Basic and Translational Research in Osteosarcoma: A Year in Review

Le Chang1, Carolyn A Meyers1, Greg Asatrian2, Jia Shen2, Michelle A Scott3 and Aaron W James1*

1Department of Pathology and Laboratory Medicine, University of California, USA
2Division of Growth and Development, School of Dentistry, University of California, USA
3Nationwide Children’s Hospital, Columbus, USA

Introduction

Osteosarcoma (OS) is the most common primary bone malignancy, predominantly afflicting children and adolescents. Despite advances in multimodal treatment, the 5 year survival rate remains at 60-70% and [1] the outcomes for metastatic disease remain poor. A lack of scientific development of targeted treatments can be attributed to the high level of genetic and cytogenetic heterogeneity within OS [2]. This brief review highlights recent advances in both basic and translational research relevant to OS in the following selected areas of: immune therapy, miRNA expression, long non-coding RNA, and novel growth factors.

Immune Therapy for Osteosarcoma

Cancer surveillance by the immune system is accomplished through the presence of antigens on cancer cells, distinguishing them from non-neoplastic cells. This tumor 'neoantigens' are recognized by the endogenous immune system and targeted for destruction. However, neoplastic cells are able to evade this immune suppression through three phases: elimination, equilibrium and escape. Mechanisms include loss of tumor antigens, downregulation of Histocompatibility Locus Antigens (HLA) and alteration of immune regulation through regulatory T cells and MDSCs (Myeloid-Derived Suppressor Cells). Therefore, reintroduction of immune control to bypass tumor cell evasion of immune surveillance presents potential for the development of novel OS therapies. Currently, studies on immune therapy that have used various immune modulating methods to increase surveillance of tumors are promising, as tumors with a significant mutation burden are particularly susceptible to heightened immune response [3].

An important area of OS immunotherapy is in the treatment of metastatic disease, as outcomes remain poor: 5-year survival is around 20% for adolescents and even less for older patients [1]. Metastatic OS becomes resistant to CD8+ T cell-mediated responses due to the upregulation of Programmed Death Receptor-1 (PD-1) and its interaction with the (PD-L1) tumor cell ligand. PD-1 acts as a key co-inhibitory receptor expressed on activated T cells, B cells, macrophages, dendritic cells and monocytes. The primary ligand for PD-1, PD-L1, is upregulated in metastatic solid OS tumors and inhibits cytokine production as well as PD-1+ CD4+ and CD8+ T cell activity. These inhibitory receptors effectively attenuate tumor-reactive T cell cytokine toxicity to the tumor. Using immunotherapy, a novel study in metastatic OS inhibited the PD-L1 ligand by using α-PD-L1 monoclonal antibodies. Lussier, et al. tested this concept in mice by implanting K7M2 tumor cells and they found that mice treated with α-PD-L1 monoclonal antibodies showed increased survival with fewer pulmonary metastases than the control. However, the mice ultimately succumbed to pulmonary metastases, which remained resistant to PD-L1 antibody therapy [4]. Therefore, they focused on overcoming the resistance of metastatic K7M2 OS cells to PD-L1 antibody therapy. Other investigators, such as Curran, et al. combined antitumor immunotherapy with α-CTLA-4 and α-PD-L1 mAb treatment in an implantable B16 melanoma mouse model and found a two-fold increase in tumor rejection [5]. CTLA-4 is another T cell checkpoint, which increases tumor tolerance by downregulating T cell activation [6]. In a further study, Lussier, et al. found that combination α-CTLA-4 and α-PD-L1 mAb treatment resulted in a synergistic effect with complete control of tumors in 60% of mice, leading to long-term survival, as compared to 0% with α-PD-L1 mAb treatment alone [7]. Additionally, mice with controlled OS were introduced with additional K7M2 cells 100 days post-initial inoculation and demonstrated complete immunity for an additional 80 days until sacrifice [7]. Therefore, combinatorial immunotherapy shows potential for increased efficacy of T cell restoration to improve outcomes in metastatic OS tumor patients and prevent tumor recurrence.

Other studies on antitumor immune response examine the effect of dendritic cells and Anti-Glucocorticoid-Induced Tumor Necrosis Factor Receptor (anti-GITR) antibodies in enhancing...
adaptive immunity. Dendritic cells have the unique ability to potentiate primary and secondary immune responses and are used as a vaccine in multiple murine tumor models. Using pure dendritic cell therapy, Fang et al. treated Sprague Dawley rats with a dendritic cell-osteosarcoma fusion tumor vaccine and both the allograft and homograft groups displayed atrophy or disappearance of tumor [8]. Another antitumor factor, Glucocorticoid-Induced Tumor Necrosis Factor Related Protein, (GITR) is a co-stimulatory molecule of the TNF receptor family expressed on activated T cells, B cells, NK cells, myeloid cells and regulatory T cells. GITR increases proliferation, activation and cytokine production of CD4+ and CD8+ cells [9] and diminishes immunosuppressive activity of regulatory T cells [10]. Kawano, et al. investigated the synergistic effect of anti-GITR antibodies and lysate pulsed dendritic cells in C3H mice, and found reduced levels of immunosuppressive cytokines and regulatory T cells and increased levels of interferon IFN-γ and increased CD8+ T lymphocyte numbers [11]. Therefore, this combination immunotherapy increases systemic immune response, including immune response to tumor antigen. Although clinical trials for OS immunotherapy have not yet been executed, a recent RCT for immunotherapy in prostate cancer showed a promising relative reduction in death for refractory patients with metastatic disease (the IMPACT study) [12]. In summary, by upregulating and priming CD8+ and CD4+ T cells as well as downregulating T cell inhibitory pathways, researchers have demonstrated another model of immunotherapy to treat metastatic OS.

miRNA Expression in Osteosarcoma

Molecular targets for osteosarcoma play a critical role as they modulate cell cycle processes including: proliferation, metabolism and apoptosis. MicroRNAs (miRNAs) play a critical role in regulating these cell processes as they can act as oncogenes or tumor suppressors by altering gene function and products. These small non-coding regulatory RNA sequences range from 18-25 nucleotides and can bind to target mRNAs using the 3' Untranslated Region (UTR) for post-transcriptional regulation [13]. Current research into miRNAs has grown as multiple genes are targeted for potential therapeutic usage. Additionally, studies involving miRNAs may help understand the pathogenesis of osteosarcomas and serve as biomarkers for prognosis. Currently miRNA gene expression in osteosarcoma has revealed several distinct miRNA in OS tumors, see [14] for an in depth review of differential miRNA expression in osteosarcoma.

Osteoblastogenesis is one relevant pathway in osteosarcoma and the RUNX2 gene encodes a master transcription factor for osteoblast differentiation. Dysregulation of RUNX2 has also been linked to osteosarcomas and Hey, et al. found an inverse correlation of expression between miR-23a and RUNX2 mRNA levels in osteosarcoma cells, indicating that miR-23a is a tumor suppressor in OS [15]. Zuo, et al. found that miR-103a also plays a role in the regulation of osteoblast differentiation by directly targeting the 3'UTR of RUNX2 mRNA to inhibit matrix mineralization and bone formation through mechanosensitive activation [16].

Another method by which miRNA regulates OS is through cellular proliferative pathways. For example, c-myc amplification in OS stimulates the MEK-ERK pathway and increases cell invasion, indicating poor prognosis [17]. MiRNAs identified by Liu et al. and Xu, et al. miR-135b [18] and miR-33b [19], respectively, have been discovered to directly repress c-MYC expression in osteosarcoma cells and inhibit cell proliferation, migration, and invasion.

In another crucial signaling network, p53, the master tumor suppressor gene regulates the expression and maturation of miRNA in several types of cancer, including OS. Wang et al. studied relevant miRNAs, including miR-192 and miR-215 and found that low levels of these two miRNAs were associated with high p53 expression and poor prognosis [20]. Therefore, miR-192 and miR-215 may have significance as biomarkers for prognosis and drug response in OS.

PI3K/Akt is another important pathway for cell proliferation and has been studied in OS pathology. Phosphatase and Tensin Homolog (PTEN) is a tumor suppressor that negatively regulates the PI3K/Akt pathway and regulation of PTEN has been a focus of many OS researchers. PTEN is inhibited by miR-17 binding to the 3'UTR and Gao, et al. induced downregulation of miR-17, thereby increasing PTEN mRNA in OS [21]. Therefore, miR-17 expression demonstrates its function as an oncogenic miRNA and its potential contribution to progression and metastasis of OS [22]. Additionally, miR-93 is significantly up-regulated in OS and is negatively correlated with PTEN expression and suppressed miR-93 signaling leads to subsequent reduced tumor growth in vivo [23]. mTOR, another component of the PI3K/Akt pathway, has been found to have increased expression in cancer. In OS, Zhang, et al. found that direct repression of mTOR by miR-10 inhibited cell proliferation and apoptosis [24].

Although many miRNAs have been identified in the pathobiology of OS, few studies have investigated delivery of miRNA applicable to gene silencing in OS cells. One study by Zhang, et al. loaded dextran nanoparticles with miR-199a-3p and the miRNA precursor, let-7a. Further examination with Western blotting and cell proliferation assay in vitro showed effective downregulation of target proteins, leading to successive inhibition of tumor cell growth and proliferation [25].

Long Non-Coding RNA Understanding in Osteosarcoma

Noncoding RNAs can be grouped into 2 classes, small noncoding RNAs (<200 bp) and long noncoding RNAs (>200bp). Long noncoding RNAs (lncRNAs) no longer function as templates for protein synthesis; however, they have intrinsic RNA-mediated functions [26]. They are involved in cell proliferation, cell cycle progression, cell growth, apoptosis, and as chemotherapy and radiation resistance in diverse cancers. Furthermore, lncRNAs are able to regulate expression of genes on epigenetic, transcriptional and post-transcriptional levels [27]. Importantly, the biologic relevance of lncRNA has already been determined in several carcinomas, including breast, gastric, non-small-cell lung and liver carcinoma [28-30].

MALAT-1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1), an evolutionarily conserved long non-coding RNA, is expressed in many normal tissues and over expressed in multiple carcinomas [31]. MALAT-1 participates in cellular differentiation, development, promotion of tumor growth and metastasis by binding to tumor suppressor gene SFPQ and releasing a proto-oncogene, PTBP2, from the SFPQ/PTBP2 complex. Dong, et al. found MALAT-1 to be a mediator of OS proliferation, migration and invasiveness, as knockdown of MALAT-1 significantly decreased growth of OS cell

Citation: Chang L, Meyers CA, Asatirian G, Shen J, Scott MA and James AW. Basic and Translational Research in Osteosarcoma: A Year in Review. SM J Orthop. 2016; 2(2): 1031.
lines and reduced expression of Matrix Metalloproteinase 9 (MMP-9), an important enzyme in cancer invasion [32]. Although the pathway in which MALAT-1 regulates proliferation and metastasis is still under investigation, knockdown of MALAT-1 reduced levels of phosphorylated PI3K and Akt, but detected no change in the products of the ERK/MAPK pathway, p-MEK1/2, p-ERK 1/2, and p-JNK. Therefore, MALAT-1 may regulate OS through the PI3K/Akt pathway. It is also known that 17β-Estradiol (E2), post-transcriptionally regulates MALAT-1 [33]. Further analysis by Fang D, et al. found high E2 concentration downregulates MALAT-1 through detachment of the SFPQ/PTBP2 complex and promotes apoptosis in OS cells [34].

LncRNA may also be relevant to OS resistance to chemotherapy. Zhang, et al. found ODRUL (Osteosarcoma Doxorubicin-Resistance Related Up-Regulated LncRNA) functions as a pro-doxorubicin-resistant molecule by inducing the expression of multidrug resistance-related-ATP-binding cassette, subfamily B member 1 (ABCBI) in OS cells. Inhibition of ODRUL inhibited OS cell proliferation, migration and partly reversed doxorubicin resistance in vitro [35]. Therefore, lncRNAs, such as ODRUL may provide a novel target for reversing drug resistance in OS as well as a biomarker to predict chemotherapy response and prognosis. Another potential OS lncRNA prognostic marker is the Highly Up-Regulated in Liver Cancer Gene (HULC), which is associated with hepatocellular carcinoma development and progression. In order to understand its potential role in OS, Sun, et al. analyzed HULC expression using Kaplan-Meier and Cox proportional regression. The study found HULC over-expression was correlated with a late clinical stage and distant metastasis and associated with shorter overall survival of OS patients [36].

Although lncRNAs have been associated with stem cell properties, there have been few studies looking into its function in cancer stem cells. Using bioinformatics analysis, Wang Y, et al. selected multiple lncRNA-mRNA pairs to investigate their role in cancer-associated stem cells. An lncRNA located at 2p21, Hypoxia-Inducible Factor-2α (HIF-2α) Promoter Upstream Transcript (HIF2PUT) was identified as a regulator of transcription factors, especially HIF-2α, a key transcription factor in stem cell pluripotency that maintains stem cell survival [37]. Overexpression of HIF2PUT inhibited cell proliferation as well as migration of CD133+ cells, a marker of cancer stem cells in OS, and increased HIF-2α mRNA expression; whereas loss of function of HIF2PUT concurrently decreased mRNA expression of HIF-2α [38]. Therefore, HIF2PUT may positively regulate HIF-2α expression and thereby its effects on cancer stem cells. Although this is new field, recent developments in lncRNA research may improve the understanding of the molecular pathway underlying the progression of OS. Importantly, lncRNA, may be another measure of prognostic and therapeutic relevance, particularly for metastatic disease.

Growth Factors in Osteosarcoma

Recent advances in the understanding of signaling molecules involved in osteosarcomas may also reveal new prognostic criteria. Several recent insights have been made into growth factors in OS, including VEGF (Vascular Endothelial Growth Factor), HDGF (Hepatoma-Derived Growth factor), and NELL-1 (Neural Epidermal Growth Factor-Like protein 1), each discussed below.

VEGF plays an important role in tumor angiogenesis and its expression may be an important biomarker in OS. In low oxygen conditions as exhibited during early OS growth, VEGF may activate pathways that induce cell proliferation, produce angiogenic factors and promote endothelial cell growth. These changes prolong the survival of malignant cells and are critical to tumor growth [39]. Recently, VEGF gene polymorphisms also have been found to increase the risk of OS. Two independent studies have found the VEGF AA genotype to be at an increased risk of OS. Li-Lian, et al. found an increased risk of OS in both SNP -2578C/A VEGF AA and CA+/AA genotypes OR 2.32 as compared to the CC genotype OR 1.68. Additionally SNP -460T/C phenotypes for VEGF CC and TC+/CC genotypes showed an increased risk as compared to the TT genotype OR 2.15 and OR 1.60, respectively [40]. These findings were corroborated by Tie, et al. who also found that the SNP -2578C/A AA genotype was associated with increased risk of OS, in particular, in male patients, patients less than 20 years old, and those with a shorter family history of cancer. The study also found that the SNP -634G/C GG genotype is correlated with increased risk of OS in female patients, patients less than 20 years old, and those with a family history of cancer [41].

In a hypoxic microenvironment, VEGF is involved in multiple pathways including the Hypoxia-Inducible Factor-1 (HIF-1) pathway. The three known forms of Hypoxia-Inducible Factors (HIF-1, HIF-2 and HIF-3) are heterodimeric transcription factors, which regulate genes in response to low oxygen levels. HIF-1 is expressed in all tissues under hypoxia and is the best-studied isoform. Accumulated HIF-1 translocates to the nucleus and binds to hypoxia-target genes including those involved in glucose metabolism (GLUT-1), erythropoiesis (erythropoietin) and angiogenesis (VEGF) [42]. The significance of Hypoxia-Inducible Factor 1 (HIF-1) in OS is not well understood, but may be of importance in the clinical pathology of OS. In a recent study by Zhao, et al., expression of HIF-1 was high in 56.82% of OS samples and HIF-1 expression was significantly associated with metastasis and poorer overall survival. Additionally, HIF-1 was found to be a prognostic biomarker (P<0.019) that may promote OS invasion by inducing VEGF-A expression [43].

A less studied growth factor, Hepatoma-Derived Growth Factor (HDGF), is in a family of growth factors called HDGF-Related Proteins (HRPs). These growth factors have a homologous N-terminal amino acid that is able to bind to the PWWP domain of DNA. HDGF has been found to be in numerous biological pathways including cellular growth, differentiation, regeneration, apoptosis and migration [44-46]. Its overexpression in multiple cancers and particularly its potential prognostic significance in Ewing’s sarcoma have led to studies on its significance in OS [47,48]. In OS, HDGF was detected by immunohistochemistry and high expression was associated with larger tumor size [49]. Human recombinant HDGF can activate the AKT/MAPK pathway resulting in OS proliferation; whereas HDGF knockdown through oligo-siRNA transfection results in decreased OS proliferation [49].

Lastly, the osteoindifferentiation factor NELL-1 has recently been shown to have potential importance in OS biology [50]. The pro-osteogenic effects of NELL-1, in which binding to integrin αβ3 leads to activation of canonical Wnt signaling and increased transcription of Runx2 are well studied [51]. Although its function in bone regeneration has been well elucidated [52], its effects on tumorigenesis are still unknown. Recently, Shen, et al. showed that OS cell lines overexpress NELL-1 in comparison to human BMSC (bone marrow mesenchymal stem cells) [50]. Likewise, primary
human OS tumors showed marked upregulations in NELL-1 protein expression in comparison to normal adult human bone tissue. Diverse expression patterns of NELL-1 were found between tumors and NELL-1 immunoreactivity was not observed to correlate with osteoid production or markers of osteogenic differentiation [50]. Although the functional importance of NELL-1 in OS is still being understood, this study suggests that NELL-1 may have alternative bioactive effects in neoplastic OS cells.

Osteosarcoma has been an important area of scientific investigation in the past few years and researchers continue to clarify and appreciate the multiple molecular mechanisms that are involved in OS development. With better understanding of the cellular pathways involved in OS, new developments in prognosis and treatment can be found with advancements in immune therapy, miRNA expression, long non-coding RNA, and novel growth factors in OS.

Acknowledgement

The present work was supported by the UCLA Daljit S and Elaine Sarkaria Fellowship award, the Orthopaedic Research and Education Foundation with funding provided by the Musculoskeletal Transplant Foundation, and NIH/NIAMS K08 AR068316-01. The authors thank AS James for his excellent technical assistance.

References

1. Mirabello L, RJ Troisi, SA Savage. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer. 2009; 115: 1531-1543.

2. Selvarajah S, Yoshimoto M, Ludkovski O, Park PC, Bayani J, Thorner P, et al. Genomic signatures of chromosomal instability and osteosarcoma progression detected by high resolution array CGH and interphase FISH. CytoGenet Genome Res. 2008; 122: 5-15.

3. Wolchok JD, Kruger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369: 122-133.

4. Lussier DM, O’Neill L, Nieves LM, McAllister MS, Holechek SA, Collins AW, et al. Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions. J Immunother. 2015; 38: 96-106.

5. Curran MA, Montalvo W, Yafta H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T cell and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A. 2010; 107: 4275-4280.

6. Sansom DM. CD28, CTLA-4 and their ligands: who does what and to whom? Immunology. 2000; 101: 169-170.

7. Lussier DM, Johnson JL, Hingorani P, Blattman JN. Combination immunotherapy with a-CTLA-4 and a-PD-L1 antibody blockade prevents immune escape and leads to complete control of metastatic osteosarcoma. J Immunother Cancer. 2015; 3: 21.

8. Fang X, Jiang C, Xia Q. Effectiveness evaluation of dendritic cell immunotherapy for osteosarcoma on survival rate and in vitro immune response. Genet Mol Res. 2015; 14: 11763-11770.

9. Watts TH. TNF/TNFR family members in costimulation of T cell responses. Annu Rev Immunol. 2005; 23: 23-68.

10. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD28(+)/CD4(-) regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol. 2002; 3: 135-142.

11. Kawano M, Tanaka K, Itokage H, Iwasaki T, Miyazaki M, Ikeda S, et al. Dendritic cells combined with anti-GITR antibody produce antitumor effects in osteosarcoma. Oncol Rep. 2015; 34: 1995-2001.

12. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EE, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010; 363: 411-422.

13. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998; 391: 806-811.

14. Chang L, Shrestha S, LaChaud G, Scott MA, James AW. Review of microRNA in osteosarcoma and chondrosarcoma. Med Oncol. 2015; 32: 613.

15. He Y, Meng C, Shao Z, Wang H, Yang S. miR-23a functions as a tumor suppressor in osteosarcoma. Cell Physiol Biochem. 2014; 34: 1485-1486.

16. Zuo B, Zhu J, Li J, Wang C, Zhao X, Cai G, et al. microRNA-103a functions as a mechanosensitive microRNA to inhibit bone formation through targeting Runx2. J Bone Miner Res. 2015; 30: 330-345.

17. Han G, Wang Y, Bi W. C-Myc overexpression promotes osteosarcoma cell invasion via activation of MEK-ERK pathway. Oncol Res. 2012; 20: 149-156.

18. Liu Z, Zhang G, Li J, Liu J, Lv P. The tumor-suppressive microRNA-135b targets c-myc in osteosarcoma. PLoS One. 2014; 9: e102621.

19. Xu N, Li Z, Yu Z, Yan F, Liu Y, Lu X. miRNA-33b suppresses migration and invasion by targeting c-Myc in osteosarcoma cells. PLoS One. 2014; 9: e115300.

20. Wang Y, Jia LS, Yuan W, Wu Z, Wang HB, Xu T, et al. Low mir-34a and miR-192 are associated with unfavorable prognosis in patients suffering from osteosarcoma. Am J Transl Res. 2015; 7: 111-119.

21. Xi Y, Chen Y. Oncogentic and Therapeutic Targeting of PTEN Loss in Bone Malignancies. J Cell Biochem. 2015; 116: 1837-1847.

22. Gao Y, Luo LH, Li S, Yang C. miR-17 inhibitor suppressed osteosarcoma tumor growth and metastasis as increasing PTEN expression. Biochem Biophys Res Commun. 2014; 444: 230-234.

23. Kawano M, Tanaka K, Itokage H, Iwasaki T, Tsumura H. microRNA-93 promotes cell proliferation via targeting of PTEN in Osteosarcoma cells. J Exp Clin Cancer Res. 2015; 34: 76.

24. Lin S, Shao NN, Fan L, Ma XC, Pu FF, Shao ZW. Effect of microRNA-101 on proliferation and apoptosis of human osteosarcoma cells by targeting miTOR. J Huazhong Univ Sci Technolog Med Sci. 2014; 34: 889-895.

25. Zhang L, Lyer AK, Yang X, Kobayashi E, Guo Y, Mankin H, et al. Polymeric nanoparticle-based delivery of microRNA-199a-3p inhibits proliferation and growth of osteosarcoma cells. Int J Nanomedicine. 2015; 10: 1077-1133.

26. Harries LW. Long non-coding RNAs and human disease. Biochem Soc Trans. 2012; 40: 902-906.

27. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into function. Nat Rev Genet. 2009; 10: 155-159.

28. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010; 464: 1071-1076.

29. Zhang EB, Kong R, Yin DD, You LH, Sun M, Han L, et al. Long non-coding RNA ANRIL indicates a poor prognosis of gastric cancer and promotes tumor growth by epigenetically silencing of miR-99a/miR-449a. Oncotarget. 2014; 5: 2276-2292.

30. Xi Y, Sun M, Liu H, Yao Y, Kong R, Chen F, et al. A critical role for the long non-coding RNA GAS5 in proliferation and apoptosis in non-small-cell lung cancer. Mol Carcinog. 2015; 54: E1-16.

31. Lin R, Maeda S, Liu C, Karin M, Edgington TS. A large non-coding RNA in osteosarcoma and chondrosarcoma. Med Oncol. 2015; 32: 613.

32. Dong Y, Yang C, Gao R, Zhou X. MALAT1 promotes the proliferation and metastasis of osteosarcoma cells by activating the PI3K/Akt pathway. Tumour Biol. 2015; 36: 1477-1486.

33. Zhao Z, Chen C, Liu Y, Wu C. 17β-Estradiol treatment inhibits breast cell
proliferation, migration and invasion by decreasing MALAT-1 RNA level. Biochem Biophys Res Commun. 2014; 445: 388-393.

34. Fang D, Yang H, Lin J, Teng Y, Jiang Y, Chen J, et al. 17ß-estradiol regulates cell proliferation, colony formation, migration, invasion and promotes apoptosis by upregulating miR-9 and thus degrades MALAT-1 in osteosarcoma cell MG-63 in an estrogen receptor-independent manner. Biochem Biophys Res Commun. 2015; 457: 500-506.

35. Zhang CL, Zhu KP, Shen GQ, Zhu ZS. A long non-coding RNA contributes to doxorubicin resistance of osteosarcoma. Tumour Biol. 2015.

36. Sun XH, Yang LB, Geng XL, Wang R, Zhang ZC. Increased expression of IncRNA HULC indicates a poor prognosis and promotes cell metastasis in osteosarcoma. Int J Clin Exp Pathol. 2015; 8: 2994-3000.

37. Covello KL, Kehler J, Yu H, Gordon JD, Arsham AM, Hu CJ, et al. HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev. 2006; 20: 557-570.

38. Siclari VA, Qin L. Targeting the osteosarcoma cancer stem cell. J Orthop Surg Res. 2010; 5: 78.

39. Mizobuchi H, Garcia-Castellano JM, Philip S, Healey JH, Gorlick R. Hypoxia markers in human osteosarcoma: an exploratory study. Clin Orthop Relat Res. 2008; 466: 2052-2059.

40. Li-Lian Z, Lin W, Lei S, Yao-Nan Z. Investigation on the role of VEGF gene polymorphisms in the risk of osteosarcoma. Pak J Med Sci. 2015; 31: 364-368.

41. Tice Z, Rai R, Zhai Z, Zhang H, Zhao Z, et al. Single nucleotide polymorphisms in VEGF gene are associated with an increased risk of osteosarcoma. Int J Clin Exp Pathol. 2014; 7: 8143-8149.

42. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A. 1995; 92: 5510-5514.

43. Zhao H, Wu Y, Chen Y, Liu H. Clinical significance of hypoxia-inducible factor 1 and VEGF-A in osteosarcoma. Int J Clin Oncol. 2015; 20: 1233-1243.

44. Clermont F, Gonzalez NS, Communi D, Franken S, Dumont JE, Robaye B. HDGF is dephosphorylated during the early steps of endothelial cell apoptosis in a caspase-dependent way. J Cell Biochem. 2008; 104: 1161-1171.

45. Enomoto H, Nakamura H, Liu W, Yoshida K, Okuda Y, Imarishi H, et al. Hepatoma-derived growth factor is induced in liver regeneration. Hepatol Res. 2009; 39: 988-997.

46. Wang CH, Davamani F, Sue SC, Lee SC, Wu PL, Tang FM, et al. Cell surface heparan sulfates mediate internalization of the PIWPP/HATH domain of HDGF via macropinocytosis to fine-tune cell signalling processes involved in fibroblast cell migration. Biochem J. 2011; 433: 127-138.

47. Yang Y, Li H, Zhang F, Shi H, Zhen T, Dai S, et al. Clinical and biological significance of hepatoma-derived growth factor in Ewing’s sarcoma. J Pathol. 2013; 231: 323-334.

48. Savola S, Klam A, Tripathi A, Niini T, Serra M, Picci P, et al. Combined use of expression and CGH arrays pinpoints novel candidate genes in Ewing sarcoma family of tumors. BMC Cancer. 2009; 9: 17.

49. Chen Z, Qiu S, Lu X. The expression and clinical significance of HDGF in osteosarcoma. Onco Targets Ther. 2015; 8: 2509-2517.

50. Shen J, LaChaud G, Khadarian K, Shrestha S, Zhang X, Soo C, et al. NELL-1 expression in benign and malignant bone tumors. Biochem Biophys Res Commun. 2015; 460: 366-374.

51. James AW, Shen J, Zhang X, Asatrian G, Goyal R, Kwak JH, et al. NELL-1 in the treatment of osteoporotic bone loss. Nat Commun. 2015; 6: 7362.

52. Zhang X, Zara J, Siu RK, Ting K, Soo C. The role of NELL-1, a growth factor associated with craniosynostosis, in promoting bone regeneration. J Dent Res. 2010; 89: 865-876.