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Bone Formation Deregulations Are the Oncogenesis Keys in Osteosarcomas

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1. Introduction

The reciprocal osteoblast-osteoclast interactions are essential in the coordinated healthy bone formation and resorption. These communications in the bone microenvironment are highly complex events and need precise regulated molecular processes to ensure constant healthy bone remodeling. (Matsuo and Irie, 2008). Malignant bone tumors seem to be mainly linked to deregulation of this osteoblast-osteoclast cooperation processes. The major malignant bone tumor in pediatrics (5% of pediatric cancers) is high-grade osteosarcoma (OS). Usually, this bone cancer is diagnosed during adolescence and represent the second most common cancer after lymphoma in this period of age. A second peak observed in life is after 50 years. During adolescence, the kids are having their puberty development and the long bones are growing then particularly fast, with a rapid cell turnover in and around the growth plate (Mathew et al., 2011; Marina et al., 2004). Then, in a not surprising way, the most common locations of OS are the long bones (frequently, distal femur, proximal tibia and humerus) and especially in these metaphyseal regions around growth plates. Furthermore, no significant improvement in prognosis for patients with OS was observed since the advent of multiagent chemotherapy, increasing the long term outcome. Even this therapeutic progress, the overall survival of the patients is now at a plateau of 70% (Le Deley et al., 2007; Mirabello et al., 2009). After increasing the patient survival, new challenges regarding chemoresistances are now appearing and are involved in the recurrence of the disease despite a successful local resection. 15 to 20% of diagnosed patients will have radiographically detectable pulmonary metastases whereas 80% will already presenting undetectable micrometastases (Bruland et al., 2009). The lack of prognostic marker at diagnosis is another key point in this cancer. The only prognostic marker is the Huvos histological grading on tumor resection after neo-adjuvant chemotherapy (Juergens et al., 1981). It is classifying patients into good responders to chemotherapy and poor responders but after 4-month-chemotherapy already done. All these epidemiologic and therapeutic characteristics initially led to develop molecular research to find new prognostic biomarkers and to define new therapeutic targets. In this context, the research focused on several genetic predisposition genes implicated in OS development even most OS tumors are sporadic cases without familial patterns. Rapidly thereafter, the OS molecular research was taking into account the worldwide well-known OS histological features, which are the presence of malignant osteoid matrix produced by the proliferating malignant osteoblastic cells. This definition underlie the fact that OS may be considered as a disease of osteoblast

dedifferentiation (Haydon et al., 2007). As the normal osteoblasts derived from multipotent mesenchymal stem cells (MSCs), the several steps of this transition may be disrupted to obtain subsequently malignant osteoblasts. Based on that, multiple research teams focused, then, on osteoblast differentiation disruptions. They demonstrated that osteosarcomagenesis may result in a deregulation of normal osteogenesis signaling pathways, such as Wnt, BMP (Bone morphogenetic protein) or FGF (Fibroblast Growth Factor) (Entz-Werlé et al., 2003; Luo et al., 2007; Lau et al., 2007), and a dysexpression of several transcription factors (like Twist, Runx2, Osterix or ATF4) (Entz-werlé et al., 2005; Tang et al., 2008). These alterations result in the blockade of cells as undifferentiated precursors. The tumorigenesis seems to be also associated with disturbed bone metabolic activities, leading to the development of a penetrating tumor into the metaphyseal region of long bones and an increase in local bone destruction rates (Costa-Rodrigues et al., 2011; Avnet et al., 2008; Lamoureux et al., 2007). Osteogenesis and osteoclastogenesis signaling pathways additionally to bone microenvironment signals disruptions seem to contribute to OS development and its local or metastatic progression.

The understanding of these deregulated molecular mechanisms in osteosarcoma has and will afford new prognostic markers and new therapeutic targets. In fact, multiple phase I and II, based on all these results, are still running and including relapsed osteosarcoma patients. The recent and increase use of zoledronic acid and targeted therapies in association with standard chemotherapy has to be considered as the emerging therapies of these last decade research in osteosarcomas (Lamoureux et al., 2007; Chou et al., 2008; Broadhead et al., 2011; PosthumaDeBoer et al., 2011). However, the major problem for targeted therapies in this bone cancer is the predominant observation of frequent deletions in karyotypic and arrayCGH (microarray based comparative genomic hybridization) approaches leading to develop therapeutic approaches by-passing the absence of protein over-expression.

2. Research methodology

In such malignant tumors, 3 main models for molecular and cellular studies are available: patient tumor collections, animal models and osteosarcoma cell lines (Kim & Helman, 2009).

To optimize such high throughput molecular researches, the tumor collections have to be integrated into clinical trials to be able to collect in parallel the clinical data and to perform informative statistical correlations. These integrated translational researches have to investigate, first, homogenously treated patient cohorts in case of correlations with response to chemotherapy before any validation as independent marker(s) in several collections. In fact, the response to neo-adjuvant chemotherapy is depending on chemotherapeutic treatment itself and the percentage of response is usually modified by any chemotherapeutic changes in the protocols. Furthermore, this malignant bone tumor is having specific key problems turning around the nature of the tumor itself and its high frequency of extended spontaneous necrosis. This histological observation is impacting especially on RNA extract quality and consequently their analyses, explaining why numerous Lab focused on DNA extracts and paraffin-embedded samples for this tumor. The high complexity of chromosomal rearrangements (Sandberg et al., 2004) is also limiting the transcriptomic screening because of the difficulties in interpreting expression results. Nevertheless, genome-wide approaches to identify OS-associated genes were performed at DNA, RNA and proteomic levels during the last decade, as well as quantitative PCRs, mutation

researches or sequencing, for example (Kubista et al., 2011; Luk et al., 2011; Smida et al., 2010; Lau, 2009). Wide epigenetic and polymorphism screening were also performed and allowed to detect complementary results (Sadikovic et al., 2008).

However, to by-pass the difficulties in tumor collections, *in vitro* and *in vivo* models were developed. As models, they allowed also to confirm the mechanistic conclusions obtained from patients tumors. The *in vitro* models are based on the establishment of patient cell lines and/or the use of commercial cell lines (SaOS or U2OS, for example) (Janeway & Walkley, 2011). Classical two-dimensional (2D) cultures can be done but it is lacking the mechanical and chemical features present in animal models and in the patient bone microenvironment. Therefore, three-dimensional assays were developed to create ideal conditions to study OS cell invasion and metastases (Xu et al., 2007). The 3D *in vitro* cell culture systems will allow live cell-based arrays, microfluidic cell culture systems and drug screening. The use of emerging microengineering approaches will provide repeatable 3D cell based assays and will allow large drug testing without the disadvantages and constraints of animal models (Tan et al., 2011; Xu & Burg, 2007).

The third research model will be based on animal models (mainly mouse, rodent or dog model). The mouse models can be transgenic mice or genetically engineered mice (with the controlled induction of gene over-expression or under-expression), subcutaneous implantation into immunocompromised mice or orthotopic models. OS may also be induced frequently by mouse irradiation and/or the use of chemical carcinogens. The emerging rodent model in OS seem to be progressively used in the recent lab studies and have promising advantages. The dog seem to be also in OS a promising model because of multiple similarities with human cancer and it is offering the possibilities to study autochthonous tumors (Mueller et al., 2007). The *in vivo* model has to re-create human conditions to optimize the mechanistic understanding of osteosarcomagenesis, reason why several models of localized or metastatic OS were developed (Jones, 2011; Janeway & Walkley, 2011; Entz-Werlé et al., 2010; Walkley et al., 2008; Dass et al., 2007; Ek et al., 2006; Dass et al., 2006; Luu et al., 2005). All these animal models are outstanding tools to perform *in vivo* target validation, drug optimization and pre-clinical studies. Furthermore, with the new technologies using bioluminescence, the drug testing and *in situ* tumor follow up is becoming easier (Sottnik et al., 2010 ; Rousseau et al., 2010).

3. Genetic predisposing disorders to OS development

By the past, the starting point in molecular research was frequently based on the correlation between congenital gene mutations and their associated risk of cancer development. For OS, multiple germline mutations are presenting a higher risk of OS. So, the Li and Fraumeni syndrome, an autosomal dominant disorder, is characterized by a germline mutation of *TP53* and a high risk of OS development. The *TP53* alterations (mutations, gene rearrangements or allelic loss) are frequently observed in sporadic OS (Smith-Sorensen et al., 1993) and usually associated with chemoresistance (Asada et al., 1998). The second most frequent germline mutation associated with OS initiation is the hereditary retinoblastoma (Toguchida, et al., 1989), whereas the loss of heterozygosity of *RB1* locus is also extremely frequent in sporadic OS. This *RB1* loss is lacking in case of p16 loss expression (Nielsen et al., 1998) and has been demonstrated as a poor prognosis biomarker (Feugeas et al., 1996). The third group of cancer predisposition syndromes is linked to mutations of *RECQ*

helicases. Among them, Rothmund-Thomson syndrome (mutation of RECQL4), Bloom syndrome (mutation of BLM gene) and Werner syndrome (mutation of WRN gene) can be listed. All these syndromes are presenting similar clinical features and exhibit predispositions to develop OS (Wang, 2005). These mutations are known to increase sensitivity to DNA-damaging agents and maybe are predisposing to bone cell dedifferentiation and consequently to develop OS. Finally, the Paget disease of bone is also a heritable disorder characterized by the increased risk of OS development (approximately 1% of patients). This adult disease is defined as a rapid bone remodeling leading to abnormal bone production (McNairn et al., 2001). The pathogenesis of the Paget disease of bone was highlighting the role of FOS gene, as well as RANK or OPG, involved in bone formation. Most of these congenital disease are characterized by the alteration of genes involved in sporadic OS and most of them are part of osteoblast-osteoclast interactions, implicated in osteosarcomagenesis, as it will be developed below.

4. The role of a defective osteogenesis in osteosarcoma development

The MSCs are bone marrow stromal cells that can differentiate into osteogenic, chondrogenic, adipogenic, neurogenic or myogenic lineages (Deng et al., 2008). The osteogenic differentiation, a tightly regulated process, is needed for bone formation. At each successive stage of differentiation, the precursors are losing their proliferative ability until their terminal differentiation in mature osteoblast (Tang et al., 2008). This osteogenic differentiation is under the control of multiple markers including in particular connective tissue growth factor (CTGF), alkaline phosphatase (ALP), Osterix, Runx2, TWIST, osteopontin (OPN), osteocalcin (OCN) and collagen IaI. During the endochondral osteogenesis of long bones, the chondrogenic cascade is also needed for the bone formation and is regulated by multiple growth factors and transcription factors such as SOX9, BMP2, BMP7, and FGF2 (Deng et al., 2008). The cross-talk and feedback cycles between these two cascades are mainly based on the BMPs, PPAR γ , Runx2 and the canonical Wnt signaling pathway. Several cell cycle genes are also interacting directly or indirectly at several steps of osteoblastic lineage with osteogenic differentiation genes and signaling pathways. Looking closely to these normal features, OS cells are really comparable to undifferentiated osteogenic precursors with a high proliferative capacity, a resistance to apoptosis and a differential expression of osteogenic markers, such as CTGF, Runx2, ALP, Osterix, Osteopontin and Osteocalcin. In fact, the late osteogenic markers, Osteocalcin and Osteopontin, and the early markers of osteogenesis, like ALP, are less expressed than in normal osteoblasts, whereas growth factors are up-regulated or down-regulated almost as in normal osteogenic cells (Luu et al., 2007; Rochet et al., 2003). Usually, malignant osteoblastic cells fail to undergo terminal osteoblastic differentiation. The aggressiveness and the metastatic potential of OS cells seem to depend on this dedifferentiation. Furthermore, OS cells seem to originate from mesenchymal stem cell which could involve at the initiation step cell cycle gene deregulations like p16/CDKN2 (Mohseny et al., 2009; Tang et al., 2008) or p53 (Tatria et al., 2006), followed by the defect of growth factor stimulation or their over-expression. Other pathways implicated in further osteoblastic differentiation will interfere later on. In the further paragraphs, the different biomarkers will be artificially classified depending on their potential and main roles in OS development. Therefore, cell cycle genes will be first described, followed by the major osteoblastic growth factors involved in OS dedifferentiation and in OS proliferation, as well as the Wnt signaling pathway. The main

transcription factors of osteoblastic differentiation will be discussed thereafter in order to understand from the very early steps to the last one how they are acting and could interfere into OS initiation step and/or metastatic spread. Most of the growth and transcription factors listed below are precisely modulated during skeletal development and will be over-expressed or under-expressed, when needed in osteoblastic cells, in osteoclasts and/or for cell-matrix interactions. Most of the deregulations characterized in OS cells are also based on this modulated expression explaining why some of these molecular factors are at once under or over-expressed depending on the model of research and the status of OS cells.

The chapters below and the figure 1 are explaining the involvement of the multiple normal osteogenic signaling pathways and their deregulations in osteosarcoma cells.

4.1 The cell cycle genes

In OS, cell cycle genes like p53, Rb, p16, MDM2, CDK4 or FOS are implicated as in most of the other cancers. In this malignant bone tumor, numerous cytogenetic studies described a variety of genetic alterations like the inactivation of Rb and/p53 pathways. These two genes are usually very frequently altered (Entz-Werlé et al., 2003) but they are functioning as co-activator of transcription factor like Runx2 (Thomas et al., 2004). MDM2 and p53 dys-expressions are also cooperating to disrupt the osteogenic differentiation into a osteoblastic precursor (Lengner et al., 2006). Because of their high frequency alterations, they were suspected to be part of the initiation step of osteosarcomagenesis, as already demonstrated in publications showing the MSC implication in the origins of OS. All these characteristics were also confirmed in genetically engineered mice where cell cycle genes are defective (Janeway & Walkley, 2011; Walkley et al., 2008).

4.2 the osteoblastic growth factors and their receptors, which are favoring the OS cell dedifferentiation

Fibroblast growth factors (FGFs) are growth factors favoring in tumor cells the increase of motility and the cell ability to microenvironment invasion. Their main receptors, FGFR1, FGFR2 and FGFR3 were described as essential osteoblastic cell surface receptors, as the inherited mutation of these genes result in skeletal dysplasia (Kan et al., 2002; Bellus et al., 2000; Bellus et al., 1996). During intramembranous ossification, FGFR4 seems to be also an important regulator of osteogenesis with involvement in preosteoblast proliferation, as well as in osteoblast functioning, explaining why it is more frequently amplified than the other FGFRs (Entz-Werlé et al., 2007a). In OS cell, alterations of the FGFs/FGFRs systems are less frequent than other genes (Entz-Werlé et al., 2007a; Mendoza et al., 2005), but they are playing a role in the activation of Runx2, which is stimulated by the activated Protein Kinase C (Kim et al., 2006), and in the modulation of OS cell interactions with the bone matrix and vessels (Georgios et al., 2011).

Connective Tissue growth Factor (CTGF), a member of the CCN family, is a modulator for osteoblast and chondrocyte differentiation and is involved in vascular endothelial cell development during endochondral ossification (Luo et al., 2004). As in uncommitted preosteoblast progenitors, CTGF is up-regulated in most of the OS cells (Luo et al., 2008; Perbal et al., 2008), contributing to maintain the undifferentiated status and may also be implicated in angiogenic pathway deregulation (mainly, VEGF and HIF1 α) during OS formation (Nishida et al., 2009).

4.3 The osteoblastic growth factors and their receptors, which are also involved in OS cell proliferation

Bone Morphogenetic Proteins (BMPs) belong to the TGF β family and are considered as pivotal growth factors of early MSC commitment to osteogenic lineage. The osteogenic BMPs include 2, 4, 6, 7 and 9 (Deng et al., 2008; Tang et al., 2008; Reddi, 1998). Even at early stages of osteogenic differentiation, these BMPs are inducing the expression of CTGF, ALP, inhibitor of DNA-binding (Id), transcription factors, like TWIST and Runx2 (Hayashi et al., 2007). The up-regulation of these specific BMPs in OS is predominantly promoting the tumor growth and OS cell proliferation (Luo et al., 2008) throughout the stimulation of most of its target genes listed above. They are also favoring the interactions between OS cells and the bone matrix.

Platelet-derived Growth factor (PDGF)/ Platelet-derived Growth factor receptor (PDGFR) signaling is preferentially playing a role in the regulation of normal osteoblastic cell proliferation and, consequently, in the deregulation of OS cell proliferation. It is also promoting malignant cell motility (Kumar et al., 2010) and it is implicated in osteoblast-osteoclast interactions (Sanchez-Fernandez et al., 2008). When the PDGF/PDGFR signaling pathway is stimulated, the patients are presenting a worst prognosis (Hassan et al., 2011; Entz-Werlé et al., 2007b).

4.4 From early to later transcriptional factors of osteoblastic differentiation

Twist, or Twist-1, and its homolog dermo1, or Twist-2, are basic helix-loop-helix (bHLH) transcription factor involved as others in osteoblastogenesis. Twist1 expression negatively regulates osteoblast differentiation and maintains osteoblastic cells in a osteoprogenitor-like state. Accordingly, Twist1 silencing enhances the osteoblast differentiation program, by acting at very early step, and mediates MSC commitment and growth (Isenman et al., 2009). This transcription factor is interacting with Id-1 and Id-2, other HLH proteins, and it is cooperating with Msx2 and BMPs. Twist1 downregulation alters usually Runx2 expression, whereas it is having at once a double effect on FGFR2 (an up-regulation or a repression activity). However, the main role of twist1 downregulation is resulting into a reduced cell apoptosis (Miraoui & Marie, 2010). These functional roles are explaining the role of Twist 1 in case of deleted and amplified gene in the OS, respectively favoring disruption of cell apoptosis and dedifferentiation of OS cells (Entz-Werlé et al., 2005 and 2007). It seem also to act at the initiation step of OS development as it was demonstrated in a double mutant mouse with Twist haploinsufficiency (Entz-Werlé et al., 2010). Dermo1 is also acting as a negative regulator of osteochondrogenesis, while promoting MSC growth and proliferation throughout the regulation of Id1 and Id-2 gene expression (Tran et al., 2010 ; Zhang et al., 2008). It is a direct transcription target of the canonical Wnt pathway and it is participating to the feedback loop of this pathway (Tran et al., 2010), but it was still yet not involved in OS development.

Runx2 is a member of the DNA-binding transcription factor family that regulates the expression of multiple genes involved in cellular differentiation and cell-cycle progression. It is genetically essential for bone development and osteoblast maturation. It is interacting with numerous transcription activators and repressors such as Rb, PTH/PTHrP, MAPLK and histone deacetylases (Deng et al., 2008; Thomas et al., 2004). It is also a critical regulator in BMP-mediated osteogenic differentiation and is having interactions during normal osteogenesis with the Wnt signaling pathway. It is physically interacting with the

hypophosphorylated form of Rb to promote terminal cell cycle exit and the differentiated osteoblastic phenotype (Thomas et al., 2004). It is acting at the pre-osteoblastic step after its activation by IHH (Indian Hedge Hog). In OS tumors, Runx2 is frequently over-expressed leading to stop osteoblast maturation and to promote metastatic spread (Thomas et al., 2004; Won et al., 2009), favoring the development of a highly aggressive disease and less differentiated OS cells (Martin et al., 2011). Controversially, other publications observed Runx2 low expression in OS cells (Entz-Werlé et al., 2010; Luo et al., 2008). These opposite results are not to be oddly considered and should provoke debate around the schedule of Runx2 activation or under-expression during osteosarcomatogenesis. In fact, a higher expression is needed in case of OS initiation and at metastatic spread but in the interval period, Runx2 could have a normal expression or could be under-expressed (Thomas et al., 2004). Its counterparts Runx1 and Runx3 are also having potential but discussed roles in osteoblastic and chondrogenic differentiation, but up to date and to our knowledge no data are available on their potential implication in OS cells.

Osterix is a zinc finger-containing transcription factor, which is, as Runx2, an important regulator of osteogenic differentiation (Deng et al., 2008), it is a downstream signal after Runx2 action in osteoblastic cell lineage differentiation and seem to be regulated by Runx3. Its normal expression in osteoblastic cells is linked to less osteolysis and suppress the cell migration. It seems to have low expression in OS cells allowing to promote tumor growth and metastases (Cao et al., 2005).

All these growth and transcription factors are more or less cooperating or interacting with the canonical Wnt signaling pathway and the metabolic pathways linked to bone angiogenesis (Broadhead et al., 2011; Wan et al., 2010; Araldi & Schipani, 2010; Deng et al., 2008; Haydon et al., 2007; Luo et al., 2004), which are described below.

4.5 Wnt osteoblastic signaling pathways

The **Wnt signaling pathway** is characterized by the binding of Wnt proteins to the cell-surface receptors of the Frizzled family and its co-receptor LRP5/6, causing, then, the activation of Dishevelled (DSH) family proteins. These activated DSH proteins are inhibiting a complex of proteins, including axin, GSK3 and APC proteins. This complex normally promotes the proteolytic degradation of β catenin. In case of Axin/GSK3/APC complex inhibition, β catenin will be stabilized and, then, able to enter the nucleus for interactions with TCF/LEF family transcription factors and to promote specific gene expression (for example, fibronectin, c-Myc or cyclin D1). During bone formation, this pathway is predominantly linked to limb development and seems to act in the terminal differentiation of osteoblast, shunting also away cells from the chondrogenic differentiation (Monaghan et al., 2001). An increase of lytic lesions is linked with the inhibition of this canonical pathway. In OS, the Wnt/ β catenin signaling is frequently activated (Entz-Werlé et al., 2003 and 2007a; Haydon et al., 2002). This up-regulation is correlated with osteoprogenitor proliferation and OS metastases (Iwaya et al., 2003), resulting from β catenin massive nuclear translocation and/or deletion of APC. The loss of APC at DNA and protein levels was also associated to a worst outcome and less response to pre-operative chemotherapy (Guimaraes et al., 2010; Entz-Werlé et al., 2007a). This Wnt up-regulation can also induce chemoresistance throughout the repression of a bone matrix proteoglycan, syndecan 2 (Dieudonné et al., 2010).

4.6 The angiogenic and metabolic signaling pathways:

The other central pathways are those involved in angiogenesis, which is essential in normal intramembranous and endochondral ossifications, but also for tumor growth and metastatic spread. A balance between pro-angiogenic and anti-angiogenic factors is required to regulate precisely angiogenesis. This balance is tipped towards the favor of neovasculature by tissue hypoxia. Usually, in tumor environment, hypoxic conditions are stimulating the deubiquitination of Von Hippel Landau (VHL) protein, which is releasing hypoxia-inducible factor-1 alpha (HIF-1 α). The stabilized form of HIF-1 α is stimulating the secretion of VEGF, which is also up-regulating via FGF and TGF α signaling. The hypoxia is also regulating processes such as apoptosis and metabolic adaptation with, in particular, the release of glycolytic enzymes. (Araldi & Schipani, 2010; Wan et al., 2010; Ishida et al., 2009). HIF-1 α upstream signals is involving mTor, RAS/MAPK cascade and PTEN. The angiogenesis is also the key in OS cell-bone matrix interactions, which are described below. Among all angiogenic markers, a focus will be done on those specifically involved in OS cells from the receptor ligands to the downstream transcription factors and their target genes. The different growth factors and their receptors are rather implicated in metastatic spread (Mizobuchi et al., 2008). In the angiogenesis studies, circulating growth factors can be estimated at plasma level and could help us to appreciate the implicated circulating factors.

Cystein-X-Cystein (CXC) chemokines and their receptors (CXCR) are proteins, containing 2 highly conserved cystein residues at N-terminus, and activating usually the chemotaxis of different cells. They can be also involved in various cellular processes such as skeletal rearrangements, cell migration and cell adhesion (Fernandez et al., 2002). In OS, the circulating CXC chemokines (CXCL4, CXCL6 and CXCL12) seem to present higher concentrations (Li et al., 2011), correlated with a worst outcome in patients. They also stimulate the specific angiogenic and hypoxic signaling pathways, which are favoring the metastatic spread. Moreover, in case of circulating OS cells, the CXCR/CXC chemokine system seems to drive the homing of these cells in the metastatic locations. So, the circulating OS cells expressing CXCR4 were preferentially re-localized in lung because of CXCL12/SDF-1 (Stromal cell-Derived Factor-1) high secretions in lung (Perissinotto et al., 2005). This metastatic homing may, then, be supported by the tricky release of CXC chemokines, which is under the control of VEGF (Oda et al. 2006).

Vascular Endothelial growth factor (VEGF)-A is one of the most important members of VEGF family. There are 5 other members VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placenta growth factor (PlGF). VEGFs are binding their receptors and consequently initiating the tyrosine kinase activation and the downstream signals. The results of this process is the vessel formation. VEGF-A was mainly involved in the angiogenic processes of OS. In this context, high levels of VEGF was regularly observed in OS and especially in metastatic forms. The VEGF combined with the proteoglycans in the bone matrix was up-regulate MMP secretion in OS and, therefore, increasing the vessel formation but also the OS cells-bone matrix interactions. It is also inducing the release via the malignant cells of anti-apoptotic factors (bcl-2 and survivin) to ensure ongoing endothelial cell proliferation and neovascularization, but it will also promote the secretion of pro-angiogenic factors like FGF or angiopoietin 1. These signaling pathway are also under control of HIF1 α /mTor stimulation (Yang et al., 2011 ; Hassan et al., 2011; Broadhead et al., 2011; Haydon et al., 2007; Oda et al., 2006).

Hepatocyte Growth factor (HGF) and its receptor (MET) regulate usually mitogenesis, motility and morphogenesis. In case of deregulation, it will contribute to cell transformation and tumor progression. HGF was also described recently as part of the angiogenic pathways (Hoot et al., 2010). A over-expression of MET, inconstantly associated with HGF high secretion, was usually observed in OS confirming its role in tumor progression, in angiogenesis stimulation and its correlation with a poor outcome (Hassan et al., 2010; Patane et al., 2006; Coltella et al., 2003). Surprising results were obtained showing deletion of MET gene associated with a poor outcome and a link with metastases (Entz-Werlé et al., 2007a). The sensivity enhancement of tumor cells in case of MET/FAS concomitant activation could explain partially this difference. In fact, the deletion of MET could then be considered much more in these OS as un marker of drug resistance, which was described in other cancers (Accordi et al., 2007).

Insulin like Growth Factor (IGF) systems play a key role in cellular metabolism, differentiation, proliferation, transformation and apoptosis, during normal and malignant growth of cells. In normal bone formation, IGF-I and II are known to have effects on cell proliferation and to be inducer of collagen synthesis (Bikle &Wang, 2011; Wang et al., 2006). The recent findings demonstrated that the lack of IGF-I or its receptor in osteoblasts is enhancing the signaling between osteoblasts and osteoclasts through RANKL/RANK or PTH. Nevertheless, their roles were recently extended to the regulation of tumor angiogenesis and postnatal vasculogenesis (Orciari et al., 2009). In OS, IGF I and II are often

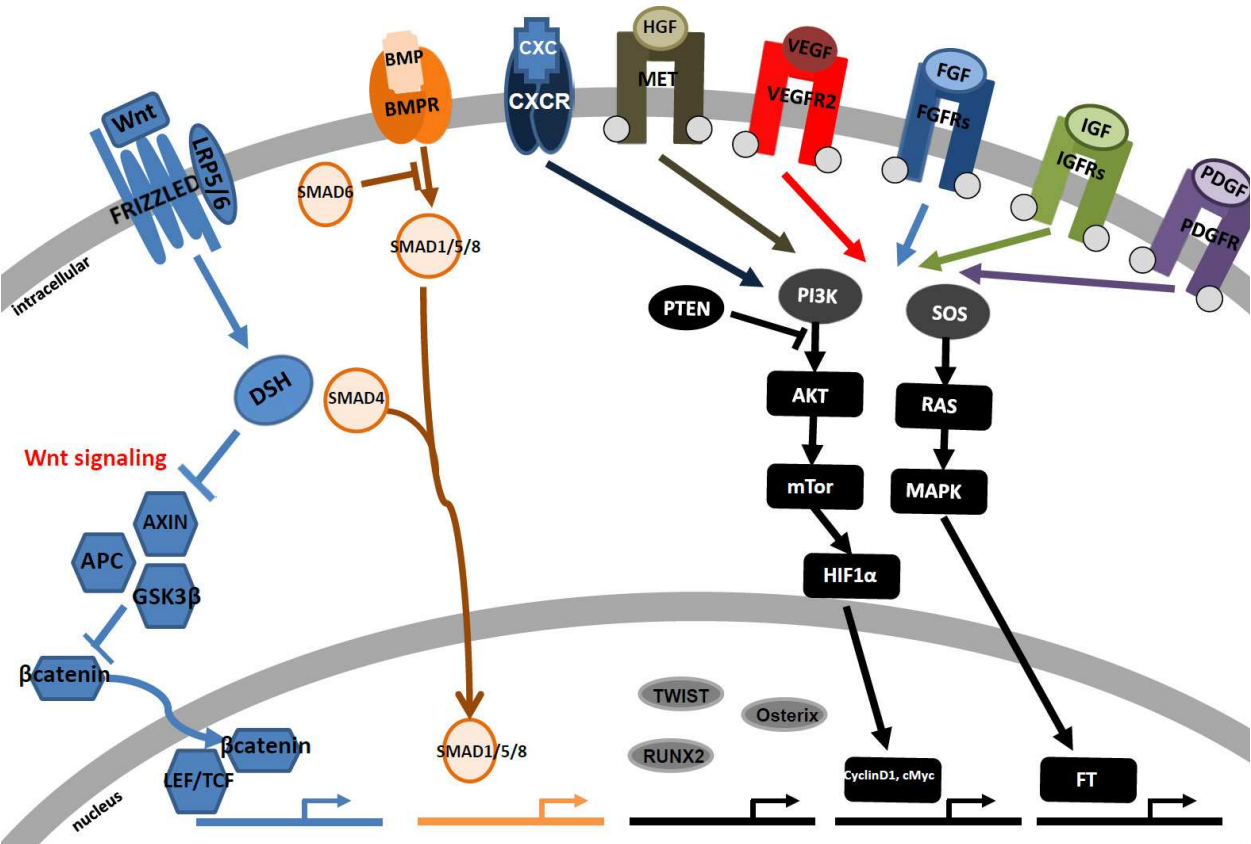


Fig. 1. A schematic summary of the major osteogenic signaling pathways involved in osteosarcomas

over-expressed. Even they are part of the osteoblastic growth regulator, they are also involved in energy homeostasis of the bone and therefore may inhibit the cell apoptosis (Hassan et al., 2011).

mTor/HIF-1 α signaling and their downstream signals are playing key roles in cell proliferation and tumor hypoxia, as described above. In OS, mTor was described as an activated protein and seem to promote concomitantly with other signals the tumor progression, whereas expression of HIF-1 was mainly associated with a metastatic disease (Zhou et al., 2010; Knowles et al., 2010; Mizobuchi et al., 2010). The downstream signals like VEGF were already described as part of OS oncogenesis. Finally, few publications involved in OS the glycolytic enzymes like pyruvate kinase, for example. However, these enzymes seem to have proliferation impact (Spoden et al., 2008).

5. Deregulation of bone destruction mechanisms and cells (osteoclasts) is part of osteosarcoma progression

Osteoclasts originate from macrophage lineage and this differentiation results from a series of molecular events associating osteoclastic signals and extracellular matrix compounds. Some of these factors are required for osteoclast proliferation and differentiation, like Macrophage-Colony Stimulating factor (M-CSF), while factors like receptor activator of nuclear $\kappa\beta$ ligand (RANKL) are more implicated in the commitment of macrophage precursors into osteoclasts and also in their survival. The RANKL/RANK activities are under the control of osteoprotegerin (OPG), which is blocking the ligand-receptor binding and subsequently the osteoclastogenesis and bone resorption (Baud'huin et al., 2011; Lamoureux et al., 2007). OPG is a potent apoptosis inhibitor of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on tumor cells. The loss of osteoclasts in the primary tumors enhances the OS metastases (Endo-Munoz et al., 2010a). The decrease of osteoclasts is consistent with a decreased antigen-presenting activity, an enhanced chemoresistance and an impaired osteoclastogenesis (Endo-Munoz et al., 2010b). Osteoclasts are also part of the vicious cycle between bone resorption and tumor cell proliferation during tumor development. In OS, the tumor secretion of bone-modulating compounds is stimulating osteoclastic bone resorption, impacting on the release of growth factors from extracellular bone matrix. The triad RANK/RANKL/OPG seem to have play a pivotal role in this vicious cycle (Lamoureux et al., 2010). The osteoclast activity is also linked to TRACP 5A and MMP9 serum levels (Avnet et al., 2008).

The bone metabolism implicate other regulators of bone turnover like **parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP)**, which have been implicated in OS progression and especially in metastatic OS spread. PTHrP could confer also chemoresistance by blocking signaling via p53, the death receptor and the mitochondrial pathways of apoptosis (Gagiannis et al., 2009).

Upstream, the **TGF β** , released from the degraded bone matrix, is acting on OS cells, stimulating the release of PTHrP, IL6 and IL11. These cytokines then stimulate osteoclasts, facilitating further invasion and release of pro-resorptive cytokines. After TGF β stimulation, PTHrP and IL11 also act on osteoblasts, stimulating increased expression of RANKL (receptor activator of nuclear factor $\kappa\beta$ ligand) and M-CSF (Endo-Munoz et al., 20010b).

The normal **bone microenvironment** and **osteosarcoma matrix** during tumor development are providing optimal conditions for tumor cell proliferation and will facilitate blood vessel

formation. Various protein fibrils (fibronectin, collagens, proteoglycans, integrins and laminins) and growth factors stored in matrix are contributing to tumor cell adhesion and spread. This microenvironment is usually convenient to allow detachment of OS cells from the tumor, adhesion to the extracellular matrix, local migration and invasion through stromal tissue until vascular extravasation. Extracellular matrix components such as **glycoaminoglycans** (GAGs) are also participating to the bone metabolism. In OS, GAGs over-expression is inhibiting OPG action, is acting as regulator of OPG availability and is having anti-tumor activity in bone microenvironment (Lamoureux et al., 2010). Another component of the matrix, named **Syndecan 2** (a cell surface heparin sulfate proteoglycan), can induce apoptosis and sensitize OS cells to the cytotoxic effect of chemotherapies (Orosco et al., 2007; Modrowski et al., 2005). The **integrins** are also taking part of the bone matrix role in OS progression and seem to participate to the mTor/HIF1 α signaling (Kim & Helman, 2009).

Finally, the bone metabolism is regulating by the **interactions between osteoclasts and osteoblasts**, which are cell-cell contacts but are also initiating paracrine mechanisms. In fact, osteoblasts synthesize a variety of molecules important in the recruitment and survival of osteoclast precursors and these proteins will regulate the later steps of osteoclastogenesis (Costa-Rodrigues et al., 2010). As with normal osteoblasts, osteoclasts will be stimulated by the OS cells. In fact, the malignant cells are usually presenting a high osteoclastogenic-triggering capacity, which is contributing to the normal bone destruction by the tumor cells and explaining partially the "vicious cycle" described above and below in the figure 2.

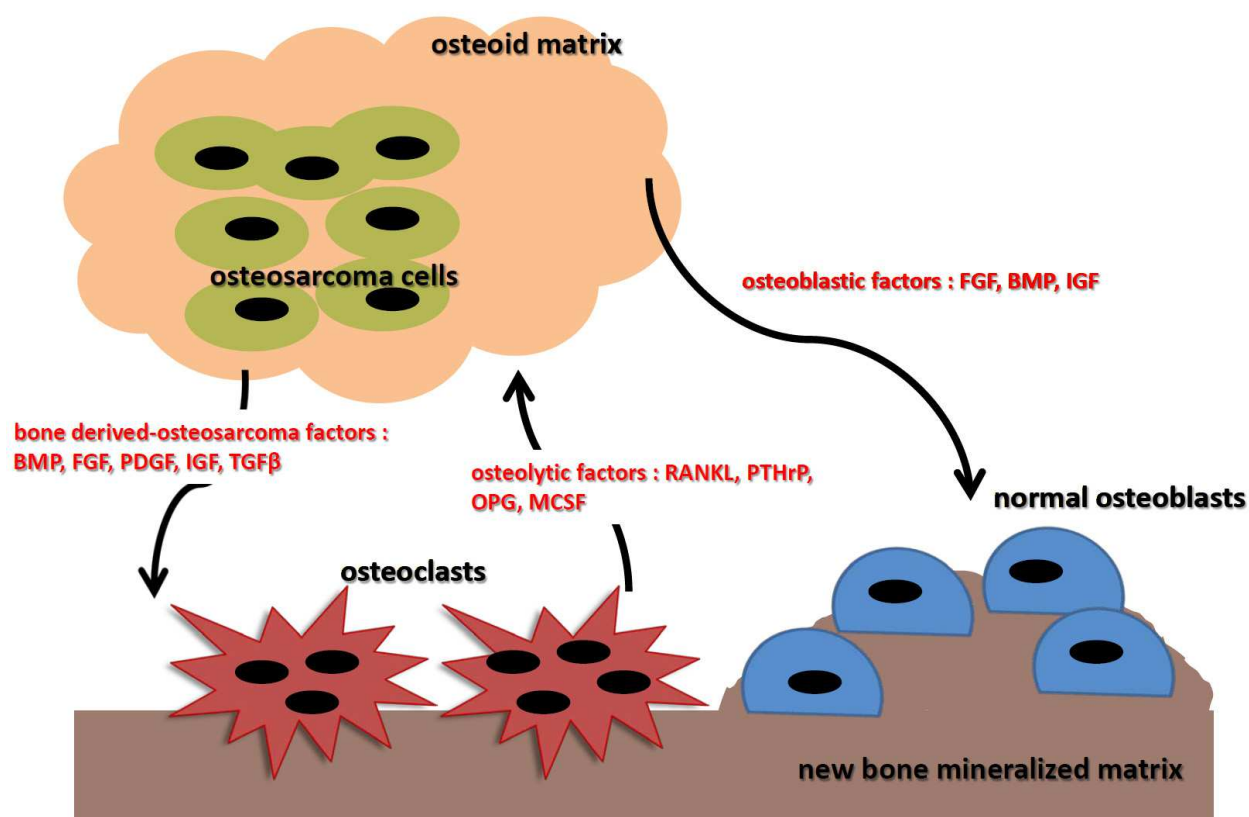


Fig. 2. A schematic summary of the bone vicious cycle involved in osteosarcoma development

6. Conclusion and future prognostic and therapeutic directions

The increase knowledge in this complex network of markers and signaling pathways involved in OS cells is now allowing to pool and extrapolate these data in a preliminary multistep osteosarcomagenesis (Figure 3).

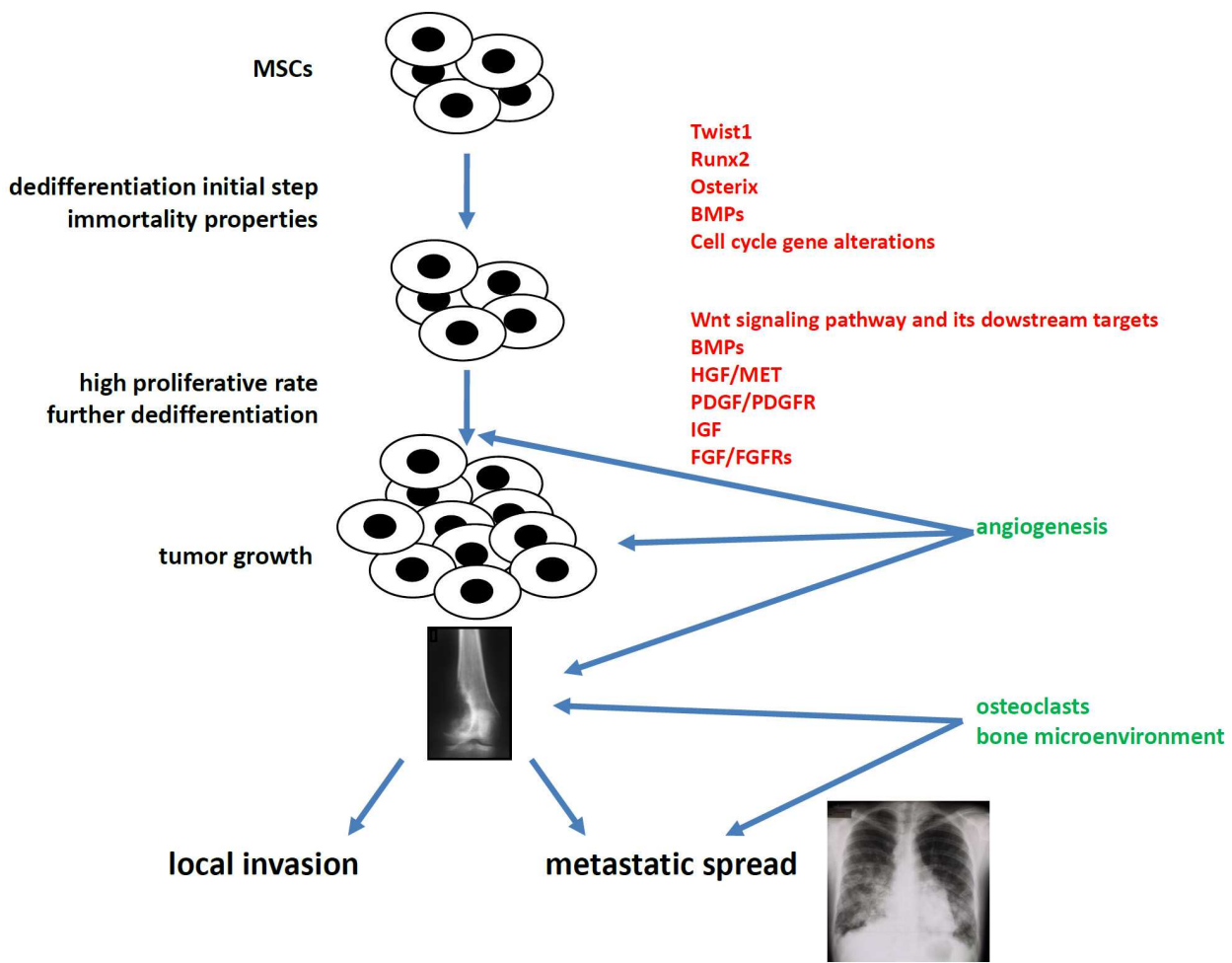


Fig. 3. An extrapolate multistep osteosarcomagenesis

Several of these biomarkers could be used as relevant prognostic factors but they have to be confirmed in larger cohorts of patients and as independent markers, like the histological Huvos grading. Among all these striking results, targeted therapies could emerge. The osteoblastic growth factors could be downregulated in case of overexpression with, for example, large spectrum inhibitors of tyrosine kinase receptors. Various PPAR γ agonists may be usable to prevent proliferation and to induce terminal differentiation of malignant cells, as well as multiple anti-angiogenic approaches (antagonists of CXCR4 or VEGFR, metronomic chemotherapy, inhibition of hypoxia signaling, for example). New drugs targeting Wnt signaling pathway are also upcoming and could be usable in the next future (Chou et al., 2008; Houghton et al., 2007).

Finally, the bone destruction pathways are now considered as the larger field of innovative targeted therapies (Lamoureux et al., 2007;). The increased use of zoledronic acid in phase I and II but also in phase III is the proof of concept.

7. References

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This book is aimed at quickly updating the reader on osteosarcoma, a dreaded primary bone cancer. Progress in management of osteosarcoma has been slow after the evolution of chemotherapy and limb salvage surgery. Research is now directed towards identifying molecular targets for systemic therapy. Availability of chemotherapy drugs and low cost implants in developing world have allowed limb salvage surgery to develop. This book looks at current basic knowledge on osteosarcoma and some of the developments in research which have the potential to change the prognosis.

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