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The canonical Wnt-beta-catenin pathway in development and chemotherapy of osteosarcoma

Chengjun Li[1], Xin Shi[1], Guangxin Zhou[1], Xiaozhou Liu[1], Sujia Wu[1], Jianning Zhao[1]

[1]Jinling Hospital, Dept Orthopedics, Nanjing Univ, Sch Med ,Nanjing 210002, Peoples R China

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1. ABSTRACT

The canonical Wnt-beta -catenin signaling pathway is a key component of normal skeletal development and disease. Alterations within this signaling pathway have been described in human and canine osteosarcoma (OS); however, debate exists as to whether or not alterations in this pathway contribute to OS development in humans. In metastatic OS, the Wnt-β-catenin pathway promotes the invasion and migration of OS cells and β-catenin acts as a biological marker of OS with the potential to metastasize to the lung. The participation of the Wnt-β-catenin pathway in OS development and metastasis is regulated by several factors, including hormones and alkaline phosphatase (ALP). This pathway is also involved in the resistance of OS to chemotherapy, especially in resistance to all three drugs used in standard chemotherapy, i.e. doxorubicin, cisplatin and methotrexate (MTX). In this review, we will summarize recent findings regarding the Wnt-β-catenin pathway in OS development and chemotherapy.

2. INTRODUCTION

The canonical Wnt-β-catenin signaling pathway passes signals from extracellular receptors through the cytoplasm and ultimately to the cell's nucleus, resulting in expression of target genes. The pathway is activated by binding of a Wnt ligand to a receptor complex which includes a member of the Frizzled protein family. The phosphoprotein dishevelled (Dsh) inhibits the activity of this multiprotein complex which also contains the proteins Axin, Adenomatous Polyposis Coli (APC), and glycogen synthase kinase-3β (GSK3β), transmitting the ligand–receptor interaction intracellularly. In the absence of a suitable ligand, this complex promotes the proteolytic degradation of the intracellular signaling molecule β-catenin. β-catenin is an obligatory, and the only nonredundant, component of the canonical Wnt pathway, involved in the control of stem cell pluripotency, cell proliferation, differentiation and migration. In normal cells, β-catenin is a tumor suppressor gene encoded by CTNNB1, which is present on the cytoplasmic side of the cell membrane and functions to support cell-cell adhesion (1,2).

In bone development, the canonical Wnt-β-catenin signaling pathway is required for osteoblast differentiation from a precursor, and for regulation of osteoblast maturation and activity (3), negatively regulating the differentiation of mesenchymal cells into a common skeletal precursor (1). Consequently, β-catenin activity is required for bone formation in both endochondral and membranous bones (4-6). In mature osteoblasts, β-catenin also regulates osteoclastogenesis and osteoclast function (7). Excessive and inadequate Wnt pathway activities are associated with the pathologic bone conditions osteopetrosis and osteoporosis, respectively (8).

Osteosarcoma (OS) is the most common primary malignant bone tumor, with a yearly incidence of approximately 6 per million children and 2 per million adults (9). OS predominantly occurs in children and adolescents, having a peak incidence in late puberty, with 50% of patients being between 10 and 20 years of age, and 60% younger than 25 years (10,11). The overall relapse-free survival rate over 5 years is approximately 65% (12). That the canonical Wnt-β-catenin signaling pathway contributes to OS has been a more recent discovery. This review will provide a summary of the canonical Wnt-β-catenin pathway and its role in the development and chemotherapy of osteosarcoma. Since β-catenin is an obligatory, and the only nonredundant, component of the canonical Wnt pathway, we will particularly stress the role of β-catenin in OS.

3. THE WNT-B-CATENIN PATHWAY IN THE DEVELOPMENT OF OSTEOSARCOMA

Alterations in the canonical Wnt-β-catenin pathway, especially involving β-catenin, have been reported in human OS primary tissues and cell lines. In human OS tissues, the expression of β-catenin is significantly higher than in normal tissues (13) or in osteoid osteoma, osteoblastoma or newly formed bone (14). In human OS cell lines, the major components of the Wnt-β-catenin pathway, including Wnt3a, β-catenin and Lef1, are upregulated compared to human fetal osteoblasts (15). This abnormal expression of components of the canonical Wnt-β-catenin pathway suggests a role of canonical Wnt-β-catenin signaling in OS development.

Based on a canine OS model, the intracellular location of β-catenin was identified within the cytoplasm of neoplastic cells (16). The β-catenin gene consists of 16 exons and importantly, the third exon encodes the NH2 domain, which contains Ser33, Ser37, Ser45, and Thr41. These residues are sites at which β-catenin is phosphorylated by GSK3β. The GSK-3β-binding domain of β-catenin corresponds to its degradation targeting box and is encoded by exon 3 of CTNNB1. Activating mutations within this region, which have been described for the adamantinomatous subtype but not the papillary subtype of OS, promote β-catenin accumulation by inhibiting its degradation, thus leading to activation of WNT signaling. Stein and colleagues further found that no mutations in exon 3 of β-catenin were detected, which is similar to human OS (16). In contrast, Bongiovanni and colleagues (17) observed nuclear β-catenin immunostaining in normal osteoblasts but absent or low expression in most canine models of OS. Cai and colleagues (18) reported similar findings in human OS tissues and cell lines. They observed the absence of nuclear β-catenin staining in about 90% of the human OS biopsies and human OS cell lines tested.

Among several histological subtypes of OS, conventional high-grade central or intramedullary osteosarcoma is the most common (75%) (19). Cai and colleagues suggested that the canonical Wnt-β-catenin pathway was inactive in conventional high-grade OS, while activation of this pathway inhibited cell proliferation or promoted osteogenic differentiation at this OS stage (18). Molecular studies on osteosarcoma are greatly hampered by the enormous genetic instability that obscures the identification of genetic loci involved in OS genesis (20). Cleton-Jansen and colleagues (21) found that the canonical Wnt-β-catenin pathway was downregulated in OS genesis of high-grade central osteosarcomas, and this difference in gene expression involved cell cycle regulation. One inhibitor of the canonical Wnt-β-catenin signaling pathway, namely dickkopf-1, was found to be required for reentry into the cell cycle of human adult stem cells from bone marrow (22). Thus, the canonical Wnt-β-catenin pathway would participate in cell cycle regulation at an early stage during OS development from stem cells. Targeting the canonical Wnt-β-catenin pathway may thus lead to promising new modalities for early prevention or therapy of OS. However, β-catenin cannot induce the malignant features and tumorigenicity conveyed by oncogenic H-RAS when introduced into partly transformed mesenchymal stem cells, even though it can foster osteogenic differentiation (23).

4. THE WNT-B-CATENIN PATHWAY IN METASTATIC OSTEOSARCOMA

Studies on the prognosis of OS found that the Wnt-β-catenin pathway can act as a biological marker of metastasis. Relapse and/or metastasis of OS occurs in 80% of cases (24). Five-year survival rates are approximately 80% with localized disease, but drop to roughly 30% if metastatic lesions are present (25). Pulmonary metastasis is the predominant site of osteosarcoma recurrence and the most common cause of death. Thus, metastatic prediction is significant in designing a therapeutic strategy. Since moderate/high cytoplasmic β-catenin expression (≥10% positive cells) is significantly associated with the development of metastasis (17), β-catenin is used as a biological marker of the metastatic potential of OS to the lung (26).

Abnormalities of the Wnt-β-catenin pathway are involved in the mechanism of metastatic OS. Expression of the Wnt receptor LRP5 is associated with metastatic disease in OS (27) and inhibition of LRP5 using a dominant-negative form of this receptor also inhibits tumor

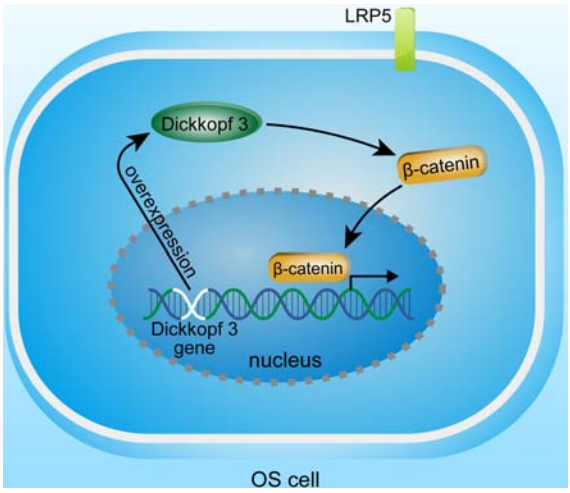

Figure 1. Abnormalities of Wnt-β-catenin signaling in metastatic OS. Overexpression of Dickkopf 3 can effectively reduce motility and invasion of OS cells by affecting intracellular β-catenin levels. Inhibition of LRP5 also inhibits tumor cell motility and invasion.

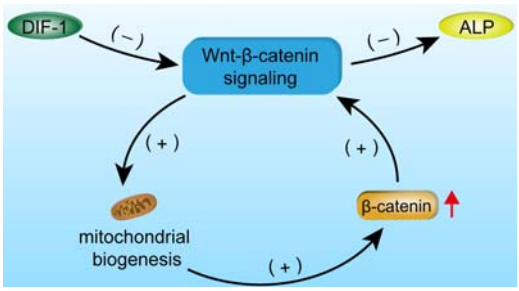

Figure 2. Abnormalities of Wnt-β-catenin signaling affect the activity of ALP. Activation of differentiation-inducing factor-1 (DIF-1) inhibits Wnt-β-catenin signaling, resulting in suppression of ALP promoter activity. Mitochondria are key in this regulation; Wnt-β-catenin signaling upregulates mitochondrial biogenesis, which in turn positively regulates β-catenin levels.

cell motility and invasion (28). Thus, LRP5 plays an important role in promoting OS metastasis. Moreover, in OS progression, intracellular β-catenin is reduced by overexpression of Dickkopf 3, a member of the gene family encoding secreted proteins that control cell fate during embryonic development (29,30), and this leads to decreased motility and invasion of OS cells (28) (Figure 1). Thus, these findings support the role of the Wnt-β-catenin pathway in promotion of metastatic OS. Blockade of Wnt/LRP5 signaling inhibits met and metalloproteinase expression and reduces tumorigenicity and metastases in animal OS models (31,32). Knockdown of the β-catenin gene also reduces the invasive ability of OS cells by-regulating MT1-MMP expression, suggestingn that β-catenin could promote the invasion of OS by regulating MT1-MMP (33,34). Moreover, the role of the Wnt-β-catenin pathway in OS metastasis is regulated by autocrine or paracrine mechanisms (35).

5. REGULATION OF THE WNT-B-CATENIN PATHWAY IN OSTEOSARCOMA

Several factors regulate promotion of the Wnt-β-catenin pathway in OS through different mechanisms. In *in vivo* experiments with rat osteoblastic OS cells (UMR 106), the canonical Wnt-β-catenin signaling pathway was found to be regulated by parathyroid hormone, in part via the cAMP-PKA pathway through differential regulation of the receptor complex proteins (FZD-1/LRP5 or LRP6) and the antagonist (36). In primary human OS, β-catenin levels increased following silencing of WIF1 by promoter hypermethylation[0]. Although WIF1 was not required for normal skeletal development, loss of WIF1 increased susceptibility to radiation-induced OS in a mouse model (37). In addition, the effect of 1,25-dihydroxyvitamin D_3 (1,25(OH)-D_3) was also associated with decreased β-catenin signaling due to inhibition of β-catenin gene activation by ligand-activated vitamin-D gene receptor signaling (38).

The etiology of high-grade central osteosarcoma in young patients is unknown. No benign or malignant precursor lesions are known. The expression of alkaline phosphatase (ALP) observed in OS recapitulates osteogenesis. In both canine and human OS, prognosis worsens with increased serum ALP concentration, correlating with shorter survival and disease-free intervals (39-42). The expression of ALP is generally used to identify cells of the osteoblastic lineage and is a hallmark of osteoblastic activity. ALP is also a transcriptional target of the Wnt-β-catenin signaling pathway, with activation of this pathway in osteoblasts being associated with increased ALP expression (43,44). Abnormalities of the Wnt-β-catenin signaling pathway could thus affect the activity of ALP. For instance, the activation of Differentiation-inducing factor-1 (DIF-1) could inhibit Wnt-β-catenin signaling, resulting in suppression of ALP promoter activity (45). DIF-1, a morphogen of Dictyostelium, inhibits cell proliferation and induces differentiation in several mammalian cells (46-48). Mitochondria play a key role in this regulation. An and colleagues (49) observed that in mouse mesenchymal C3H10T1/2 cells, Wnt-β-catenin signaling upregulates mitochondrial biogenesis, which in turn positively regulates β-catenin levels (Figure 2). Furthermore, they found that both basal and Wnt-3-stimulated ALP activity was significantly suppressed in a human OS cell line devoid of mitochondrial DNA compared to that of mitochondria-intact cells (49). These findings further suggest that Wnt-β-catenin signaling participates in positive feedback with energy metabolism during Wnt-induced osteoblastic differentiation of stem cells. Even though expression of the canonical Wnt-β-catenin pathway is abnormal in OS, Wnt-β-catenin expression does not correlate with serum ALP concentration in canine OS (50).

6. THE ROLE OF THE WNT-B-CATENIN PATHWAY IN CHEMOTHERAPY RESISTANCE OF OSTEOSARCOMA

Treatment with neoadjuvant and adjuvant chemotherapy in addition to radical surgery has been demonstrated to significantly improve the prognosis for osteosarcoma patients. An approximately 70% long-term

event-free survival rate for osteosarcoma patients can currently be achieved by using the standard three-drug chemotherapy protocol that includes doxorubicin, cisplatin and high-dose methotrexate (51). Nevertheless, multi-drug resistance and poor clinical outcome are the main problems in 50% of osteosarcoma patients (52). Therefore, identifying the mechanisms of action of chemotherapeutic agents could improve targeted therapy for OS patients. The Wnt-β-catenin pathway is involved in OS chemotherapy resistance, especially to standard three-drug chemotherapy.

Tumor stem cells possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. Thus, tumor stem cells are tumorigenic compared to other non-tumorigenic cancer cells. By giving rise to new tumors, tumor stem cells can make tumors persist as a distinct population, and cause relapse and tumor metastasis. The poor prognosis of OS could be partly due to a failure to target these tumor stem cells, and development of specific therapies targeted at tumor stem cells therefore holds hope for improvement of survival and quality of life of cancer patients, especially for sufferers of metastatic disease. Salinomycin, which has been found to target tumor stem cells (53), inhibits OS by selectively targeting its stem cells both *in vitro* and *in vivo* without severe side effects (54). The canonical Wnt-β-catenin pathway is involved in the inhibitory mechanism of salinomycin (54), suggesting that the Wnt-β-catenin pathway may participate in tumor stem cell-targeting therapy for OS.

6.1 Doxorubicin

Small interfering RNAs (siRNAs) are small double-stranded RNA molecules 20–25 base pairs in length, which interfere with the expression of specific genes with complementary nucleotide sequences. Integrative approaches coupling protein interaction maps to siRNA screening data have suggested that the components that constitute the Wnt-β-catenin signaling machinery in a given cell type are highly variable (55). Verkaar and colleagues (56) confirmed that small molecule-mediated cell-type-specific activation of Wnt-β-catenin signaling can be achieved. The siRNA-mediated silencing of β-catenin can suppress chemosensitivity of the human OS cell line MG-63 to doxorubicin, an anthracycline antibiotic which is widely used for the treatment of many different cancers including OS. Following knockdown of the β-catenin gene, chemoresistance to doxorubicin was reduced via the NF-κB pathway (33). Zhang and colleagues (34) reported similar findings *in vitro* with U2-OS cells. Wnt-β-catenin signaling targeting T-cell factor represses syndecan-2, a key modulator of apoptosis and chemosensitivity in OS cells, contributing to the resistance of OS to doxorubicin (57,58).

6.2 Cisplatin

Cisplatin is widely used in the treatment of a variety of pediatric and adult solid tumors including OS, due to its therapeutic advantages such as high efficiency, mild side effects and easy administration. Cisplatin is a DNA-damaging agent that forms cisplatin-DNA adducts and kills cells via several mechanisms, including induction of apoptosis (59). Although high-grade OS can be considered as a cisplatin-responsive tumor, it may present an inherent or acquired resistance to this drug which severely limits its clinical efficacy (60). Thus, resistance to cisplatin leads to poor response to chemotherapy and treatment failure. Cisplatin resistance is multifactorial, with several different mechanisms that can be involved simultaneously. One of these mechanism involves Wnt-β-catenin signaling. Overexpression of TWIST in human OS cells significantly reduces cell survival against cisplatin by reducing β-catenin levels via a phosphatidylinositol 3-kinase (PI3K)-dependent pathway (61). The PI3K/Akt pathway regulates several apoptosis-related downstream targets (62-64), resulting in cell growth, survival and cisplatin resistance. The Wnt-β-catenin signaling pathway participates in cisplatin resistance through interactions with the PI3K/Akt pathway.

6.3 Methotrexate

Methotrexate (MTX) is another common constituent of chemotherapeutic regimens for high-grade osteosarcoma, together with doxorubicin, cisplatin and ifosfamide (65,66). MTX is a potent inhibitor of dihydrofolate reductase (DHFR), an enzyme which plays a key role in intracellular folate metabolism and is essential for DNA synthesis and cell growth (67,68). However, MTX resistance is a problem in OS chemotherapy and one of the mechanisms underlying MTX resistance is associated with Wnt-β-catenin signaling. Ma and colleagues (15) found that knocking down β-catenin increased the sensitivity of Saos2 cells to MTX-induced cell death. Thus, Wnt-β-catenin signaling may contribute to MTX resistance.

6.4 COX-2 inhibitors

Cyclo-oxygenase (COX)-2 inhibitors have been found to have anticancer effects that could reduce the occurrence of cancers and pre-cancerous growths (69-71). In particular, celecoxib has been shown to act as an inhibitor of proliferation in several tumor cell types (72,73). The antitumor effects of celecoxib depend on its COX-2-inhibiting potency, especially its regulation of the prostaglandin pathways (74). COX-2-related mechanisms have been identified that several cell signaling pathways activate COX-2 expression, including the PI3K/Akt or Wnt-β-catenin pathways (75,76). In particular, the Wnt-β-catenin pathway is a classical pathway that has been suggested as a COX-2-related target of nonsteroidal anti-inflammatory drugs (NSAIDs) in cancer cells (77). Studies have established that high levels of β-catenin correlate with tumorigenesis in several tumour types, suggesting that it could be a downstream target of COX-2 inhibitors (78-80). In the human OS cell line MG-63, β-catenin was identified as a downstream target of COX-2 inhibitors, and celecoxib was found to inhibit β-catenin-dependent survival (81).

In summary, the Wnt-β-catenin signaling pathway contributes to resistance to all three drugs used in standard chemotherapy. Thus, in the mechanism of chemotherapy resistance, the Wnt-β-catenin signaling pathway provides a key interaction point with other pathways such as the PI3K/Akt pathway. Increased knowledge of Wnt-β-catenin signaling in chemotherapy

resistance of OS would significantly improve the efficiency of OS chemotherapy and the clinical outcome of OS patients.

7. CONCLUSION

The Wnt-β-catenin signaling pathway is a key component of normal skeletal development and disease. Alterations in this signaling pathway have been described in both human and canine OS. However, debate exists as to whether such alterations actually contribute to human OS development. These conflicting reports indicate that additional research is necessary to clarify the role of Wnt signaling in OS development. In metastatic OS, the Wnt-β-catenin pathway promotes invasion and migration of OS cells. In particular, β-catenin can act as a biological marker of the metastatic potential of OS to the lung. Thus, further studies on the prediction of OS metastasis by β-catenin would be promising. Participation of the Wnt-β-catenin pathway in OS development and metastasis is regulated by several factors, including hormones and ALP. This suggests that specific hormones and Wnt-β-catenin signaling form a network in OS regulation. The Wnt-β-catenin pathway is involved in OS chemotherapy resistance, especially in resistance to all three drugs used in standard chemotherapy, i.e. doxorubicin, cisplatin and MTX. Taken together, these findings suggest that the Wnt-β-catenin pathway is significant in OS development and chemotherapy response, since it can promote OS development, metastasis and resistance to chemotherapy. Therefore, inhibition or regulation of this pathway would be a promising target for new OS therapies.

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9. REFERENCES

1. Logan CY, Nusse R: The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20,781–810. (2004)

2. Lie DC, Colamarino SA, Song HJ, Désiré L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR, Gage FH: Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 437 (7063),1370–5. (2005)

3. Hartmann C: A Wnt canon orchestrating osteoblastogenesis. *Trends Cell Biol* 16,151–158.(2006)

4. Day TF, Guo X, Garrett-Beal L, Yang Y: Wnt/b-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell* 8, 739–750(2005)

5. Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C: Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev Cell* 8,727–738. (2005)

6. Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F: Sequential roles of Hedgehog and Wnt signaling in osteoblast development. *Development* 132,49–60. (2005)

7. Kubota T, Michigami T, Ozono K: Wnt signaling in bone metabolism. J Bone Miner Metab. 27(3),265-71. (2009)

8. Glass DA, Karsenty G: In vivo analysis of Wnt signaling in bone. *Endocrinology* 148,2630-2634. (2007)

9. Raymond AK, Ayala AG, Knuutila S: Conventional osteosarcoma World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. *IARC Press, Lyon* 264–270.270(2006)

10. Meyers PA, Gorlick R: Osteosarcoma. *Pediatr Clin North Am* 44,973–89. (2002)

11. Picci P: Osteosarcoma (osteogenic sarcoma) *Orphanet J Rare Dis* 2,6. (2007)

12. Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jürgens H, Winkler K: Prognostic factors in highgrade osteosarcoma of the extremities or trunk, an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 20(3),776–790 (2002)

13. Zhou L, Park BH, Park JH, Jang KY, Park HS, Wagle S, Lee KB, Kim JR: Overexpression of the prolyl isomerase PIN1 promotes cell growth in osteosarcoma cells. *Oncol Rep*;29(1),193-8. (2013)

14. Yang JZ, Zhang XH, Liu JR, Ding Y, Gao F, Wang Y: Expression and significance of N-cadherin and β-catenin protein in osteosarcoma. *Zhonghua Zhong Liu Za Zhi*;32(8),586-9. (2010)

15. Ma Y, Ren R, Han EQ, Li H, Chen D, Jacobs JJ, Gitelis S, O'Keefe RJ, Konttinen YT, Yin G, Li TF: Inhibition of the Wnt-β-catenin and Notch signaling pathways sensitizes osteosarcoma cells to chemotherapy. *Biochem Biophys Res Commun*. pii, S0006-291X(12)02478-3. (2013)

16. Stein TJ, Holmes KE, Muthuswamy A, Thompson V, Huelsmeyer MK: Characterization of β-catenin expression in canine osteosarcoma. *Vet Comp Oncol* 9(1),65-73. (2011)

17. Bongiovanni L, Mazzocchetti F, Malatesta D, Romanucci M, Ciccarelli A, Buracco P, De Maria R, Palmieri C, Martano M, Morello E, Maniscalco L, Della Salda L: Immunohistochemical investigation of cell cycle and apoptosis regulators (Survivin, β-Catenin, P53, Caspase 3) in canine appendicular osteosarcoma. *BMC Vet Res* 8,78. (2012)

18. Cai Y, Mohseny AB, Karperien M, Hogendoorn PC, Zhou G, Cleton-Jansen AM: Inactive Wnt/beta-catenin pathway in conventional high-grade osteosarcoma. *J Pathol* 220(1),24-33. (2010)

19. Fletcher CDM, Unni KK, Mertens F: Osteogenic tumours World Health Organization classification of tumours Pathology and genetics of tumours of soft tissue and bone. *IARC Press, Lyon* 259–290. (2002)

20. Hogendoorn PCW, Bove´e JVMG, Karperien M, Cleton-Jansen AM: Skeletogenesis, Genetics. *Nature Encyclopedia of the Human Genome* 306–313. (2003)

21. Cleton-Jansen AM, Anninga JK, Briaire-de Bruijn IH, Romeo S, Oosting J, Egeler RM, Gelderblom H, Taminiau AH, Hogendoorn PC: Profiling of high-grade central osteosarcoma and its putative progenitor cells identifies tumourigenic pathways. *Br J Cancer* 101(11),1909-18. (2009)

22. Gregory CA, Singh H, Perry AS, Prockop DJ: The Wnt signaling inhibitor dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow. *J Biol Chem* 278(30),28067-78. (2003)

23. Piperdi S, Austin-Page L, Geller D, Ahluwalia M, Gorlick S, Gill J, Park A, Zhang W, Li N, Chung SH, Gorlick R: β-Catenin Does Not Confer Tumorigenicity When Introduced into Partially Transformed Human Mesenchymal Stem Cells. *Sarcoma* 2012,164803. (2012)

24. Mirabello L, Troisi RJ, Savage SA: Osteosarcoma incidence and survival rates from 1973 to 2004, data from the Surveillance, Epidemiology, and End Results Program. *Cancer* 115,1531–1543. (2009)

25. Gorlick R, Khanna C: Osteosarcoma. J *Bone Miner Res* 25,683–691. (2010)

26. Iwaya K, Ogawa H, Kuroda M, Izumi M, Ishida T, Mukai K: Cytoplasmic and/or nuclear staining of beta-catenin is associated with lung metastasis. *Clin Exp Metastasis* 20(6),525-9. (2003)

27. Hoang BH, Kubo T, Healey JH, et al: Expression and potential role of low-density lipoprotein receptor-related protein 5 (LRP5) and Wnt signaling components in osteosarcoma. *Proc Am Assoc Cancer Res* 44,921.(2003)

28. Hoang BH, Kubo T, Healey JH, Yang R, Nathan SS, Kolb EA, Mazza B, Meyers PA, Gorlick R: Dickkopf 3 inhibits invasion and motility of Saos-2 osteosarcoma cells by modulating the Wnt-beta-catenin pathway. *Cancer Res* 64(8),2734-9. (2004)

29. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C: Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391,357-62. (1998)

30. Krupnik VE, Sharp JD, Jiang C, et al: Functional and structural diversity of the human Dickkopf gene family. *Gene* 238,301-13. (1999)

31. Guo Y, Rubin EM, Xie J, et al: Dominant negative LRP5 decreases tumorigenicity and metastasis of osteosarcoma in an animal model. *Clin Orthop Relat Res* 466, 2039-45. (2008)

32. Guo Y, Zi X, Koontz Z, et al: Blocking Wnt/LRP5 signaling by a soluble receptor modulates the epithelial to mesenchymal transition and suppresses met and metalloproteinases in osteosarcoma Saos-2 cells. *J Orthop Res* 25, 964-71. (2007)

33. Zhang F, Chen A, Chen J, Yu T, Guo F: SiRNA-mediated silencing of beta-catenin suppresses invasion and chemosensitivity to doxorubicin in MG-63 osteosarcoma cells. *Asian Pac J Cancer Prev* 12(1),239-45. (2011)

34. Zhang F, Chen A, Chen J, Yu T, Guo F: Influence of β-catenin small interfering RNA on human osteosarcoma cells. *J Huazhong Univ Sci Technolog Med Sci* 31(3),353-8. (2011)

35. Chen K, Fallen S, Abaan HO, Hayran M, Gonzalez C, Wodajo F, MacDonald T, Toretsky JA, Uren A: Wnt10b induces chemotaxis of osteosarcoma and correlates with reduced survival. *Pediatr Blood Cancer* 51(3),349-55. (2008)

36. Kulkarni NH, Halladay DL, Miles RR, Gilbert LM, Frolik CA, Galvin RJ, Martin TJ, Gillespie MT, Onyia JE: Effects of parathyroid hormone on Wnt signaling pathway in bone. *J Cell Biochem* 95(6),1178-90. (2005)

37. Kansara M, Tsang M, Kodjabachian L, Sims NA, Trivett MK, Ehrich M, Dobrovic A, Slavin J, Choong PF, Simmons PJ, Dawid IB, Thomas DM: Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. *J Clin Invest* 119(4),837-51. (2009)

38. Yang H, Zhang Y, Zhou Z, Jiang X, Shen A: Snail-1 regulates VDR signaling and inhibits 1,25(OH)-D3 action in osteosarcoma. *Eur J Pharmacol* 670(2-3),341-6. (2011)

39. Ehrhart NP, Dernell WS, Hoffmann WE, Weigel RM, Powers BE, et al: Prognostic importance of alkaline phosphatase activity in serum from dogs with appendicular osteosarcoma, 75 cases (1990-1996). *J Am Vet Med Assoc* 213,1002–1006. (1998)

40. Garzotto CK, Berg J, Hoffmann WE, Rand WM: Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. *J Vet Int Med* 14,587–592. (2000)

41. Bacci G, Longhi A, Versari M, Mercuri M, Briccoli A, et al: Prognostic factors for osteosarcoma of the extremity treated with neoadjuvant chemotherapy. *Cancer* 106,1154–1161. (2006)

42. Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, 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* 20,776–790. (2002)

43. Rawadi G, Vayssiere B, Dunn F, Baron R, Roman-Roman S: BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. *J Bone Miner Res* 18,1842–1853. (2003)

44. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, et al: LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 107,513–523. (2001)

45. Matsuzaki E, Takahashi-Yanaga F, Miwa Y, Hirata M, Watanabe Y, Sato N, Morimoto S, Hirofuji T, Maeda K, Sasaguri T: Differentiation-inducing factor-1 alters canonical Wnt signaling and suppresses alkaline phosphatase expression in osteoblast-like cell lines. *J Bone Miner Res* 21(8),1307-16. (2006)

46. Miwa Y, Sasaguri T, Kosaka C, Taba Y, Ishida A, Abumiya T, Kubohara Y: Differentiation-inducing factor-1, a morphogen of Dictyostelium, induces G1 arrest and differentiation of vascular smooth muscle cells. *Circ Res* 86,68–75. (2000)

47. Takahashi-Yanaga F, Taba Y, Miwa Y, Kubohara Y, Watanabe Y, Hirata M, Moromoto S, Sasaguri T: Dictyostelium differentiation-inducing factor-3 activates glycogen synthase kinase-3 beta and degrades cyclin D1 in mammalian cells. *J Biol Chem* 278,9663–9670. (2003)

48. Mori J, Takahashi-Yanaga F, Miwa Y, Watanabe Y, Hirata M, Morimoto S, Shirasuna K, Sasaguri T: Differentiationinducing factor-1 induces cyclin D1 degradation through the phosphorylation of Thr286 in squamous cell carcinoma. *Exp Cell Res* 310,426–433. (2005)

49. An JH, Yang JY, Ahn BY, Cho SW, Jung JY, Cho HY, Cho YM, Kim SW, Park KS, Kim SY, Lee HK, Shin CS: Enhanced mitochondrial biogenesis contributes to Wnt induced osteoblastic differentiation of C3H10T1/2 cells. *Bone* 47(1),140-50. (2010)

50. Piskun CM, Muthuswamy A, Huelsmeyer MK, Thompson V, Stein TJ: Wnt/β-catenin expression does not correlate with serum alkaline phosphatase concentration in canine osteosarcoma patients. *PLoS One* 6(10),e26106. (2011)

51. Chou AJ, Gorlick R: Chemotherapy resistance in osteosarcoma, current challenges and future directions. *Expert Rev Anticancer Ther* 6,1075–1085. (2006)

52. Limmahakhun S, Pothacharoen P, Theera-Umpon N,et al: Relationships between serum biomarker levels and clinical presentation of human osteosarcomas. *Asian Pac J Cancer Prev* 12, 1717-22. (2011)

53. Huczynski A: Salinomycin – a New Cancer Drug Candidate. *Chem Biol Drug Des* 79,235–238. (2012)

54. Tang QL, Zhao ZQ, Li JC, Liang Y, Yin JQ, Zou CY, Xie XB, Zeng YX, Shen JN, Kang T, Wang J: Salinomycin inhibits osteosarcoma by targeting its tumor stem cells. *Cancer Lett* 311(1),113-21. (2011)

55. Major MB, Roberts BS, Berndt JD, Marine S, Anastas J, et al: New regulators of Wnt/beta-catenin signaling revealed by integrative molecular screening. *Sci Signal* 1,ra12. (2008)

56. Verkaar F, van der Stelt M, Blankesteijn WM, van der Doelen AA, Zaman GJ: Discovery of novel small molecule activators of β-catenin signaling. *PLoS One* 6(4),e19185. (2011)

57. Dieudonné FX, Marion A, Haÿ E, Marie PJ, Modrowski D: High Wnt signaling represses the proapoptotic proteoglycan syndecan-2 in osteosarcoma cells. *Cancer Res* 70(13),5399-408. (2010)

58. Dieudonné FX, Marion A, Marie PJ, Modrowski D: 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(10),2118-29. (2012)

59. Wang D, Lippard SJ: Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 4(4),307–320. (2005)

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

61. Wu J, Liao Q, He H, Zhong D, Yin K: TWIST interacts with β-catenin signaling on osteosarcoma cell survival against cisplatin. *Mol Carcinog* (Epub ahead of print) (2012)

62. Lai VK, Ashraf M, Jiang S, Haider K: MicroRNA-143 is a critical regulator of cell cycle activity in stem cells with co-overexpression of Akt and angiopoietin-1 via transcriptional regulation of Erk5/cyclin D1 signaling. *Cell Cycle* 11, 767–777. (2012)

63. Sun H, Lesche R, Li DM, Liliental J, Zhang H, et al: PTEN modulates cell cycle progression and cell survival by regulating phophatidylin- ositol 3,4,5- triphosphateand Akt/protein kinase B signaling pathway. *Proc Natl Acad Sci U S A* 96, 6199–6204. (1999)

64. del Peso L, González-García M, Page C, Herrera R, Nuñez G: Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science* 278, 687–689. (1997)

65. Bacci G, Ferrari S, Bertoni F et al: Long-term outcome for patients with nonmetastatic osteosarcoma of the extremity treated at the Istituto Ortopedico Rizzoli according to the Istituto Ortopedico Rizzoli/osteosarcoma-2 protocol, an updated report. *J Clin Oncol* 18, 4016–4027. (2000)

66. Bacci G, Briccoli A, Ferrari S et al: Neoadjuvant chemotherapy for osteosarcoma of the extremities with synchronous lung metastases, treatment with cisplatin, adriamycin and high dose of methotrexate and ifosfamide. *Oncol Rep* 7, 339–346. (2000)

67. Bertino JR, Göker E, Gorlick R et al: Resistance mechanisms to methotrexate in tumours. *Stem Cells* 14, 5–9. (1996)

68. Slansky JE, Li Y, Kaelin WG et al: A protein synthesis-dependent increase in E2F1 mRNA correlates with growth regulation of the dihydrofolate reductase promoter. *Mol Cell Biol* 13, 1610–1618. (1993)

69. Steinbach G, Lynch PM, Phillips RK, et al: The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 342, 1946 – 1952. (2000)

70. Koki AT, Masferrer JL: Celecoxib, a specific COX-2 inhibitor with anticancer properties. *Cancer Control* 9(2 suppl), 28 – 35. (2002)

71. Arun B, Goss P: The role of COX-2 inhibition in breast cancer treatment and prevention. *Semin Oncol* 31(2 suppl 7), 22 – 29. (2004)

72. Cho SJ, Kim N, Kim JS, et al: The anti-cancer effect of COX-2 inhibitors on gastric cancer cells. *Dig Dis Sci* 52, 1713 – 1721. (2007)

73. Soh JW, Kazi JU, Li H, et al: Celecoxib-induced growth inhibition in SW480 colon cancer cells is associated with activation of protein kinase G. *Mol Carcinog* 47, 519 – 525. (2008)

74. Amir M, Agarwal HK: Role of COX-2 selective inhibitors for prevention and treatment of cancer. *Pharmazie* 60, 563-570. (2005)

75. Thun MJ, Henley SJ, Patrono C: Nonsteroidal anti-inflammatory drugs as anticancer agents, mechanistic, pharmacologic and clinical issues. *J Natl Cancer Inst* 94, 252-266. (2002)

76. Cui W, Hu SX, Tang ZY, et al: In-vivo effects and mechanisms of celecoxib-reduced growth of cyclooxygenase-2 (COX-2)-expressing versus COX-2-deleted human HCC xenografts in nude mice. *Anticancer Drugs* 19, 891-897. (2008)

77. Boon EM, Keller JJ, Wormhoudt TA, et al: Sulindac targets nuclear β-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. *Br J Cancer* 90, 224-229. (2004)

78. Buchanan FG, DuBois RN: Connecting COX-2 and Wnt in cancer. *Cancer Cell* 9, 6-8. (2006)

79. Kim SH, Song SH, Kim SG, et al: Celecoxib induces apoptosis in cervical cancer cells independent of cyclooxygenase using NF-κB as a possible target. *J Cancer Res Clin Oncol*130, 551-560. (2004)

80. Zhang H, Ye Y, Bai Z, et al: The COX-2 selective inhibitor-independent COX-2 effect on colon carcinoma cells is associated with the Delta1/Notch1 pathway. *Dig Dis Sci* 53,2195-2203. (2008)

81. Xia JJ, Pei LB, Zhuang JP, Ji Y, Xu GP, Zhang ZP, Li N, Yan JL: Celecoxib inhibits β-catenin-dependent survival of the human osteosarcoma MG-63 cell line. *J Int Med Res* 38(4),1294-304. (2010)

Abbreviations, OS,osteosarcoma; ALP,alkaline phosphatase; MTX,methotrexate; Dsh,Dishevelled; Apc,Adenomatous Polyposis Coli; GSK3β,glycogen synthase kinase-3β; 1,25(OH)-D3,1,25-dihydroxyvitamin D3; DIF-1,differentiation-inducing factor-1; siRNA,small interfering RNA; PI3K,phosphatidylinositol 3-kinase; COX,cyclo-oxygenase; NSAIDs,nonsteroidal anti-inflammatory drugs

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Send correspondence to: Jianning Zhao, Jinling Hosp, Dept Orthopedics, Nanjing Univ, Sch Med, Nanjing 210002, Peoples R China, Tel: 025-80860951, Fax: 025-80860951, E-mail: zhaojianning0207@yeah.net