Wnt Pathway in Osteosarcoma, from Oncogenic to Therapeutic

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ABSTRACT

Osteosarcoma is the most common malignant bone tumor in children and adolescents. Although pathologic characteristics of this disease are clear and well established, much remains to be understood about this tumor, particularly at the molecular signaling level. Secreted signaling molecules of the Wnt family have been widely investigated and found to play a central role in controlling embryonic bone development, bone mass, and postnatal bone regeneration. A variety of studies also suggest that Wnt signaling pathway is closely associated with bone malignancies, including breast or prostate cancer induced bone metastasis, multiple myeloma, as well as osteosarcoma. Here, we provide an overview of the role of Wnt signaling pathway in osteosarcoma development and progression, highlighting the aberrant activation of Wnt pathway in this bone malignancy. We also discuss the potential therapeutic applications for the treatment of osteosarcoma targeting Wnt pathway. J. Cell. Biochem. 115: 625–631, 2014.   © 2013 Wiley Periodicals, Inc.

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Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. Based on the statistics from the American Cancer Society, the 5-year survival rate for patients with localized OS is in the range of 60–80% following modern treatment. However, this survival rate for patients whose cancer has already metastasized at the time it is found is only about 15–30% (http://www.cancer.org/cancer/osteosarcoma). OS is characterized by morphologically abnormal osteoblastic cells producing aberrant osteoids. Approximately 15–20% of patients present with radiographically detectable metastases, and the most frequent site for metastatic presentation is the lung. Studies have demonstrated diverse genetic alterations in OS cells including structural abnormalities, gain and/or loss of chromosomes, mutation in tumor suppressor genes, and epigenetic modifications

[Kansara and Thomas, 2007]. More effective treatment strategies are urgently needed to inhibit local tumor growth and tumor cells from spreading to the lung and to improve patient survival rates. As such, understanding the molecules and their signaling mechanisms involved in the tumor development and metastasis are of key importance for developing new and effective therapeutic approaches.

Secreted Wnt glycoproteins are important regulators of cellular differentiation and function. Abnormal Wnt signaling leads to developmental defects and human diseases affecting either tissue development or homeostasis. Human and mouse genomes encode 19 Wnt and 18 Wnt genes, respectively [Akiyama, 2000]. Wnt ligands are unique, in that they can activate several different receptor-mediated signal transduction pathways. Wnt pathway is highly conserved across

species, including *Caenorhabditis elegans*, *Drosophila melanogaster*, Zebrafish, *Xenopus laevis*, chicken, mouse, and human. Wnt pathway has been found to play a central role in controlling embryogenesis. In bone development, Wnt signaling is required for limb bud initiation, early limb patterning, and, finally, late limb morphogenesis events [Hartmann and Tabin, 2000]. Since the original description of the causal mutations in the low-density lipoprotein receptor related protein 5 (LRP-5) gene that were identified in patients with osteoporosis pseudoglioma syndrome (OPPG) [Gong et al., 2001] and in kindreds with autosomal dominant high bone mass [Boyden et al., 2002], considerable attention has been focused on the key role of Wnt/β-catenin signaling pathway in the regulation of bone mass [Kato et al., 2002; Hartikka et al., 2005]. More recently, a striking finding that Wnt pathway also plays a crucial role in bone regenerative process (e.g., fracture repair) has been uncovered [Chen and Alman, 2009]. Available published data strongly support the notion that modulation of this pathway may provide a promising therapeutic approach to enhance bone formation [Chen et al., 2007]. Furthermore, a variety of studies have also demonstrated that Wnt pathway is critically involved in bone malignancies, such as breast or prostate cancer induced bone metastasis, multiple myeloma, Ewing sarcoma, as well as OS [Tian et al., 2003; Uren et al., 2004; Chen et al., 2011]. In this review, we will focus on the Wnt signaling pathway and its involvement in OS development and metastasis. We will also discuss the possibility to develop new therapeutic approaches targeting this Wnt pathway.

WNT SIGNALING PATHWAY

The Wnt proteins are a large family of secreted cysteine-rich glycoproteins. Cell signaling cascades provoked by Wnt proteins have been well conserved through evolutionary processes among a variety of species. Wnt proteins act on target cells by binding to Frizzleds (Fzs), seven-span transmembrane receptor proteins, and LRP-5/6, single-span transmembrane co-receptor proteins. Wnts activate at least four distinct intracellular signaling cascades: the Wnt/β-catenin pathway, the Wnt/Ca^{2+} pathway, the Wnt/planar cell polarity (Wnt/PCP) pathway, or the Wnt/protein kinase A (Wnt/PKA) pathway [Nelson and Nusse, 2004].

THE CANONICAL WNT PATHWAY

Among the four Wnt pathways in literature, the canonical Wnt pathway (Wnt/β-catenin pathway) is most extensively studied, which regulates the ability of the β-catenin protein to drive activation of specific target genes and regulate a diverse array of biological processes. At least 7 of 19 Wnt proteins, including Wnt-1, Wnt-2, Wnt-3, Wnt-3a, Wnt-4, Wnt-8, and Wnt-10b, have been reported to activate this pathway. The canonical Wnt pathway affects cellular functions by regulating β-catenin levels and subcellular localization. The canonical Wnt pathway is initiated by the binding of appropriate Wnt ligands to the Fzs and LRP-5/6 co-receptor. In the absence of appropriate Wnt ligands, β-catenin is phosphorylated in its NH2-terminal degradation box, polyubiquitinated by βTRCP1 (a component of ubiquitin E3 ligase) or βTRCP2 complex for the following proteasome-mediated degradation by a multi-protein complex comprising glycogen synthase kinase 3β (GSK-3β), adenomatous polyposis coli (APC), and axin [Eastman and Grosschedl, 1999;

Behrens, 2000]. In the presence of an appropriate Wnt ligand, binding of Wnt to receptor complex leads to the activation of the intracellular protein, Disheveled (Dvl), an intracellular mediator that plays a central role in transducing the signal from the receptor complex. The activation of Dvl leads to the inhibition of GSK-3β, results in the collapse of the multi-protein complex [Seto and Bellen, 2004]. Hence, β-catenin cannot be targeted for degradation and it accumulates and translocates to the nucleus, where in concert with members of the T cell factor/lymphoid enhancer factor (TCF/LEF) family, activates the transcription of a wide range of genes, including c-myc and cyclin D1 (CCND1) (Fig. 1).

The complexity of Wnt intracellular signaling pathways parallels the complexity observed in the diversity of Wnt receptors. To date, there are 10 human Fz receptors. It should be emphasized that, although the role of Fz in acting as a receptor for Wnts is long established, LRP-5 and its closely related homologue, LRP-6, are two important molecules in mediating the Wnt/β-catenin pathway [Gordon and Nusse, 2006], in that both of them act as co-receptors for Wnt proteins, and this canonical Wnt pathway can be antagonized by secreted proteins from the Dickkopf (Dkk) family that bind with high affinity to LRP-5 or LRP-6 and thereby directly prevent Wnt binding [Bafico et al., 2001].

THE NON-CANONICAL WNT PATHWAYS

The Non-canonical Wnt pathway functions in a β-catenin-independent manner [Gordon and Nusse, 2006]. Non-canonical Wnt signals are transduced through Fz family receptors and co-receptors, such as tyrosine-protein kinase transmembrane receptor (ROR-2, a member of the receptor tyrosine kinase-like orphan receptor [ROR] family) and receptor-like tyrosine kinase (RYK), but not LRP-5 or LRP-6. There are three other non-canonical Wnt signaling pathways that have been described in the literature.

In the Wnt/Ca^{2+} pathway, Wnt protein (e.g., Wnt-5a) binds to a Fz receptor and co-receptor such as Knypek or ROR-2. This binding stimulates heterotrimeric G proteins, increases intracellular calcium levels, decreases cyclin GMP (cGMP) levels, and activates protein kinase C (PKC) or calcium/calmodulin-dependent protein kinase II (CaMKII) to induce nuclear factor of activated T cells (NF-AT) and other transcription factors [Wang and Malbon, 2003; Kohn and Moon, 2005]. Wnt/Ca^{2+} pathway can affect cell adhesion and cell movement during gastrulation. Wnt/Ca^{2+} pathway also inhibits the canonical Wnt pathway by promoting GSK-3-independent β-catenin degradation. In the Wnt/PCP pathway, Wnt protein signaling through Fz receptors mediates asymmetric cytoskeletal organization, and the polarization of cells by inducing modifications to the actin cytoskeleton. Two independent pathways, which are initiated by Dvl, trigger the activation of the small GTPases Ras homolog gene family (Rho) and Ras-related C3 botulinum toxin substrate (Rac). Activation of Rho requires Daam-1 and leads in turn to the activation of the Rho-associated kinase (ROCK). Rac activation is independent of Daam-1 and stimulates c-Jun N-terminal Kinase (JNK) and nemo-like kinase (NLK) signaling cascades [Habas and Dawid, 2005] (Fig. 2). In addition, the Wnt/PKA pathway activates cyclin AMP (cAMP) response element binding protein (CREB), and thus stimulates CREB-mediated transcription in a PKA-dependent manner.

So far, Wnt-3, Wnt-3a, Wnt-7a, Wnt-8, and Wnt-10b have been shown to mediate the canonical Wnt pathway. Wnt-5a and Wnt-11
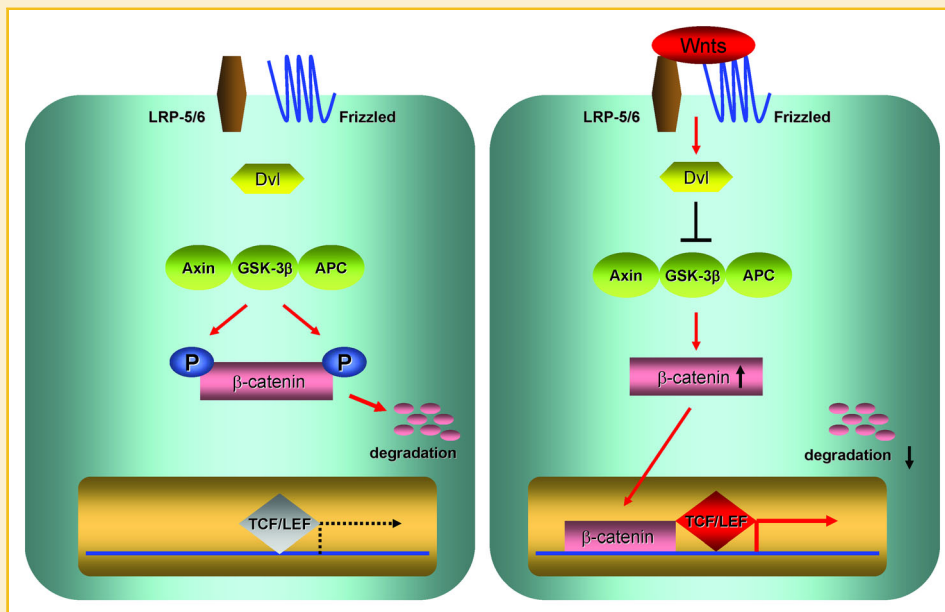
Fig. 1. An overview of the canonical Wnt pathway. In the absence of Wnt ligand, β-catenin is targeted for phosphorylation and degradation by a multi-protein complex comprising GSK-3β, APC, and axin. In the presence of an appropriate Wnt ligand, Wnt bind to the receptor Fz and co-receptor LRP-5/6, and this binding leads to the activation of the intracellular protein, Dvl, an intracellular mediator that plays a central role in transducing the signal from the receptor complex. The activation of Dvl leads to the inhibition of GSK-3β, results in the disassociation of the multi-protein complex and the intracellular accumulation of β-catenin. Hence, β-catenin cannot be targeted for degradation and it accumulates and translocates to the nucleus, where in concert with members of the TCF/LEF family, activates the transcription of a wide range of genes, including c-myc and cyclin D1.

can mediate non-canonical Wnt pathway. Other Wnt ligands, such as Wnt-1 and Wnt-4 can signal through either canonical or non-canonical Wnt pathway. Although the specificity between Wnt molecules and Fz receptors remains unclear, it is known that different Wnt ligands will preferentially activate one of these four canonical or non-canonical Wnt pathways.

WNT SIGNALING IN OSTEOSARCOMA

The Wnt pathway is clearly important in many forms of human cancer, especially in epithelial cancer types in which gain- or loss-of-function events appear to contribute to both inherited cancer risk and somatic carcinogenesis. In particular, β-catenin is required for differentiation and proliferation of cells in a wide variety of tissues, including bone, brain, heart, mammary gland, skin, intestine, and the reproductive system. As such, aberrant activation of Wnt/β-catenin pathway is closely associated with a variety of human cancers including OS.

WNT LIGANDS AND RECEPTORS IN OSTEOSARCOMA

In OS, overexpression of numerous Wnt components including Wnt ligands and Frizzled and LRP receptors highlighted the implications of aberrant Wnt/β-catenin signaling in the development and progression of OS. Hoang et al. examined Wnts, Fzs, and LRP-5 expression using reverse transcription polymerase chain reaction (RT-PCR) in four OS cell lines (U2OS, HOS, 143B, and Saos-2). Of the Wnt-related genes, highly transforming Wnt-1 was expressed by 2 out of 4 cell lines. Three out of 4 cell lines expressed Wnt-4, while all

cell lines expressed weakly transforming Wnt-5a and 7a. None of these cell lines showed any detectable expression of Wnt-11. Of the receptors, while Fz-3 was only expressed by 143B, Fz-1, Fz-2, Fz-4, Fz-5, Fz-9, and LRP-5 were expressed by all [Hoang et al., 2004a]. Interestingly, 22 out of 44 (50%) OS patient samples express LRP-5, which correlated significantly with tumor metastasis [Hoang et al., 2004a], suggesting that expression of LRP-5 is a common event in OS. In another study, Chen et al. [2008] detected expression of multiple Wnt ligands (i.e., Wnt-2b, Wnt-3, Wnt-5a, Wnt-5b, and Wnt-14) and receptors (i.e., Fz-1, Fz-2, Fz-3, Fz-6, Fz-7, LRP-5, and LRP-6) in two human OS cell lines MG63 and HOS. In two murine OS cell lines K7M2 and K12 cells, Wnt-2, Wnt-3, Wnt-5a, Wnt-6, Wnt-7a, Wnt-8a, Wnt-10b, Wnt-15, Fz-1, Fz-5, LRP-5, and LRP-6 were all detected. Immunohistochemistry studies of 44 human OS samples demonstrated that Wnt-10b was observed in 75%, with a trend toward decreased survival in these patients [Chen et al., 2008].

Using quantitative real-time PCR (qPCR), Ma et al. [2013] showed that Wnt-3a is highly upregulated in Saos-2 tumor cells, as compared to human fetal osteoblasts (hFOB). Using a Microarray approach, Modder et al. [2011] reported that Wnt-10b, but not Wnt-3a upregulated the tumor necrosis factor-α (TNF-α, known inducers of NFκB), NFκB activity, and genes involved in Notch pathway (Notch-1 and Jagged-1) in human U2OS OS cells. Recently, Guo et al. [2007] demonstrated that Saos-2 cells transfected with a dominant-negative soluble LRP-5 resulted in an increase in E-cadherin and decrease in N-cadherin, which are epithelial cell marker and mesenchymal biomarker, respectively. This led to the reversal of the epithelial-to-mesenchymal transition (EMT), a hallmarker of OS.
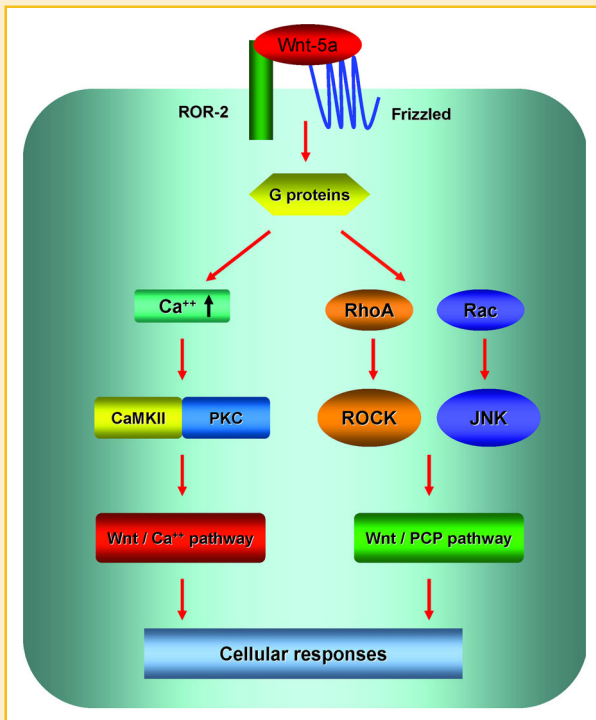
Fig. 2. An overview of the non-canonical Wnt signaling pathway. In the Wnt/Ca²⁺ pathway, Wnt-5a binds to a Fz receptor and co-receptor ROR-2. This binding activates G proteins and increases intracellular calcium levels or decreases cGMP. Hence, the protein kinase C (PKC) or calcium/calmodulin-dependent protein kinase II (CaMKII) was activated. In the Wnt/PCP pathway, Wnt binds to its Fz receptor and triggers the activation of the small GTPases Ras homolog gene family (e.g., RhoA) and Ras-related C3 botulinum toxin substrate (Rac). Activation of RhoA leads to the activation of the Rho-associated kinase (ROCK). Rac activation stimulates c-Jun N-terminal Kinase (JNK) signaling cascade.

The increase in E-cadherin also caused a corresponding decrease in Slug and Twist, the Wnt-responsive transcriptional repressors shown to promote cancer progression and metastasis, suggesting that LRP-5 promotes EMT and OS invasiveness. The same research group also showed that dominant-negative LRP-5 (DNLRP5) caused in vivo anti-tumor activity in DNLRP5-transfected 143B cells injected into a nude mouse model. Interestingly, DNLRP5 was associated with a decreased primary and metastatic tumor burden in the same orthotopic xenograft model.

Based on these studies, overexpression of multiple Wnt ligands and receptors clearly suggested an autocrine or paracrine mode of Wnt pathway in OS. In particular, LRP-5 is significantly involved in OS disease progression as reflected in the tendency for tumors that express this receptor to metastasize.

β-CATENIN AND TCF/LEF-DEPENDENT TRANSCRIPTION IN OSTEOSARCOMA

The involvement of β-catenin in tumorigenesis was first established in colorectal cancer, where it is found to form a complex with the APC tumor-suppressor. Recent studies demonstrated that deregulation of β-catenin signaling has been found to be a common occurrence in OS. Haydon et al. [2002] revealed cytoplasmic and/or nuclear accumulation of β-catenin in 33 of 47 (70.2%) patient samples. When analyzed against other clinicopathologic parameters, β-catenin accumulation correlated only with younger age (26.4 vs. 39.8 years). Similarly, Iwaya et al. [2003] also showed cytoplasmic and/or nuclear staining of β-catenin in both LM8 and Dunn OS cells. Interestingly, LM8 cells with a high metastatic potential to the lung displayed much stronger staining intensity than in the original Dunn cells, suggesting β-catenin is a biological marker of lung metastasis. Notably, using sequencing analysis, both two research groups didn't detect any mutations in the exon 3 in the β-catenin gene, although this site harbors the majority of β-catenin mutations identified in other human malignancies [Haydon et al., 2002; Iwaya et al., 2003].

Furthermore, β-catenin and its transcriptional factor, LEF-1, were also shown to be consistently upregulated in human OS Saos-2 cells compared to hFOB [Ma et al., 2013]. Knocking down β-catenin increased the Saos-2 sensitivity to methotrexate (MTX) induced cell death. Consistently, the expression level of β-catenin protein correlated with the invasiveness of OS, and chemical inhibition of the Wnt/β-catenin signaling enhanced MTX mediated death of Saos-2 cells. In another study, exogenous Wnt-3a and Wnt-10b treatment induced Dvl phosphorylation and β-catenin stabilization in two murine OS cell lines K7M2 and K12. Interestingly, both recombinant Wnt-3a and Wnt-10b ligands significantly increased TCF-4 dependent transcriptional activity and cell motility of metastatic K7M2 greater than non-metastatic K12 cells [Chen et al., 2008].

SILENCING OF WNT INHIBITORY FACTOR 1 (WIF-1)

Wnt signaling pathway is tightly controlled by secreted antagonists that either bind Wnt receptors, exemplified by the Dkk [Niehrs, 2006] and sclerostin, or directly bind Wnt ligands, such as the Wnt inhibitory factor 1 (Wif-1), the secreted frizzled-related protein (SFRP) family, and Cerberus [Kawano and Kypta, 2003]. Recently, Wif-1 was shown to be epigenetically silenced due to promoter hypermethylation, and this silencing activate β-catenin in 5 human OS cell lines (143B, G292, HOS, Saos-2, and SJSA) and in primary human OS samples [Kansara et al., 2009]. In vitro studies showed that Wif-1 not only suppressed β-catenin levels, but also inhibited tumor cell growth, while inducing differentiation of human and mouse primary osteoblasts. Importantly, targeted deletion of mouse Wif-1 accelerated development of radiation-induced OS in vivo [Kansara et al., 2009]. These findings suggest that de-repression of Wnt signaling by targeting secreted Wnt antagonists in osteoblasts may increase susceptibility to OS. Hoang and his group also provided evidence indicating epigenetic silencing of Wif-1 in OS [Rubin et al., 2010]. They reported downregulation of Wif-1 mRNA in several OS cell lines as compared with normal human osteoblasts, and this is closely associated with its promoter hypermethylation.

NON-CANONICAL WNT PATHWAY IN OSTEOSARCOMA

The receptor tyrosine kinase ROR-2 regulates cell migration by acting as a receptor or co-receptor for Wnt-5a, activation of ROR-2 by Wnt-5a inhibits β-catenin mediated canonical Wnt pathway but stimulate the non-canonical Wnt/JNK pathway [Mikels and Nusse, 2006]. It has been shown that Wnt-5a/ROR-2 signaling involves the activation of Src-family protein tyrosine kinases (SFK), leading to matrix

metalloproteinase 13 (MMP-13) expression, and that constitutively active Wnt-5a/ROR-2 signaling confers invasive properties on OS cells in a cell-autonomous manner [Enomoto et al., 2009].

Furthermore, Lu et al. [2012] recently showed Wnt-5a was detected in 34/42 (81.0%) and ROR-2 was detected in 31/42 (73.8%) OS patient samples, significantly higher than in osteochondroma samples (16.7% and 25.0%). Expression of these proteins was positively correlated and both correlated with Enneking surgical stage and tumor metastasis. Thus, Wnt-5a and ROR-2 were more highly expressed in more severe disease states, and therefore may play a coordinated role in the occurrence and progression of OS.

TARGETING WNT PATHWAY AS A THERAPEUTIC APPROACH FOR OSTEOSARCOMA

A variety of studies have demonstrated the role of aberrantly activated Wnt pathway in the development and progression of OS. Therefore, the Wnt pathway can be targeted at multiple levels for therapeutic purposes [McQueen et al., 2011; Hoang, 2012]. Several strategies have been devised to exploit the Wnt pathway for therapeutic purpose.

RECOMBINANT WNT PATHWAY INHIBITORS

It is clear that activation of Wnt pathway plays an important role in the biology of OS. Hence, strategies targeting to inactivate this pathway using recombinant Wnt inhibitors might represent a useful therapeutic approach to inhibit OS. One good example is Dkk-3, which is also known as reduced expression in immortalized cells, has been implicated as a tumor suppressor exhibiting downregulation in several cancer cell lines [Tsuji et al., 2000]. Hoang et al. [2004b] found that recombinant Dkk-3 was able to inhibit invasion and motility of Saos-2 OS cells by blocking the cytosolic accumulation of β-catenin. More recently, Hoang's group further demonstrated Dkk-3 had inhibited tumorigenesis and metastasis, in that inoculation of Dkk-3-transfected 143B cell lines into nude mice showed significantly decreased tumor growth and less metastatic pulmonary nodules (88.7%) compared to the control vector. This antitumor effect was mediated through Dkk-3 induced downregulation of anchorage-independent growth and cellular motility [Lin et al., 2013].

Promoter hypermethylation induced epigenetic silencing of Wif-1 has been closely associated with OS development [Kansara et al., 2009]. Preclinical studies demonstrated that recombinant Wif-1 exerts potent anticancer effect, as 143B tumor cells over-expressing Wif-1 displayed a significant decrease in tumor growth and induced much less lung metastasis [Rubin et al., 2010]. These findings suggested that reexpression of Dkk-3 (or Wif-1) in Dkk-3 (or Wif-1)-deficient OS tumors may prove to be of a valuable preventive or therapeutic strategy.

ANTIBODY–BASED THERAPEUTICS

As aberrant Wnt pathway activation by increased expression of Wnt ligands and receptors is a frequent event in OS, this presents an opportunity to develop antibodies against the overexpressed Wnt and Fz proteins as potential OS therapeutics. Monoclonal antibody against Wnt-1 triggered apoptosis in several cancer lines including sarcoma, and effectively blocked tumor growth in mice [He et al., 2004]. Mikami et al. [2005] also observed decreased β-catenin

and significant apoptosis induction in monoclonal anti-Wnt-1 antibody treated SJSA-1 OS cells and tumor cells that had metastasized to the lung. These preliminary results are encouraging, but their in vivo efficacy as antitumor agents is required to be rigorously evaluated.

SMALL MOLECULES INHIBITORS

Small molecule inhibitors hold great promise for the delay of OS tumorigenesis and metastasis. Besides being readily available in abundance, natural compounds have diverse structural, and stereochemical characteristics and are thus valuable sources for exploring molecular diversity in drug development. Leow et al. reported anticancer effects of two such inhibitors, curcumin and PKF118-310. Both two compounds suppressed intrinsic and activated β-catenin/TCF transcriptional activities, and induced a dose-dependent decrease in OS cell migration and invasion. The antiproliferative effect of PKF118-310 is attributed to PKF118-310-induced apoptosis and G2/M phase arrest. Moreover, these anticancer effects correlated with the decreased expression of Wnt target genes involving CCND1, c-myc, and survivin. Besides inactivation of Wnt pathway, Li et al. provided evidence that curcumin also inhibits proliferation and invasion of OS cells through downregulation of Notch-1 signaling, thus adding another mechanism in this antitumor effect [Xia et al., 2010]. Dihydroartemisinin (DHA) is a semi-synthetic derivative of artemisinin and is widely used as an intermediate in the preparation of other artemisinin-derived anti-malarial drugs. DHA also exhibits antitumor activity against a wide spectrum of cancer cells. Most recently, Liu et al. reported that DHA can antagonize Wnt signaling through its stimulation of catalytic activity of GSK-3β. DHA not only inhibits proliferation, migration, and invasion, but also induces apoptosis in human OS cells. In addition, DHA prevents OS formation and maintains intact bone structure in athymic mice [Liu et al., 2013]. Ji et al. [2011] further showed that DHA exerts its anticancer effect against OS through its induction of apoptosis and cell cycle arrest.

WNT TARGET GENES AS THERAPEUTIC TARGETS

Aberrant β-catenin mediated, TCF/LEF dependent transcriptional activity is considered to drive cancer formation by altering expression of a limited set of targets genes controlling cell proliferation, differentiation, migration, and apoptosis. Some of these target genes, such as c-myc and CCND1 are directly implicated in driving OS formation. High level oncogene c-myc amplification and expression was shown in numerous cancers. In the MG63 OS cell line, transfection of an antisense c-myc fragment induced cell cycle arrest and enhanced apoptosis. Using a conditional transgenic mouse model, Arvanitis et al. [2008] showed that myc inactivation caused proliferative arrest and promoted differentiation in OS. Moreover, transient myc inactivation appears to cause epigenetic changes in tumor cells that render them insensitive to myc-induced tumorigenesis [Jain et al., 2002]. CCND1 is an important regulator of the cell cycle that is highly expressed in human cancers as an indirect consequence of Wnt signaling [Tetsu and McCormick, 1999]. Silencing of CCND1 using lentivirus mediated RNA interference (RNAi) enhanced the cytotoxicity of doxorubicin (DXR) in the drug-resistant human OS MG63 cells [Zhang et al., 2012]. In another study,

Gli-2 was aberrantly overexpressed in human OS biopsy specimens. Knockdown using RNAi prevented OS growth and promoted the arrest of OS cells, and this antitumor effect is asslociated with protein level of CCND1 [Nagao et al., 2011].

Other Wnt target genes such as CD44 and Cyclooxygenase 2 (Cox-2) are also likely to contribute to cancer formation or progression and might be amenable to therapeutic intervention. Cox-2 inhibitor, celecoxib, was shown to reduce cytosolic and nuclear β-catenin and inhibit survival in OS MG63 cells [Xia et al., 2010].

CONCLUSIONS

The Wnt signaling plays a key role in bone development and regeneration. This pathway also plays an important role in bone malignant diseases, such as breast or prostate cancer induced bone metastasis, multiple myeloma, Ewing sarcoma, and OS. In OS, overexpression of numerous Wnt components including Wnt ligands, Fzs, and LRP receptors, as well as the epigenetic silencing of genes encoding endogenous Wnt pathway inhibitors, such as Wif-1, highlighted the implications of aberrant Wnt signaling in the development and progression of this bone malignancy. So far, our knowledge concerning how Wnt pathway regulates OS is still not fully understood, and there are many issues to be addressed. For example, the specificity of interaction between Wnt ligands and Fz receptors is unknown, and the role of non-canonical Wnt signaling in OS is poorly investigated. It also seems important to uncover which of the Wnt ligands are critical to mediate canonical or non-canonical Wnt pathway in OS. In addition, OS development is a complex process involving multiple molecular signaling pathways, thus another attention should focus on how these pathways interact with Wnt signal.

Nevertheless, the complexity of the Wnt pathway makes it amenable to therapeutic intervention at many levels, ranging from inhibition of ligand-receptor interactions at the cell surface to blockade of β-catenin and inactivation of target genes. The development of therapeutics specifically targeting the aberrant Wnt pathway in OS cells is still in its infancy, with no drugs currently in late-stage clinical trials that we are aware of. However, existing drugs, especially small molecule inhibitors seem to hold great promise for the treatment of OS. Future challenges will be to identify small molecules with improved selectivity for the β-catenin-TCF/LEF interaction, to limit the potential for side effects resulting from disruption of other β-catenin complexes that are essential for maintaining cell adhesion and regulation of the Wnt pathway in non-cancer tissues.

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