The handle http://hdl.handle.net/1887/20397 holds various files of this Leiden University dissertation.

Author: Anninga, Jakob Klaas
Title: Clinical and molecular features of high-grade osteosarcoma
Issue Date: 2013-01-09
Summary and concluding remarks
In this thesis 7 chapters are presented, describing clinical, pathological and molecular studies related to the most common primary bone tumour, osteosarcoma. **Chapter 1, the general introduction**, is an overview of epidemiology including incidence, age distribution, localization in the skeleton, risk factors and survival. The objective of this description is to gain more insight in the clinico-pathological behavior of osteosarcoma, based on epidemiologic information (1). The incidence pattern is age dependent. Osteosarcoma in children under 5 years of age is rare, less than 2% of all osteosarcomas occur in this age group. A steep rise in incidence occurs during puberty, peaking at an incidence of 8.6 cases/10^6 population up to 20 years of age, followed by a low rate of on average 1.7/10^6 population during adulthood (25–59 years of age) (2). A non-unified second peak occurs in people of 60+ years, reaching 4.9/10^6 new cases yearly. Remarkably, this 2nd incidence peak is absent in the Asian people (3). This peak has suggested to be due to secondary osteosarcomas, after radiation or as complication of Paget’s disease. The different distribution and histology of osteosarcomas in patients older than 60 years of age suggests indeed a distinctive biological behaviour. An adequate treatment is of utmost importance for survival of patients that has not improved the past 2-3 decades. Contemporary treatment consists of pre-and postoperative (neo-adjuvant) chemotherapy and radical surgery. If no clear resection margins can be obtained, the patient has a very high risk of being incurable.

With respect to the prognosis of patients with osteosarcoma, the chances for survival after incomplete surgery are less than 15% (4). Hence locations where complete resection is impossible, for example axial or pelvic site, have strong influence on outcome. Axial site is more often present in older patients, therefore age can be biased by the site of the primary tumour with respect to prognosis. For resectable disease, metastases at diagnosis, proximal site and large (≥ 1/3 extremity length) size of the primary tumour are the most important adverse prognostic factors (4-6). Two treatment related factors are also of favourable prognostic importance, these are good histologic response on pre-operative chemotherapy and presence of chemotherapy induced toxicity (7). Other factors, such as pathological fracture at diagnosis, type of surgery, age and gender were of minor prognostic importance. Genetic risk factors, like the Li-Fraumeni syndrome, the (heritable or bilateral) Retinoblastoma, the helicase-mutation syndromes and other diseases in their context to osteosarcoma are discussed in this chapter.

The pathology of osteosarcoma was discussed, with emphasis on the unconventional subtypes of high-grade osteosarcoma and the low-grade osteosarcoma variants. This was chosen because these variants contribute to only 5% of all osteosarcomas but, were overrepresented in the hands and feet (discussed in chapter 6).

In **chapter 2** the literature of chemotherapeutic treatment of localized, non-metastatic osteosarcoma of the extremities was reviewed. One of the main conclusions was that there are not more than 4 effective cytostatic drugs, where efficacy is defined as an response rate (RR) in phase-II trials of 20% or more. These 4 drugs are doxorubicin (RR 43%), ifosfamide (RR 33%), methotrexate (RR 32%) and cisplatin (RR 26%). Meta-analysis demonstrated that 2-drug regimens (mainly consisting of doxorubicin and cisplatin) are inferior to regimens containing 3 or more drugs. According to this analysis there was no survival benefit of 4-drug regimens compared to 3-drug regimens. Therefore a 3-drug combination such as
methotrexate, doxorubicin (a.k.a. adriamycin) and cisplatin, a regimen referred to as MAP, is considered the best induction regimen and should be used as standard treatment for osteosarcoma in clinical protocols. The debate remains whether adding a high responsive drug, like ifosfamide, to MAP should be reserved for non-responding patients or in cases of progressive disease. Furthermore, it was concluded that investigating more of this type of conventional drug regimens would not be advantageous.

Therefore, we started a study in osteosarcomas to investigate if genome wide gene expression provides a better insight into the biology of this tumour. Gene expression pattern of 25 high-grade osteosarcoma biopsies were correlated to the outcome of disease or response to neo-adjuvant treatment. In addition we investigated if drug targets from such expression data could be determined. The results of this study were presented in chapter 3 and showed that nearly 3000 genes were significantly differentially expressed in osteosarcoma, compared with non-malignant cells (osteoblastomas, mesenchymal stem cells and mesenchymal stem cells differentiated into osteoblasts). Gene expression profiles could not be correlated to either response to treatment or survival. Analysis at a single gene level proved to be not useful in osteosarcomas, because this tumour has a highly complex karyotype, that diminishes the reliability of single genes to predict the clinical determinants of malignant diseases, unless thousands of samples are used (8). Therefore, pathway analysis was chosen as a method for further analysis of malignant transformation of the mesenchymal stem cell, the presumed precursor of osteosarcoma (9).

At pathway level, we found down-regulation of the Wnt3a/β-catenin signalling (reflected by downregulation of Axin and CCND1), upregulation of the Wnt5a/alternative signalling, overexpression of the cell cycle genes and a disturbed p53/apoptotic pathway (reflected by downregulation of BBC3/PUMA) in osteosarcomas.

The statistical background for the choice for pathways analysis is described in chapter 4. This paper describes the algorithm for the association of the expression of groups of genes with clinical variables. The groups of genes can be clustered based on pathways, as defined for example in the Gene Ontology data base (http://amigo.geneontology.org) (10). The Global Test can test the statistical significance of a certain pathway, attributed to a clinical variable of interest, for instance survival. The test is based on the Cox proportional hazards model, with the possibility to adjust for the presence of co-variables. In this paper, the expression profile of the patients, whose biopsies were analyzed in chapter 3, were tested. Using this model it was found that pathways, involving the cell cycle, DNA repair and apoptosis were associated with survival. It was further concluded that using the Cox model, survival data are not lost and can be adjusted for the presence of co-variates, which allows to improved performance of this test.

In order to establish molecular targets for osteosarcoma treatment, the epidermal growth factor receptor HER2 was mentioned as a candidate and is the subject of research, described in chapter 5. Her2 is highly expressed in 25% of the breast cancer patients, and its related tumorigenic effects (11, 12) can be reverted by the monoclonal antibody trastuzumab (Herceptin®) (13). Based on the presumed overexpression of Her2 in osteosarcomas (14-16), a phase-II study with trastuzumab was initiated in osteosarcomas (www.cancer.gov/clinical_trials: MSKCC-99097/NCI-T98-0083 and COG-AOST0121). However, in our study no
membranous (3+) HER2 overexpression was found, which is a prerequisite for trastuzumab treatment (17, 18). Neither HER2 mRNA was overexpressed at the gene level, nor FISH analysis showed HER2-gene amplification in the single sample that stained moderate (2+) positive membrane staining. We concluded that HER2-gene amplification or membranous HER2 protein overexpression is absent in human osteosarcoma, and that we cannot support the principle to treat osteosarcomas with Herceptin.

After we had confirmed the complexity of osteosarcoma at molecular level with the gene-expression study, another question was whether there is also clinical evidence for heterogeneity of osteosarcomas. To answer this question, we studied the clinico-pathological features of osteosarcomas with a rare localization, i.e. the small tubular and flat bones of the hands and feet. The results of this study are described in chapter 6. In total 40 patients with osteosarcomas of the hands or feet, obtained from the merged Dutch (10/1733) and the Rizzoli Institutional databases (30/2488) were described, representing only 0.95% of all osteosarcomas, present in both databases. Compared with the usual sites (around the knee or humerus), osteosarcomas in hands or feet occurred in older patients (mean age 42 years), with a male predominance (male female ratio=1.7:1), patients had a longer delay before the definitive diagnoses was made, and had a higher proportion of low grade (30%) and intermediate grade (5%) of malignancy compared to osteosarcoma at conventional sites, that show low-grade malignancy in 1%-2% (19). Overall cumulative incidence of death (CID) of the whole group was 80%, however worse in patients with location in the hands (4y CID 38%) than in the feet (2.5 CID 11%), and no deaths were observed in patients with low-or intermediate grade osteosarcomas. It was concluded that high-grade osteosarcoma of the hands or feet are a peculiar subgroup of osteosarcomas, and that high-grade tumours have a similar prognosis as osteosarcoma in the long tubular bones of the skeleton. It is recommended to treat high-grade osteosarcomas of the distal extremities in the same way as those tumours at conventional sites.

The last part of this thesis, chapter 7, deals with osteosarcoma as systemic disease, which occurs as synchronous metastases (metastases at diagnosis, in 16% of the newly diagnosed patients (4, 20-24) or as recurrent or relapsed disease (metachronous metastases), which occurs in 45% of all patients treated for localized osteosarcoma (4, 20-26). A study was done to determine prognostic factors in 88 patients with pulmonary (n=26 synchronic, n=62 metachronic) metastases from the Leiden University Medical Center data base. Overall survival of the patients with resectable metastatic osteosarcoma was 23%, not worse for patients with synchronous versus metachronous metastases. Survival was determined only by resectability of the metastases, even if surgery was more often than once required. Poor prognostic factors for survival in patients who underwent surgery were high (5 or more) number of metastases (HR 1.29), whereas favorable prognostic factors were necrotic metastases (HR 0.17) and female gender (HR 0.41). Although it would suggest that pre-operative chemotherapy could induce necrotic metastases, the trend towards better survival for patients who received chemotherapy, found in this study was not significant ($\chi^2$ p=0.04). Overall, it was concluded that cure can be achieved in a subset of patients with (synchronous or metachronous) metastases by aggressive surgical treatment, but the role of chemotherapy remains elusive.
DISCUSSION

From the above chapters it can be concluded that high-grade osteosarcoma cannot be considered as one disease, but is a heterogeneous tumour at clinical, pathological and genomic level. This may be the reason that contributes to the lack of improvement in survival during the past 3 decades. One of the important findings in this thesis was that there are only 4 effective drugs against high-grade osteosarcoma, i.e. doxorubicin, methotrexate, cisplatin and ifosfamide. After relapse, the treatment options become even more limited, because re-using the same drugs questions their efficacy, and adds to the cumulative toxicity of these drugs (27), like cardiac (28), hearing loss (29), renal damage (30, 31), fertility problems (32, 33) or second malignancies (34). Treatment with the monoclonal antibody Herceptin could not be supported by us and others, because there is no membranous HER2-receptor overexpression on osteosarcomas, as is shown by us and others (35-38). Array analysis revealed up-regulation of cell cycle genes and a disturbed Wnt- and p53/apoptotic signalling as most important abnormal pathways in osteosarcomas compared with non-malignant cells. Upregulation of cell cycle genes is not surprising in cancer cells, neither disturbance of the apoptotic pathway. In order to think of the Wnt-signalling as potential therapeutic target for osteosarcoma, the Wnt-pathway in general and as far relevant for osteosarcoma will shortly be discussed shortly in the next paragraph.

Wnt-pathway

The Wnt signalling plays an important role in developmental biology and in cancer (39, 40). Due to the numerous Wnt-ligands (n=19), Wnt-receptors (Frizzled: Fzd's n=10), co-receptors (n=8) and modulators, like Wnt-inhibitors (Dickkopfs, Wnt-inhibiting factors, soluble Fzd-related proteins and proteoglycans) the downstream signals after ligand-receptor binding are pleiotropic and tissue specific, spatial-and time dependent (39, 41, 42). That means that the effect of Wnt signalling in the bone marrow niche (reservoir mesenchymal stem cells (MSC) is different from Wnt signalling in cartilage of tubular bones or in flat bones, or in other tissues. For an extensive discussion about these topics, the reader is referred to some excellent reviews (39, 40, 43, 44).

Modern insights in these complexities have replaced the old distinction of canonical and non-canonical by β-catenin dependent and β-catenin independent respectively, and an overview of both pathways is given in Figure 1. In summary, the β-catenin dependent (or canonical) pathway stabilizes cytoplasmatic β-catenin after binding of the Wnt3a (or other “canonical” Wnts) with the Fzd2/7 receptor, by inhibiting the proteosomal degradation of the continuously formed β-catenin (Fig.1 A) (40). Due to the rising cytoplasmatic concentration, β-catenin shuttles to the nucleus, and activates transcription factors for proliferation (de-repression) (45) or induces differentiation (co-activation) of for instance Runx2 (46, 47), a master gene for osteogenesis. The β-catenin independent signalling is activated after binding of Wnt5a with either Fzd2 (Fig.1, B) or Fzd4 (Fig.1 C), with or without the co-receptor ROR2 or with ROR2 as a single receptor (Fig.1 E). The oncogenic transcription factor Jun-N-terminal kinase (JNK)
(48) is activated after Wnt5a-Fzd2 binding mediated by the small GTP-ases Rho and Rac (Fig.1 C), and is called the *Wnt/PCP pathway* (41, 49). The other β-catenin independent signalling, the *Wnt/Ca2+ pathway* (Fig.1 D), activates the transcription factors NEMO or NFAT, which inhibit β-catenin dependent proliferation (50, 51) and activate osteogenesis (52, 53) respectively. Other modulations of the Wnt5a signalling are shown in Fig.1 by the red circles and are at the level of competitive inhibition of Wnt3a binding with Fzd receptors (Fig.1 F), via the ubiquitin ligase Siah2 (54, 55) or directly via ROR2 activation (56).

Bone development is an complex process, in which Wnt's play an important role in multiple ways (see Fig.2) (for reviews, see (44, 47, 57)). In the early MSC stage Wnt3a/β-catenin is required for lineage fate decision (57-59) and stimulates the proliferation of stem cells to maintain an adequate number of progenitor cells. Furthermore, Wnt3a signalling prevents osteo-chondrogenic progenitors from developing into cartilage and differentiate into osteoblasts (46, 60, 61), but for the definitive differentiation into the osteogenic lineage, Wnt3a needs temporary be downregulated (62, 63), which is mediated via Wnt5a and Dkk1 (62-64). Finally, the β-catenin pathway is required for definitive differentiation of precursor cells into osteocytes (63, 65).
FIGURE 1.
Wnt signalling pathways.

This figure shows a number of different Wnt-signalling pathways, as well as possible cross-talks between the β-catenin dependent (“canonical”, column A), and β-catenin independent (“non-canonical”, column B-E) pathways. The Wnt3a-activated (“on”) β-catenin dependent pathway under column A and the β-catenin repressive (“off”) situation in column F. See text for details. The β-catenin independent signalling is divided into the Wnt/PCP/Jnk and Wnt/Ca2+ pathways. Recently, insights have added a Wnt-Ror2 pathway has been added to these pathways, which inhibits the canonical pathway at 4 possible levels. Firstly, by competitive inhibition with the Wnt3a receptor binding (Red circle 1), by increased degradation of β-catenin via ROR2 activated Siah-2 activity (red circle 2), by repression ofTCF3 transcription via NEMO (red circle 3), or by directly inhibition of β-catenin-TCF binding (red circle 4). Another cross-talk is β-catenin activation, when Wnt5a binds to the Frizzled4 (Fzd4) receptor. The different and complex outcomes of the various Wnt signalling pathways are the result of binding of “canonical” (represented by Wnt3a) or “non-canonical” (represented by Wnt5a) Wnt-ligands to multiple receptors (Fzd’s), either with or without different co-receptors (Lrp’s, ROR’s or Ryk’s), modulated by inhibitors, such as the Dickkopf’s (Dkk’s), the Wnt–inhibiting factors (WIF’s), the soluble Frizzled related proteins (sFRP’s), or Sclerostin (SOST). LRP5,6 lipoprotein related protein 5,6; Dvl Dishevelled; APC Adenomatous Polyposis Coli; GSK-3β Glycogen Synthase Kinase-3β; CK1 Casein Kinase-1α; CamKII Calcium/Calmoduline dependent Kinase II; JNK c-Jun N-terminal Kinase; Rock2 Rho-associated Kinase; β-TrCP is a ubiquitin ligase; TCF1/3 T-cell factor 1/3; Lef1 Lymphoid Enhancer Factor 1; Gro Groucho.
FIGURE 2.
Wnt-signaling in stages of osteoblastic development.

The two Wnt-signalling pathways are represented by Wnt3a as Wnt3a/β-catenin dependent and Wnt5a as Wnt/β-catenin independent pathways. The orange and purple line at the bottom of figure 2 are the hypothetical levels of each of the Wnt-pathways, showing a inhibition of the β-catenin pathway, by Wnt5a and probably Dkk1. In the early mesenchymal stem cell (MSC) stage, β-catenin stimulates the proliferative activity of the cell, and induces the MSC to the osteo-chondrogenic lineage, thereby suppressing the adipogenic lineage (ASC = adipogenic stem cell). In a later stage of the osteochondrogenic progenitor cell, Wnt3a stimulates proliferation of the progenitor cells to ensure a pool of these cells, inhibits chondrogenic differentiation (by suppressing SOX9 and IHH) and starts the induction of the osteogenesis by activating the transcriptionfactor Runx2. To continue osteoblastogenesis, Wnt3a needs to be downregulated, which is done by Wnt5a, and probably Dkk1. For the terminal differentiation of the osteoblasts into osteocytes, Wnt3a has to be upregulated again, in order to activate transcription factors is necessary for differentiation to osteocytes. Disruption of this tightly regulated signalling in normal bone development leads to hyperproliferation, defective differentiation commitment control, resulting in genetic instability and osteosarcomagenesis.

In normal osteogenesis, other signalling systems, such as bone morphogenetic proteins (BMP) and Hedgehog (IHH) are required but are not further discussed here.
Wnt-signaling in stages of osteoblastic development. The two Wnt-signalling pathways are represented by Wnt3a as Wnt3a/β-catenin dependent and Wnt5a as Wnt/β-catenin independent pathways. The orange and purple line at the bottom of figure 2 are the hypothetical levels of each of the Wnt-pathways, showing a inhibition of the β-catenin pathway, by Wnt5a and probably Dkk1. In the early mesenchymal stem cell (MSC) stage, β-catenin stimulates the proliferative activity of the cell, and induces the MSC to the osteo-chondrogenic lineage, thereby suppressing the adipogenic lineage (ASC = adipogenic... systems, such as bone morphogenetic proteins (BMP) and Hedgehog (IHH) are required but are not further discussed here.
Wnt-signalling, cancer and osteosarcoma

In our array study we found evidence for down-regulation of the Wnt3α/β-catenin pathway and up-regulation of the alternative, Wnt5α pathway. This is in contrast to the activating β-catenin deregulation, which is the driving force for tumorigenesis in most types of epithelial cancers, for example in colon cancer (66), ovarian cancer (67), prostate cancer (68) or lung cancer (69). Wnt3α/β-catenin overexpression has been reported in osteosarcoma, either directly (70, 71) or indirectly by inhibition of the Wnt ligand (72, 73) or due to overexpression of the co-receptor LRP5 (74). However, overexpression of β-catenin, as seen in the Gardner syndrome, did not result in an increased incidence of osteosarcomas (75), whereas in the benign osteoblastomas clear expression of β-catenin was observed (76). Absent nuclear β-catenin staining was observed in only one other study of osteosarcoma (77). Recently, Mathushansky reported that inactivation of the β-catenin dependent Wnt pathway was tumorigenic in the high-grade undifferentiated pleomorphic sarcoma (78). It was shown that the mesenchymal stem cell was the progenitor of the undifferentiated sarcoma and that down-regulation of the Wnt/β-catenin dependent pathway failed to commit the stem cell to differentiation into mature connective tissue lineages. In addition Wnt5α was defective in regulating a commitment-viability checkpoint, as is known that this non-canonical pathway mediates anti-apoptotic signalling (79). In another study the Wnt/β-catenin signalling was downregulated in Rhabdomyosarcoma cell line, blocking the normal myogenic differentiation and increasing resistance to apoptosis (80). Restoration of the Wnt3α activation resulted in myogenic differentiation.

Another example of the contribution of an inactive Wnt3α/β-catenin signalling is reported in Retinoblastoma’s (81). Wnt signalling re-activation significantly decreased the viability of the retinoblastoma cells by p53-induced cell cycle arrest. The authors concluded that the Wnt-pathway acted as a tumour suppressor in the retinoblastoma cells lines, and that loss of Wnt signalling contributed to the tumorigenesis in the retina.

Inactivity of the Wnt3α/β-catenin signalling in our study has been confirmed by Cai et al. (76). Restoration of the Wnt3α/β-catenin pathway by inhibition of GSK-3β, that phosphorylates β-catenin, demonstrated differentiation into bone of 2 of 4 osteosarcoma cell lines.

What exactly the role of the downregulation of the Wnt3α/β-catenin pathway in the tumorgenesis of osteosarcoma is, remains difficult to explain. The hypothesis is that, similar as in undifferentiated sarcomas and rhabdomyosarcomas, bone-progenitor cells will not be able to complete osteogenesis and remain in continuous proliferative state (as was shown by the upregulated cell cycle genes). The overexpressed Wnt5α signalling on the other hand drives the osteo-progenitor cells into the direction of osteogenesis (82). However, due to the disturbed apoptotic regulation these cells lack a differentiation commitment check, that results in progressive genomic instability, which is the hallmark of osteosarcoma (83, 84). However, this is still hypothesis, and it would be a challenge to study Wnt-signalling in the normal osteogenesis and in the disturbed osteogenesis, such as in osteosarcoma, or in other pathologic conditions.
Summary and concluding remarks

Wnt signalling and potential therapies
Given the observations that the Wnt3a/β-catenin pathway was inactive in osteosarcomas and that 2 of 4 osteosarcoma cell lines differentiated into normal bone after inhibition of GSK3β, it could be argued that therapy, aiming to inhibit proteosomal degradation of β-catenin might be of advantage in patients with the Wnt-pathway in the off-state. One of the most promising compounds to interfere with the proteosomal activity is bortezomib (85, 86). This drug has shown to restore normal bone development in Multiple Myeloma patients (87, 88), irrespective the response on treatment (89). Although the mechanism is not completely resolved, it has been suggested in these patients that bortezomib inhibits the Wnt3a antagonist Dkk1 (87), induced differentiation of osteoblasts via stabilization of β-catenin (86), or via bortezomib induced apoptosis of the tumour cells (90). In mice that were treated with bortezomib, inhibition of cell proliferation and increased apoptosis of the osteosarcoma cells was observed, resulting in regression of the tumour (85). Bortezomib has been used in clinical phase-1 (91), phase-II (92) or phase-III studies (93), is tolerated well with few side effects. Even in combination with other drugs, that might be used in (refractory) osteosarcoma, or in older patients, bortezomib can be used safely (94-96). Therefore, bortezomib might one of the few agents worth for future evaluation in osteosarcoma therapy, preferably in a window phase in patients with absent Wnt3a/β-catenin dependent signalling.
CONCLUDING REMARKS

It can be concluded from this thesis that high-grade osteosarcoma is at clinical, pathological and molecular level a heterogeneous disease. To treat high-grade osteosarcoma adequately, neo-adjuvant chemotherapy should be combined with radical surgery, irrespective the localization of the tumour. An adequate chemotherapy regimen for osteosarcoma consists at least of 3 out of 4 effective cytostatic agents, i.e. methotrexate, doxorubicin and cis-platin. A fourth active agent, ifosfamide, should possibly be reserved for patients with refractory disease or patients with relapse. Patients with metastatic pulmonary osteosarcoma should receive surgery in case of resectable disease, whereas the use of chemotherapy is these patients can be considered, but is not of proven value. Patients with irresectable metastatic osteosarcoma should be offered phase-I and phase-II studies, because no response can be expected from other conventional cytostatic drug combinations. With respect to new drug developments, the use of the monoclonal antibody trastuzumab against HER2 is not supported by us, because we were not able to demonstrate overexpression of the HER2 receptor on osteosarcoma cells. At molecular level, a disturbed Wnt signalling was found in addition to abnormal cell cycle regulation and a disturbed p53/apoptotic pathway. This combination of these pathway abnormalities might be oncogenic. Failure of the mesenchymal stem cell to differentiate into the osteoblastic lineage, due to abnormal proliferation and lack of differentiation commitment results in chromosomal instability, which is the hallmark of osteosarcoma. In patients with an inactive Wnt3a/β-catenin signalling the proteosome inhibitor bortezomib might be a candidate drug, to explore its suggested differentiation inducing properties. More research should be directed to study Wnt signalling in normal and disturbed osteogenesis, in order to clarify the mechanisms by which Wnt3a has its effects in osteosarcoma.
REFERENCES


Summary and concluding remarks

42. Verkaar F, Zaman GJ: A model for signaling specificity of Wnt/Frizzled combinations through co-receptor recruitment. FEBS Lett 584:3850-3854, 2010
46. Day TF, Guo X, Garrett-Beal L, et al.: Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8:739-750, 2005
60. Hill TP, Spater D, Taketo MM, et al.: Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8:727-738, 2005
63. Marcellini S, Henriquez JP, Bertin A: Control of osteogenesis by the canonical Wnt and BMP pathways in vivo: Cooperation and antagonism between the canonical Wnt and BMP pathways as cells differentiate from osteochondroprogenitors to osteoblasts and osteocytes. Bioessays, 2012


