Chemotherapy

Elucidation of the mechanisms of chemotherapy resistance and implementation of strategies to overcome it will be pivotal to improve the survival for OSA patients. Here we will give some important pathways in the pathogenesis of the OSA when discovered by finding treatment to OSA.

Mechanisms of the chemotherapy resistance by ABCB1 to doxorubicin

In the past 20 years, several studies have shown that OSA patients may be inherently resistant to doxorubicin or may become unresponsive to this drug during the chemotherapeutic treatment (Chou et al., 2006). Although resistance to doxorubicin in human tumor cells may be caused by different mechanisms, including increased efflux, more efficient intracellular detoxification, alterations of topo-isomerase II and increased DNA repair, the most relevant mechanism of doxorubicin resistance in OSA has been demonstrated to be the ATP-binding cassette (ABC) transporters mediated drug efflux (Chou et al., 2006). In particular, high expression of ABCB1 protein (also known as MDR1 or P-glycoprotein) has bee n demonstrated to be responsible for doxorubicin resistance in human OSA cell lines and to be associated with an adverse clinical outcome in high-grade, non-metastatic OSA patients treated with conventional chemotherapy protocols (Baldini et al., 1995; Chan et al., 1997; Pakos et al., 2003; Serra et al., 2003). However, a few studies did not confirm this evidence (Gorlick et al., 1999; Schwartz et al., 2007) and have been recently discussed (Serra et al., 2007). A possible strategy to overcome the clinical doxorubicin resistance may, therefore, be based on the use of ABC transporter inhibitors (in particular, of ABCB1), with the aim of reverting tumor cells toward a drug sensitive phenotype.

Reversion of chemotherapy resistance by inhibition of P-glycoprotein (P-gp) expression may overcome the chemotherapy resistance observed in many cancer types and may allow for improved therapeutic ratio. SiRNA specific for ABCB1 (MDR1) mRNA might restore

sensitivity to chemotherapy in tumor cell lines known to overexpress the MDR1 gene (Perez et al., 2011).

However, there are many other kinds of pathway or mechanisms that may affect the chemotherapy sensitivity, including Type-I insulin-like growth factor receptor (IGF1R), beta-catenin, calpain-6 levels (Luk et al., 2011; Zhang et al., 2011; Marion et al., 2012).

HMGB1-mediated autophagy as a novel therapeutic resistance for OSA

Autophagy is a catabolic process critical to maintaining cellular homeostasis and responding to cytotoxic insult. Autophagy is recognized as "programmed cell survival" in contrast to apoptosis or programmed cell death. Upregulation of autophagy has been observed in many types of cancers and has been demonstrated to both promote and inhibit antitumor drug resistance depending to a large extent on the nature and duration of the treatment-induced metabolic stress as well as the tumor type. Cisplatin, doxorubicin and methotrexate are commonly used anticancer drugs in OSA, the most common form of childhood and adolescent cancer. Inhibition of both HMGB1 and autophagy increase the drug sensitivity of OSA cells in vivo and in vitro (Huang et al., 2012).

The alkylating agents cisplatin and anthracycline, the antibiotic doxorubicin and the antimetabolite met hotrexate significantly increase protein and mRNA expression of HMGB1 in human p53-deficient OSA cell lines (e.g., MG-63 and SaOS-2). Aside from p53, the expression of HMGB1 is regulated by other transcription factors such as c-Myc and Kruppel-like factor (KLF)-4 in various cell types (Huang et al., 2012). Suggesting that targeting c-Myc and Kruppel-like factor (KLF)-4 may be a novel method to deal with the chemotherapy resistance of OSA.

The inflammasome regulates the release of caspase activation-dependent cytokines, including interleukin (IL)-1β, IL-18 and high-mobility group box 1 (HMGB1). PKR deficiency significantly inhibited the secretion of IL-1β, IL-18 and HMGB1 in E. coli-induced peritonitis (Lu et al., 2012). Anticancer agents including doxorubicin, cisplatin, and methotrexate each induced HMGB1 upregulation in human OSA cells, and RNA interference-mediated knockdown of HMGB1 restored the chemosensitivity of OSA cells in vivo and in vitro (Huang et al., 2012). Mechanistic investigation revealed that HMGB1 increased drug resistance by inducing autophagy, an intracellular self-defense mechanism known to confer drug resistance. HMGB1 bound to the autophagy regulator Beclin1 and regulated the formation of the Beclin1-PI3KC3 (PI3KC3, phosphatidylinositol-3-kinase class 3) complex that facilitates autophagic progression (Huang et al., 2012).

The human high mobility group protein B1 (HMGB1) has attracted considerable interest among oncologists because it sensitises cancer cells to the anticancer drug cisplatin by shielding cisplatin-DNA adducts from nucleotide excision repair (Pil et al., 1992; Zamble et al., 1996; He et al., 2000; Kartalou et al., 2001; Jung et al., 2003; Kasparkova et al., 2003).

High mobility group box 1 protein (HMGB1) is a significant contributor to drug resistance in OSA cells (Apetoh et al., 2004). Thus, these findings provide a novel mechanism of OSA resistance to therapy facilitated by HMGB1-mediated autophagy and provide a new target for the control of drug-resistant OSA patients.

What's more, overexpression of plasma membrane multi-drug resistance protein 1 (MRP-1) can lead to multidrug resistance. The expression of mitochondrial MRP-1 in untreated human normal and cancer cells and tissues was examined by differential centrifugation and western blotting and immunofluorescence microscopy. The efflux activity of mitochondrial MRP-1 was more efficient (55-64%) than that of plasma membrane MRP-1 (11-22%; $p<0.001$) (Roundhill et al., 2012). Induced MRP-1 expression resulted in a preferential increase in mitochondrial MRP-1, suggesting selective targeting to this organelle. Therefore, the mechanisms of chemotherapy resistance of the OSA ware not as simple as we discussed before. And only with more studies can we find out the details of mechanisms in the resistance of the OSA. And we believe it will not take a long time in the future.

Mechanisms of the OSA Metastasis

Classical high-grade OSA of the extremity has more of a tendency to metastasize, unlike low-grade parosteal OSAs. Primary OSA is a highly aggressive tumor that metastasizes by hematogenous dissemination. At diagnosis, nearly all patients will have microscopic metastases (Wolf et al., 1999). Despite resection and chemotherapy, 30%-40% of patients with localized disease will experience relapse, usually within 3 years (Longhi et al., 2006). Thus, it's very important for us to recover the mechanisms of metastatic of the OSA to help us improve the prognosis of the OSA. The lung is the most common site of metastatic disease, however, extrapulmonary sites are increasingly affected in treated patients. This may be because of change in the natural history of the disease by multiagent chemotherapy or longer survival times of these patients (Wolf et al., 1999; Kim et al., 2004; Akasbi et al., 2012). Novel strategies to improve treatment of metastatic patients require a better understanding of the processes involved, like angiogenesis, migration and the immune response.

Whole genome expression analysis of both the cells and the host showed that angiogenesis and migration-related genes matrix metalloproteinase 19 (Mmp-19) and erythroblastosis virus E26 oncogene homologue 1 (Ets-1) were overexpressed in transformed MSCs compared to normal MSCs (Mohseny et al., 2012).

mRNA expression microarray and N-linked glycoproteomic analyses were performed on two commonly used isogenic pairs of human metastatic OSA cell lines, namely HOS/143B and SaOS-2/LM7 (Flores et al., 2012). CCL5 has been reported to stimulate directional migration and invasion of human cancer cells (Huang et al., 2009; Kulbe et al., 2004). CCL5 directed human OSA cell migration (U2OS and MG63 cells) (Wang et al., 2012). They also found that CCL5 increased invasive ability of human OSA cells through Matrigel basement

membrane matrix (Wang et al., 2012). Interaction of CCL5 with its specific receptor CCR on the surface of cancer cells has been reported to induce cancer migration (Luboshits et al., 1999; Chuang et al., 2009). Therefore, CCL5 and CCR5 interaction is very important in migration activity of OSA cells. According to their experiments results (Wang et al., 2012), αvβ3 integrin up-regulation, activated MEK and ERK signaling pathways and NF-κB activation are involved in the CCL5-mediated migration of the OSA cell lines cells.

Investigating the host response, embryos injected with transformed MSCs showed decreased expression of immune response-related genes, especially major histocompatibility complex class 1 (mhc1ze), as compared to embryos injected with normal MSCs (Mohseny et al., 2012).

The biological markers CXCR4, HER2 and CD44 are also involved in tumor growth and the homing of cancer cells to distant site s (Ma et al., 2012). CCN3 influence the ability of metastatic cancers to colonize and grow in bone (Ouellet et al., 2012).

Summary

Past years have brought many new concepts regarding the pathogenesis and biology of metastatic OSA tumors. Some of them have been well studied, confirmed and widely accepted. Others are still under investigation. Combining the findings of studies concerning different mechanisms in the process of neoplastic cell dissemination and tumor growth prompts us to make new steps in our way of thinking about malignancy and is a prerequisite for improving treatment results.

Localised OSA will be cured in 50% of patients with cisplatin and doxorubicin (Whelan et al., 2012). Large randomised trials can be conducted in this rare cancer. Failure to improve survival over 20 years argues for concerted collaborative international efforts to identify and rapidly test new treatments.

References

Abarzua F, Sakaguchi M, Takaishi M, et al (2005). Adenovirus-mediated overexpression of REIC/Dkk-3 selectively induces apoptosis in human prostate cancer cells through activation of c-Jun-NH2-kinase. *Cancer Res*, **65**, 9617-22.

Abramson LP, Stellmach V, Doll JA, et al (2003). Wilms' tumor growth is suppressed by antiangiogenic pigment epithelium-derived factor in a xenograft model. *J Pediatr Surg*, **38**, 336-42.

Akasbi Y, Arifi S, Lahlaidi K, et al (2012). Renal metastases of a femur osteosarcoma: a case report and a review of the literature. *Case Rep Urol*, **2012**, 2591-3.

Apetoh L, Ghiringhelli F, Tesniere A, et al (2007). Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med*, **13**, 1050-9.

Bacci G, Rocca M, Salone M, et al (2008). High grade osteosarcoma of the extremities with lung metastases at presentation: treatment with neoadjuvant chemotherapy and simultaneous resection of primary and metastatic lesions. *J Surg Oncol*, **98**, 415-20.

Baldini N, Scotlandi K, Barbanti-Brodano G, et al (1995). Expression of P-glycoprotein in high-grade osteosarcomas in relation to clinical outcome. *N Engl J Med*, **333**, 1380-5.

Barker N, Clevers H (2006). Mining the Wnt pathway for cancer therapeutics. *Nat Rev Drug Discov*, **5**, 997-1014.

Bielack SS, Carrle D, Hardes J, et al (2008). Bone tumors in adolescents and young adults. *Curr Treat Options Oncol*, **9**, 67-80.

Broadhead ML, Dass CR, Choong PF (2009). In vitro and in vivo biological activity of PEDF against a range of tumors. *Expert Opin Ther Targets*, **13**, 1429-38.

Brunet A, Bonni A, Zigmond MJ, et al (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell*, **96**, 857-68.

Cai D, Latham VM, Jr, Zhang X, et al (2006). Combined depletion of cell cycle and transcriptional cyclin-dependent kinase activities induces apoptosis in cancer cells. *Cancer Res*, **66**, 9270-80.

Cardone MH, Roy N, Stennicke HR, et al (1998). Regulation of cell death protease caspase-9 by phosphorylation. *Science*, **282**, 1318-21.

Chan HS, Grogan TM, Haddad G, et al (1997). P-glycoprotein expression: critical determinant in the response to osteosarcoma chemotherapy. *J Natl Cancer Inst*, **89**, 1706-15.

Chen JK, Taipale J, Cooper MK, et al (2002). Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev*, **16**, 2743-8.

Chen YN, Sharma SK, Ramsey TM, et al (1999). Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. *Proc Natl Acad Sci USA*, **96**, 4325-9.

Chou AJ, Gorlick R (2006). Chemotherapy resistance in osteosarcoma: current challenges and future directions. *Expert Rev Anticancer Ther*, **6**, 1075-85.

Choy E, Hornicek F, MacConaill L, et al (2012). High-throughput genotyping in osteosarcoma identifies multiple mutations in phosphoinositide-3-kinase and other oncogenes. *Cancer*, **118**, 2905-14.

Chuang JY, Yang WH, Chen HT, et al (2009). CCL5/CCR5 axis promotes the motility of human oral cancer cells. *J Cell Physiol*, **220**, 418-26.

Cooper MK, Porter JA, Young KE, et al (1998). Teratogen-mediated inhibition of target tissue response to Shh signaling. *Science*, **280**, 1603-7.

Corbit KC, Aanstad P, Singla V, et al (2005). Vertebrate Smoothened functions at the primary cilium. *Nature*, **437**, 1018-21.

Crescenzi E, Palumbo G, Brady HJ (2005). Roscovitine modulates DNA repair and senescence: implications for combination chemotherapy. *Clin Cancer Res*, **11**, 8158-71.

Dawson DW, Volpert OV, Gillis P, et al (1999). Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science*, **285**, 245-8.

De Blasio A, Messina C, Santulli A, et al (2005). Differentiative pathway activated by 3-aminobenzamide, an inhibitor of PARP, in human osteosarcoma MG-63 cells. *FEBS Lett*, **579**, 615-20.

Dieudonne FX, Marion A, Marie PJ, et al (2012). Targeted inhibition of T-cell factor activity promotes syndecan-2 expression and sensitization to doxorubicin in osteosarcoma cells and bone tumors in mice. *J Bone Miner Res*, **27**, 2118-29.

Dorfman HD, Czerniak B (1995). Bone cancers. *Cancer*, **75**, 203-10.

Ek ET, Dass CR, Choong PF (2006). PEDF: a potential molecular therapeutic target with multiple anti-cancer activities. *Trends Mol Med*, **12**, 497-502.

Ek ET, Dass CR, Choong PF (2006). Pigment epithelium-derived factor: a multimodal tumor inhibitor. *Mol Cancer Ther*, **5**, 1641-6.

Ek ET, Dass CR, Contreras KG, et al (2007). Pigment epithelium-derived factor overexpression inhibits orthotopic osteosarcoma growth, angiogenesis and metastasis. *Cancer Gene Ther*, **14**, 616-26.

Evans DB, Hipskind RA, Bilbe G (1996). Analysis of signaling pathways used by parathyroid hormone to activate the c-fos gene in human SaOS2 osteoblast-like cells. *J Bone Miner Res*, **11**, 1066-74.

Ferrari S, Smeland S, Mercuri M, et al (2005). Neoadjuvant chemotherapy with high-dose Ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. *J Clin Oncol*, **23**, 8845-52.

Flores RJ, Li Y, Yu A, et al (2012). A systems biology approach reveals common metastatic pathways in osteosarcoma. *BMC Syst Biol*, **6**, 50.

Florio M, Hernandez MC, Yang H, et al (1998). Id2 promotes apoptosis by a novel mechanism independent of dimerization to basic helix-loop-helix factors. *Mol Cell Biol*, **18**, 5435-44.

Foukas AF, Deshmukh NS, Grimer RJ, et al (2002). Stage-IIB osteosarcomas around the knee. A study of MMP-9 in surviving tumour cells. *J Bone Joint Surg Br*, **84**, 706-11.

Fu W, Ma L, Chu B, et al (2011). The cyclin-dependent kinase inhibitor SCH 727965 (dinacliclib) induces the apoptosis of osteosarcoma cells. *Mol Cancer Ther*, **10**, 1018-27.

Gong C, Liao H, Wang J, et al (2012). LY294002 induces G0/G1 cell cycle arrest and apoptosis of cancer stem-like cells from human osteosarcoma via down-regulation of PI3K activity. *Asian Pac J Cancer Prev*, **13**, 3103-7.

Gorlick R, Huvos AG, Heller G, et al (1999). Expression of HER2/erbB-2 correlates with survival in osteosarcoma. *J Clin Oncol*, **17**, 2781-8.

Guo Y, Xie J, Rubin E, et al (2008). Frzb, a secreted Wnt antagonist, decreases growth and invasiveness of fibrosarcoma cells associated with inhibition of Met signaling. *Cancer Res*, **68**, 3350-60.

Gurney A, Axelrod F, Bond CJ, et al (2012). Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci USA*, **109**, 11717-22.

Halin S, Wikstrom P, Rudolfsson SH, et al (2004). Decreased pigment epithelium-derived factor is associated with metastatic phenotype in human and rat prostate tumors. *Cancer Res*, **64**, 5664-71.

Harting MT, Blakely ML (2006). Management of osteosarcoma pulmonary metastases. *Semin Pediatr Surg*, **15**, 25-9.

Haydon RC, Deyrup A, Ishikawa A, et al (2002). Cytoplasmic and/or nuclear accumulation of the beta-catenin protein is a frequent event in human osteosarcoma. *Int J Cancer*, **102**, 338-42.

He ML, Wu Y, Zhao JM, et al (2013). PIK3CA and AKT gene polymorphisms in susceptibility to osteosarcoma in a Chinese population. *Asian Pac J Cancer Prev*, **14**, 5117-22.

He Q, Liang CH, Lippard SJ (2000). Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin. *Proc Natl Acad Sci USA*, **97**, 5768-72.

Heikkila P, Teronen O, Hirn MY, et al (2003). Inhibition of matrix metalloproteinase-14 in osteosarcoma cells by clodronate. *J Surg Res*, **111**, 45-52.

Hengartner MO (2000). The biochemistry of apoptosis. *Nature*, **407**, 770-6.

Himelstein BP, Asada N, Carlton MR, et al (1998). Matrix metalloproteinase-9 (MMP-9) expression in childhood osseous osteosarcoma. *Med Pediatr Oncol*, **31**, 471-4.

Hirotsu M, Setoguchi T, Sasaki H, et al (2010). Smoothened as a new therapeutic target for human osteosarcoma. *Mol Cancer*, **9**, 5.

Hoeflich KP, Luo J, Rubie EA, et al (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature*, **406**, 86-90.

Hsieh JC, Kodjabachian L, Rebbert ML, et al (1999). A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature*, **398**, 431-6.

Hu B, Mitra J, van den Heuvel S, et al (2001). S and G2 phase roles for Cdk2 revealed by inducible expression of a dominant-negative mutant in human cells. *Mol Cell Biol*, **21**, 2755-66.

Huang CY, Fong YC, Lee CY, et al (2009). CCL5 increases lung cancer migration via PI3K, Akt and NF-kappaB pathways. *Biochem Pharmacol*, **77**, 794-803.

Huang J, Liu K, Yu Y, et al (2012). Targeting HMGB1-mediated autophagy as a novel therapeutic strategy for osteosarcoma. *Autophagy*, **8**, 275-7.

Huang J, Ni J, Liu K, et al (2012). HMGB1 promotes drug resistance in osteosarcoma. *Cancer Res*, **72**, 230-8.

Huangfu D, Anderson KV (2005). Cilia and Hedgehog responsiveness in the mouse. *Proc Natl Acad Sci USA*, **102**, 11325-30.

Huangfu D, Liu A, Rakeman AS, et al (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature*, **426**, 83-7.

Hughes DP (2009). Strategies for the targeted delivery of therapeutics for osteosarcoma. *Expert Opin Drug Deliv*, **6**, 1311-21.

Iavarone A, Garg P, Lasorella A, et al (1994). The helix-loop-helix protein Id-2 enhances cell proliferation and binds to the retinoblastoma protein. *Genes Dev*, **8**, 1270-84.

Itoh Y (2006). MT1-MMP: a key regulator of cell migration in tissue. *IUBMB Life*, **58**, 589-96.

Jin J, Cai L, Liu ZM, et al (2013). miRNA-218 inhibits osteosarcoma cell migration and invasion by down-regulating of TIAM1, MMP2 and MMP9. *Asian Pac J Cancer Prev*, **14**, 3681-4.

Jin S, Pang RP, Shen JN, et al (2007). Grifolin induces apoptosis via inhibition of PI3K/AKT signalling pathway in human osteosarcoma cells. *Apoptosis*, **12**, 1317-26.

Jung Y, Lippard SJ (2003). Nature of full-length HMGB1 binding to cisplatin-modified DNA. *Biochemistry*, **42**, 2664-71.

Kager L, Zoubek A, Potschger U, et al (2003). Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. *J Clin Oncol*, **21**, 2011-8.

Kansara M, Thomas DM (2007). Molecular pathogenesis of osteosarcoma. *DNA Cell Biol*, **26**, 1-18.

Kartalou M, Essigmann JM (2001). Recognition of cisplatin adducts by cellular proteins. *Mutat Res*, **478**, 1-21.

Kasparkova J, Delalande O, Stros M, et al (2003). Recognition of DNA interstrand cross-link of antitumor cisplatin by HMGB1 protein. *Biochemistry*, **42**, 1234-44.

Kawano Y, Kypta R (2003). Secreted antagonists of the Wnt signalling pathway. *J Cell Sci*, **116**, 2627-34.

Kido A, Tsutsumi M, Iki K, et al (1999). Overexpression of matrix metalloproteinase (MMP)-9 correlates with metastatic potency of spontaneous and 4-hydroxyaminoquinoline 1-oxide (4-HAQO)-induced transplantable osteosarcomas in rats. *Cancer Lett*, **137**, 209-16.

Kim MR, Zhou L, Park BH, et al (2011). Induction of G (2)/M arrest and apoptosis by sulforaphane in human osteosarcoma U2-OS cells. *Mol Med Rep*, **4**, 929-34.

Kim SJ, Choi JA, Lee SH, et al (2004). Imaging findings of extrapulmonary metastases of osteosarcoma. *Clin Imaging*, **28**, 291-300.

Kim SM, Lee H, Park YS, et al (2012). ERK5 regulates invasiveness of osteosarcoma by inducing MMP-9. *J Orthop Res*, **30**, 1040-4.

Koyama T, Suzuki H, Imakiire A, et al (2004). Id3-mediated enhancement of cisplatin-induced apoptosis in a sarcoma cell line MG-63. *Anticancer Res*, **24**, 1519-24.

Kulbe H, Levinson NR, Balkwill F, et al (2004). The chemokine network in cancer-much more than directing cell movement. *Int J Dev Biol*, **48**, 489-96.

Lambert LA, Qiao N, Hunt KK, et al (2008). Autophagy: a novel mechanism of synergistic cytotoxicity between doxorubicin and roscovitine in a sarcoma model. *Cancer Res*, **68**, 7966-74.

Lengner CJ, Steinman HA, Gagnon J, et al (2006). Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling. *J Cell Biol*, **172**, 909-21.

Lin YC, You L, Xu Z, et al (2006). Wnt signaling activation and WIF-1 silencing in nasopharyngeal cancer cell lines. *Biochem Biophys Res Commun*, **341**, 635-40.

Liu B, Shi ZL, Feng J, et al (2008). Celecoxib, a cyclooxygenase-2 inhibitor, induces apoptosis in human osteosarcoma cell line MG-63 via down-regulation of PI3K/Akt. *Cell Biol Int*, **32**, 494-501.

Longhi A, Errani C, De Paolis M, et al (2006). Primary bone osteosarcoma in the pediatric age: state of the art. *Cancer Treat Rev*, **32**, 423-36.

Lu B, Nakamura T, Inouye K, et al (2012). Novel role of PKR in inflammasome activation and HMGB1 release. *Nature*, **488**, 670-4.

Luboshits G, Shina S, Kaplan O, et al (1999). Elevated expression of the CC chemokine regulated on activation, normal T cell expressed and secreted (RANTES) in advanced breast carcinoma. *Cancer Res*, **59**, 4681-7.

Luk F, Yu Y, Walsh WR, et al (2011). IGF1R-targeted therapy and its enhancement of doxorubicin chemosensitivity in human osteosarcoma cell lines. *Cancer Invest*, **29**, 521-32.

Ma Q, Zhou Y, Ma B, et al (2012). The clinical value of CXCR4, HER2 and CD44 in human osteosarcoma: A pilot study. *Oncol Lett*, **3**, 797-801.

Maik-Rachline G, Shaltiel S, Seger R (2005). Extracellular phosphorylation converts pigment epithelium-derived factor from a neurotrophic to an antiangiogenic factor. *Blood*, **105**, 670-8.

Mankin HJ, Hornicek FJ, Rosenberg AE, et al (2004). Survival data for 648 patients with osteosarcoma treated at one institution. *Clin Orthop Relat Res*, **429** 286-91.

Marion A, Dieudonne FX, Patino-Garcia A, et al (2012). Calpain-6 is an endothelin-1 signaling dependent protective factor in chemoresistant osteosarcoma. *Int J Cancer*, **130**, 2514-25.

Matsubara K, Fukushima S, Akane A, et al (1992). Increased urinary morphine, codeine and tetrahydropapaveroline in parkinsonian patient undergoing L-3, 4-dihydroxyphenylalanine therapy: a possible biosynthetic pathway of morphine from L-3, 4-dihydroxyphenylalanine in humans. *J Pharmacol Exp Ther*, **260**, 974-8.

Matsumoto K, Ishikawa H, Nishimura D, et al (2004). Antiangiogenic property of pigment epithelium-derived factor in hepatocellular carcinoma. *Hepatology*, **40**, 252-9.

McNeill H, Woodgett JR (2010). When pathways collide: collaboration and connivance among signalling proteins in development. *Nat Rev Mol Cell Biol*, **11**, 404-13.

Messerschmitt PJ, Garcia RM, Abdul-Karim FW, et al (2009). Osteosarcoma. *J Am Acad Orthop Surg*, **17**, 515-27.

Meyer B, Murua Escobar H, Hauke S, et al (2004). Expression pattern of the HMGB1 gene in sarcomas of the dog. *Anticancer Res*, **24**, 707-10.

Mialou V, Philip T, Kalifa C, et al (2005). Metastatic osteosarcoma at diagnosis: prognostic factors and long-term outcome-the French pediatric experience. *Cancer*, **104**, 1100-9.

Mirabello L, Troisi RJ, Savage SA (2009). Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer*, **115**, 1531-43.

Mohseny AB, Xiao W, Carvalho R, et al (2012). An osteosarcoma zebrafish model implicates Mmp-19 and Ets-1 as well as reduced host immune response in angiogenesis and migration. *J Pathol*, **227**, 245-53.

Moll UM, Zaika A (2001). Nuclear and mitochondrial apoptotic pathways of p53. *FEBS Lett*, **493**, 65-9.

Murray AR, Ma JX (2010). PEDF as a treatment for cervical cancer. *Cancer Biol Ther*, **9**, 975-7.

Nagao H, Ijiri K, Hirotsu M, et al (2011). Role of GLI2 in the growth of human osteosarcoma. *J Pathol*, **224**, 169-79.

Nelson AR, Fingleton B, Rothenberg ML, et al (2000). Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol*, **18**, 1135-49.

Ouellet V, Siegel PM (2012). CCN3 modulates bone turnover and is a novel regulator of skeletal metastasis. *J Cell Commun Signal*, **6**, 73-85.

Pakos EE, Ioannidis JP (2003). The association of P-glycoprotein with response to chemotherapy and clinical outcome in patients with osteosarcoma. A meta-analysis. *Cancer*, **98**, 581-9.

Perez J, Bardin C, Rigal C, et al (2011). Anti-MDR1 siRNA restores chemosensitivity in chemoresistant breast carcinoma and osteosarcoma cell lines. *Anticancer Res*, **31**, 2813-20.

Pil PM, Lippard SJ (1992). Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. *Science*, **256**, 234-7.

Rasola A, Sciacovelli M, Chiara F, et al (2010). Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition. *Proc Natl Acad Sci USA*, **107**, 726-31.

Rengan R, Mick R, Pryma D, et al (2012). A phase I trial of the HIV protease inhibitor nelfinavir with concurrent chemoradiotherapy for unresectable stage IIIA/IIIB non-small cell lung cancer: a report of toxicities and clinical response. *J Thorac Oncol*, **7**, 709-15.

Ritter J, Bielack SS (2010). Osteosarcoma. *Ann Oncol*, **21**, 320-5.

Romashkova JA, Makarov SS (1999). NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature*, **401**, 86-90.

Roundhill EA, Burchill SA (2012). Detection and characterisation of multi-drug resistance protein 1 (MRP-1) in human mitochondria. *Br J Cancer*, **106**, 1224-33.

Roy HK, Olusola BF, Clemens DL, et al (2002). AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. *Carcinogenesis*, **23**, 201-5.

Rubin EM, Guo Y, Tu K, et al (2010). Wnt inhibitory factor 1 decreases tumorigenesis and metastasis in osteosarcoma. *Mol Cancer Ther*, **9**, 731-41.

Rubin LL, de Sauvage FJ (2006). Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov*, **5**, 1026-33.

Ruiz i Altaba A (1997). Catching a Gli-mpse of Hedgehog. *Cell*, **90**, 193-6.

Santamarina M, Hernandez G, Zalvide J (2008). CDK redundancy guarantees cell cycle progression in Rb-negative tumor cells independently of their p16 status. *Cell Cycle*, **7**, 1962-72.

Sasaki K, Hitora T, Nakamura O, et al (2011). The role of

MAPK pathway in bone and soft tissue tumors. *Anticancer Res*, **31**, 549-53.
Schwartz CL, Gorlick R, Teot L, et al (2007). Multiple drug resistance in osteogenic sarcoma: INT0133 from the Children's Oncology Group. *J Clin Oncol*, **25**, 2057-62.
Scrace SF, Kierstan P, Borgognoni J, et al (2008). Transient treatment with CDK inhibitors eliminates proliferative potential even when their abilities to evoke apoptosis and DNA damage are blocked. *Cell Cycle*, **7**, 3898-907.
Senderowicz AM (2003). Small-molecule cyclin-dependent kinase modulators. *Oncogene*, **22**, 6609-20.
Serra M, Picci P, Ferrari S, et al (2007). Prognostic value of P-glycoprotein in high-grade osteosarcoma. *J Clin Oncol*, **25**, 4858-60.
Serra M, Scotlandi K, Reverter-Branchat G, et al (2003). Value of P-glycoprotein and clinicopathologic factors as the basis for new treatment strategies in high-grade osteosarcoma of the extremities. *J Clin Oncol*, **21**, 536-42.
Shida D, Takabe K, Kapitonov D, et al (2008). Targeting SphK1 as a new strategy against cancer. *Curr Drug Targets*, **9**, 662-73.
Smeland S, Muller C, Alvegard TA, et al (2003). Scandinavian Sarcoma Group Osteosarcoma Study SSG VIII: prognostic factors for outcome and the role of replacement salvage chemotherapy for poor histological responders. *Eur J Cancer*, **39**, 488-94.
Strasser A, O'Connor L, Dixit VM (2000). Apoptosis signaling. *Annu Rev Biochem*, **69**, 217-45.
T L, L FH, Ak R, Knigge U, U FR (2014). Primary hyperparathyroidism in young people. When should we perform genetic testing for multiple endocrine neoplasia 1 (MEN-1)? *J Clin Endocrinol Metab*, (in press).
Taipale J, Cooper MK, Maiti T, et al (2002). Patched acts catalytically to suppress the activity of Smoothened. *Nature*, **418**, 892-7.
Tang QL, Xie XB, Wang J, et al (2012). Glycogen synthase kinase-3beta, NF-kappaB signaling, and tumorigenesis of human osteosarcoma. *J Natl Cancer Inst*, **104**, 749-63.
Teo H, Ghosh S, Luesch H, et al (2010). Telomere-independent Rap1 is an IKK adaptor and regulates NF-kappaB-dependent gene expression. *Nat Cell Biol*, **12**, 758-67.
Uchibori M, Nishida Y, Nagasaka T, et al (2006). Increased expression of membrane-type matrix metalloproteinase-1 is correlated with poor prognosis in patients with osteosarcoma. *Int J Oncol*, **28**, 33-42.
Uehara H, Miyamoto M, Kato K, et al (2004). Expression of pigment epithelium-derived factor decreases liver metastasis and correlates with favorable prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer Res*, **64**, 3533-7.
Vadas M, Xia P, McCaughan G, et al (2008). The role of sphingosine kinase 1 in cancer: oncogene or non-oncogene addiction? *Biochim Biophys Acta*, **1781**, 442-7.
van Lohuizen M, Verbeek S, Scheijen B, et al (1991). Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. *Cell*, **65**, 737-52.
Vivanco I, Sawyers CL (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*, **2**, 489-501.
Wang SW, Wu HH, Liu SC, et al (2012). CCL5 and CCR5 interaction promotes cell motility in human osteosarcoma. *PLoS One*, **7**, e35101.
Weeraratna AT, Jiang Y, Hostetter G, et al (2002). Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell*, **1**, 279-88.
Whelan JS, Jinks RC, McTiernan A, et al (2012). Survival from high-grade localised extremity osteosarcoma: combined results and prognostic factors from three European Osteosarcoma Intergroup randomised controlled trials. *Ann Oncol*, **23**, 1607-16.
Wissmann C, Wild PJ, Kaiser S, et al (2003). WIF1, a component of the Wnt pathway, is down-regulated in prostate, breast, lung, and bladder cancer. *J Pathol*, **201**, 204-12.
Wolf R, Wolf RF, Hoekstra HJ (1999). Recurrent, multiple, calcified soft tissue metastases from osteogenic sarcoma without pulmonary involvement. *Skeletal Radiol*, **28**, 710-3.
Woodgett JR (2012). Can a two-faced kinase be exploited for osteosarcoma? *J Natl Cancer Inst*, **104**, 722-3.
Wu B, Crampton SP, Hughes CC (2007). Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration. *Immunity*, **26**, 227-39.
Wu Z, Min L, Chen D, et al (2011). Overexpression of BMI-1 promotes cell growth and resistance to cisplatin treatment in osteosarcoma. *PLoS One*, **6**, e14648.
Yao C, Wu S, Li D, et al (2012). Co-administration phenoxodiol with doxorubicin synergistically inhibit the activity of sphingosine kinase-1 (SphK1), a potential oncogene of osteosarcoma, to suppress osteosarcoma cell growth both in vivo and in vitro. *Mol Oncol*, **6**, 392-404.
Yu W, Shun MC, Anderson K, et al (2006). alpha-TEA inhibits survival and enhances death pathways in cisplatin sensitive and resistant human ovarian cancer cells. *Apoptosis*, **11**, 1813-23.
Yu Y, Luk F, Yang JL, et al (2011). Ras/Raf/MEK/ERK pathway is associated with lung metastasis of osteosarcoma in an orthotopic mouse model. *Anticancer Res*, **31**, 1147-52.
Zamble DB, Mu D, Reardon JT, et al (1996). Repair of cisplatin--DNA adducts by the mammalian excision nuclease. *Biochemistry*, **35**, 10004-13.
Zhang F, Chen A, Chen J, et al (2011). SiRNA-mediated silencing of beta-catenin suppresses invasion and chemosensitivity to doxorubicin in MG-63 osteosarcoma cells. *Asian Pac J Cancer Prev*, **12**, 239-45.
Zi X, Guo Y, Simoneau AR, et al (2005). Expression of Frzb/secreted Frizzled-related protein 3, a secreted Wnt antagonist, in human androgen-independent prostate cancer PC-3 cells suppresses tumor growth and cellular invasiveness. *Cancer Res*, **65**, 9762-70.