

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Osteosarcoma: From Molecular Biology to Mesenchymal Stem Cells

---

Matthew L. Broadhead, Saumiyar Sivaji,  
Zsolt Balogh and Peter F.M. Choong

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/67371>

---

## Abstract

Osteosarcoma is the most common primary malignant tumour of bone. Currently, despite treatment with multi-agent chemotherapy and limb salvage surgery, the five-year survival rate for osteosarcoma remains at 70%. The pathogenesis of osteosarcoma is complex and involves alterations in cellular apoptosis, adhesion, migration, invasion and molecular signalling. Research most recently has focused on the molecular basis of the disease with the goal of identifying novel therapeutic targets. To this end, mesenchymal stem cells (MSCs) have been identified to play a role in sarcomagenesis. MSC transformation may give rise to tumours, whereas interactions of MSCs with osteosarcoma cells in the tumour microenvironment may cause increased cell proliferation. This is in stark contrast to the role of MSCs as a promising source for tissue repair and regeneration. In order to utilize MSCs for biological reconstruction in the setting of osteosarcoma, further research is necessary to delineate the role of MSCs in osteosarcoma transformation and progression.

**Keywords:** osteosarcoma, pathogenesis, mediators, mesenchymal stem cell, MSC

---

## 1. Introduction

Osteosarcoma is the most common primary malignancy that arises from bone. While relatively rare, with an annual incidence of 1–3 cases per million [1], it is fatal if left untreated. Osteosarcoma has a bimodal distribution affecting patients in the 2nd and 3rd decade of life and those after the 6th decade of life [2]. It is the sixth most common paediatric cancer and is the second-highest cause of cancer-related death in this age group [3, 4].

Current treatment protocols for osteosarcoma combine neoadjuvant chemotherapy, surgery and adjuvant chemotherapy. The five-year survival rate for patients diagnosed with osteosarcoma remains at 60–75% [5]. The medical and surgical treatments of osteosarcoma can cause significant morbidity for the patient. Chemotherapy agents are systemically toxic and surgery, in the form of amputation or limb salvage, require a prolonged period of rehabilitation. Despite the advent of multi-agent chemotherapeutic regimens, the prognosis for osteosarcoma has not significantly improved; hence, there is a real need to optimize current strategies and to develop novel approaches for treatment.

Our understanding of osteosarcoma has traditionally been based upon anatomical and histological principles. Primary osteosarcoma arises in the metaphysis of long bones, most commonly, within the medullary cavity. The most common sites for osteosarcoma are the distal femur, proximal tibia and proximal humerus. The occurrence of osteosarcoma in sites other than long bones increases with age. The tumour typically breaks through the cortex of the bone into surrounding soft tissues, around which a pseudocapsule forms [6].

Histologically, osteosarcoma is a malignant mesenchymal cell tumour, characterized by pleomorphic spindle-shaped cells, capable of producing an osteoid matrix. Tumour cells metastasize primarily via the haematogenous route. There are various subtypes of osteosarcoma, including the intramedullary 'classic' osteosarcoma already described, periosteal osteosarcoma, parosteal osteosarcoma, small cell osteosarcoma and telangiectatic osteosarcoma.

Current standards for staging and surgical resection of osteosarcoma rely on this anatomical knowledge [1]. However, recent advances in molecular biology have provided insight into the molecular pathogenesis of the disease. Through the identification of specific mediators of osteosarcoma progression and tumour pathways, novel approaches for targeting osteosarcoma are being developed.

This chapter will outline our current understanding of the molecular pathogenesis of osteosarcoma with some reference to the development of novel treatment agents. The environmental, genetic and molecular alterations that underlie osteosarcomagenesis will be discussed with further emphasis on the role of mesenchymal stem cells (MSCs). MSCs have been identified as playing a role in not only sarcomagenesis but also the progression of disease. This role of MSCs in osteosarcoma contrasts with their ability to differentiate into the various cell types of connective tissue for tissue repair. This chapter discusses MSC origin, differentiation and transformation in sarcomagenesis. The interactions between MSCs and osteosarcoma cells are outlined. A number of research models that utilize MSCs in order to replicate the human condition will be discussed along with the potential use of MSCs in biologic reconstruction.

## 2. Pathogenesis of osteosarcoma

The pathogenesis of osteosarcoma is a complex process, which is not completely understood and involves tumorigenesis from mesenchymal cells, alterations in cellular apoptosis, adhesion, migration and invasion, as well as tumour-induced osteolysis and angiogenesis. Various genetic and molecular alterations underlie these processes. It is hoped that by targeting

the deranged molecular signalling of these pathways that novel treatment agents could be developed that enhance the efficacy of conventional chemotherapeutics and possibly reduce patient morbidity.

## **2.1. Environmental factors**

Physical, biological and chemical agents have been implicated in osteosarcoma pathogenesis. There is a well-documented risk of osteosarcoma following exposure to ultraviolet and ionizing radiation, which occurs in 2-3% of cases. The first identified case of radiation exposure association with osteosarcoma was found in female watch-makers working with radium [7]. Nevertheless, only 2% of osteosarcoma cases are associated with radiation exposure [8] and it is not thought to contribute significantly to paediatric disease. Samartz et al., have identified that radiation-related-sarcoma formation can even occur in those with low-level radiation exposure. Of children who received radiotherapy for treatment of a solid tumour, 5.4% develop a secondary neoplasm and only 25% of these are sarcomas [9]. A latent period of 10–20 years between radiation exposure and osteosarcoma formation has been observed [10]. Methylcholanthrene and chromium salts [11], beryllium oxide [12], zinc beryllium silicate [13], asbestos and aniline dyes [14] are among the chemical agents associated with osteosarcoma formation.

## **2.2. Familial and chromosomal abnormalities**

Amplifications of chromosomes 6p21, 8q24 and 12q14, and loss of heterozygosity of 10q21.1, are among the most common genomic alterations in osteosarcoma [15]. Numerical chromosomal abnormalities associated with osteosarcoma include loss of chromosomes 9, 10, 13 and 17, as well as gain of chromosome 1 [4]. Osteosarcoma has been reported in patients with Werner syndrome, Rothmund-Thompson syndrome, Bloom syndrome, Li-Fraumeni syndrome, and hereditary retinoblastoma [14]. In particular, Werner, Rothmund-Thompson and Bloom [16] syndromes are characterized by genetic defects in the RecQ helicase family. DNA-helicases separate double stranded DNA prior to replication [17, 18].

Paget osteosarcoma occurs in approximately 1% of patients with Paget's disease [19]. These tumours are characteristically high grade pleomorphic intramedullary tumours. Loss of heterozygosity of chromosome 18q is a recognized genetic anomaly contributing to tumorigenesis: the specific region located between loci D18S60 and D18S42 contains the tumour suppressor locus [20]. This region also encodes for receptor activator of nuclear factor kappa B (RANK), a peptide which is a mediator of osteoclastic activity [21].

## **2.3. Tumour suppressor gene dysfunction**

The p53 mutation is the most common genetic aberrancy in malignancy, and is a causative factor in the transformation and proliferation of osteosarcoma cells [22]. Here, it is found to be mutated in 22% of cases [4]. The presence of p53 mutation in osteosarcoma was initially identified in the autosomal dominant Li-Fraumeni syndrome, which is a syndrome characterised by a predisposition to forming multiple malignancies, such as osteosarcoma, rhabdomyosarcoma and breast cancer.

Normally, p53 is a vital protein in cell cycle arrest, cellular senescence and DNA damage response and repair [23]. It is regulated by mouse double minute 2 homolog (MDM2), a protein that inhibits p53 activation via multiple methods including the ubiquitin degradation pathway and competitively binding to the amino terminus of p53 (instead of transcriptional co-activators) [24]. Transcriptional activation of p21 (cyclin-dependent kinase inhibitor) mediates p53 activity, where its expression results in cellular arrest in either the G1 or G2 phase. This can be either temporary, until the source of the cellular stress has been removed or subsided, or can be irreversible, which is known as cellular senescence. Cellular senescence is stimulated by the presence of oncogene activation or presence of DNA damage. Its ability to arrest the cell cycle in the G1/G2 phase is dependent on its response to stressful stimuli [25].

Mutation in the retinoblastoma gene (Rb1) is the most common mutation found in osteosarcomas whereby greater than 70% of cases are associated with an alteration in Rb gene. The association between hereditary retinoblastoma and osteosarcoma has been localised to this mutation, where it acts as a dysfunctional tumour suppressor. Normally, Rb1 is found on chromosome 13, which encodes for a nuclear protein allowing sequestration of transcription factors and acts as a tumour suppressor. This protein is vital in regulation of cell cycle progression from the G1 to S phase of the cell cycle. Hypophosphorylation of Rb protein allows it to bind to E2F transcription factor which inhibits cellular progression from G1 into the S phase. Once pRb is phosphorylated, it releases E2F, allowing continuation of the cell cycle. Additional biological characteristics include regulating DNA replication, apoptosis, cellular differentiation, as well as DNA damage response and repair [26–28].

## 2.4. Transcription and growth factors

Osteosarcoma cells produce a number of transcription and growth factors that contribute towards continued tumour cell growth and proliferation. During transcription single-stranded messenger RNA (mRNA) is formed from double-stranded DNA. Transcription factors bind to promoter sequences for specific genes to initiate the process. Transcription is usually a tightly regulated process and deregulation can lead tumour formation. Growth factors may act via both autocrine and paracrine mechanisms and overexpression or constitutive activation may lead to accelerated osteosarcoma cell proliferation.

The activator protein 1 complex (AP-1) is a regulator of transcription that controls cell proliferation, differentiation and bone metabolism. AP-1 is comprised of Fos and Jun proteins, products of the c-fos and c-jun proto-oncogenes, respectively. Upregulation of Fos and Jun is seen in high-grade osteosarcomas [29, 30] and is also associated with a propensity to develop metastatic lesions [31].

Myc is a transcription factor that acts in the nucleus to stimulate cell growth and division. Myc amplification has been implicated in osteosarcoma pathogenesis and resistance to chemotherapeutics. Overexpression of Myc in bone marrow stromal cells leads to osteosarcoma development and loss of adipogenesis [32]. This factor is amplified in U2OS osteosarcoma cell line variants with the highest resistance to doxorubicin and gain of Myc was found in SaOS-2 methotrexate-resistant variants [33].



In addition to Myc, transforming growth factor beta 1 (TGF- $\beta$ 1) has been shown to be over-expressed in high grade osteosarcomas [34]. Smad activation was implicated downstream of TGF- $\beta$  with an inability to phosphorylate the Rb protein.

Insulin-like growth factor (IGF)-I and IGF-II are overexpressed by osteosarcomas. Activation of the IGF-1R receptor leads to the activation of phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. This leads to accelerated cell proliferation and inhibition of apoptosis [35].

Connective tissue growth factor (CTGF) is a potent stimulator for the proliferation of osteosarcoma cells, leading to increased expression of type I collagen, alkaline phosphatase, osteopontin and osteocalcin, markers for bone cell differentiation and maturation [36]. CCN3, a related protein, is overexpressed in osteosarcoma and is associated with a worse prognosis [37].

The wingless-type (Wnt) canonical pathway, is a specific cascade that occurs within the Wnt family of glycoproteins and has been identified in the molecular basis of osteosarcoma formation. The Wnt family is essential in cellular differentiation and cell fate determination, and in the context of osteosarcomas, directing mesenchymal stem cells down the osteogenic lineage. Through this pathway, bone morphogenic protein 2 (BMP-2) is the key factor in osteogenesis. Another factor has been identified to inhibit the Wnt cascade, and histologically has been identified at the peripheries of osteosarcomas, Dickkopf 1 (DKK1). A secreted antagonist of Wnt pathway is low density lipoprotein receptor related protein 5 (LRP-5) which has been correlated with metastatic disease in osteosarcoma, independent of the histological type. When LRP-5 is expressed, the Wnt pathway is activated resulting in the up-regulation of a number of genetic factors including matrix metalloproteinases (MMP) which have been known to be involved in metastatic activity of cancers. Hoang et al. have analysed osteosarcoma patients expressing LRP-5, who were metastases free at time of diagnosis to have a lower probability of an event-free survival [38].

Stromal cell derived factor-1 (SDF-1), also known as C-X-C motif chemokine 12 (CXCL-12), [39] is a ligand for CXCR-4 and a part of the cxc chemokine family, where CXCR-4 has been implicated in various cancer types. SDF-1/CXCL-12 is a chemokine that has a paracrine effect within the interstitial space stimulation migration of pluripotent cells as well as tumour cells. The interaction between CXCR-4 and SDF-1/CXCL-12 has an important role in cancer progression as it promotes osteosarcoma cell migration and angiogenesis [40]. Within osteosarcoma the level of CXCR-4 mRNA is low however the SDF-1/CXCL-12/CXCR4 combination is required in osteosarcoma cell proliferation. Tumour promotion occurs by SDF-1/CXCL-12 in a paracrine manner, stimulating cellular growth and survival. Besides tumour promotion CXCR-4 is involved in metastatic spread of tumour cells into areas where SDF-1/CXCL-12 is expressed. This factor is important in angiogenesis as it promotes endothelial cells into the tumour microenvironment [39].

## 2.5. Osteosarcoma invasion

Degradation of the extracellular matrix by osteosarcoma cells allows for invasion of surrounding tissues by the primary tumour mass. Matrix metalloproteinases (MMPs) and the urokinase plasminogen activator (uPA) system are the effectors of this matrix breakdown.

The MMPs include collagenases, gelatinases and stromelysins. Collagenases break down collagen types I, II and III. Gelatinases break down collagen type IV, while stromelysins break down collagen types III, IV and V as well proteoglycans [41].

The urokinase plasminogen activator (uPA) system has been studied extensively with relation to osteosarcoma invasion. When uPA binds to its receptor uPAR it becomes active. Activated uPA then cleaves plasminogen to form plasmin. Plasmin is both responsible for direct breakdown of the extracellular matrix but also for further activation of pro-MMPs [42, 43].

uPA levels possess prognostic significance in osteosarcoma. An inverse relationship exists between survival time and uPA levels in osteosarcoma [44]. The downregulation of uPAR in a clinically relevant murine model of osteosarcoma resulted in limited primary tumour growth and inhibited metastatic spread [45].

## **2.6. Osteoclasts and osteosarcoma-induced osteolysis**

Substantial osteolysis may result from osteosarcoma growth. This osteolysis at the tumour site is the result of interactions between osteosarcoma cells, osteoclasts, osteoblasts and the bone matrix. Growth factors such as transforming growth factor beta (TGF- $\beta$ ) are released from degraded bone matrix and stimulate the release of tumoral cytokines that induce osteoclastic resorption of bone. Among the osteoclast-stimulating cytokines are parathyroid hormone-related protein (PTHrP), interleukin-6 (IL-6) and interleukin-11 (IL-11) [46, 47]. Further growth factors are then released from the bone matrix, leading to a cycle of osteolysis, osteoclast activation and osteosarcoma invasion.

The critical involvement of osteoblasts in the osteolytic process is a surprising finding. Among the other factors that osteosarcoma cells release are the osteoblast-stimulating factors endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) [48, 49]. Osteoblast stimulation by these factors leads to increased expression of receptor activator of nuclear factor  $\kappa$ B ligand (RANKL). RANKL is a key regulator of osteoclast differentiation and activity. Osteosarcoma cells have been noted to produce RANKL independently also [50].

## **2.7. Osteosarcoma angiogenesis**

Tumour neovascularization is required for continued osteosarcoma growth and progression. Osteosarcoma cells obtain the necessary oxygen and nutrients for cellular proliferation from the neovasculature and gain access to these vessels in order to metastasize.

The process of angiogenesis is regulated by a balance between pro-angiogenic and anti-angiogenic regulators. Loss of tumour suppressor gene function and oncogene activation pushes this balance toward neoangiogenesis. The hypoxic and acidotic environment that surrounds the primary tumour also promotes vascular proliferation. Such conditions lead to de-ubiquitination of the von Hippel Lindau protein. Von Hippel Lindau protein releases hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ). HIF-1 $\alpha$  upregulates vascular endothelial growth factor (VEGF) [51]. VEGF is pro-angiogenic through stimulation of the processes of endothelial cell proliferation,

migration and maturation. An immature, irregular and leaky vasculature is thus formed in and around the tumour.

Anti-angiogenic factors are downregulated in osteosarcoma. These include thrombospondin 2, transforming growth factor beta (TGF- $\beta$ ) [52], troponin I, reversion-inducing cysteine rich protein with Kazal motifs (RECK) [53] and pigment epithelial derived factor (PEDF) [54]. Downregulation of such molecules may lead to increased invasion through predominately avascular zones, such as the growth plate [55, 56].

Osteosarcoma is a particularly vascular tumour. However, the true significance of vascular density is yet to be fully elucidated. While vascular tumours may be more likely to lead to increased rate of metastasis, increased osteosarcoma microvascular density may offer a survival advantage attributed to improved tumour penetration by intravenously delivered chemotherapeutics [57].

### 3. Mesenchymal stem cell origin and differentiation

The defining features that characterise stem cells as a group are the ability to self-renew and the ability to differentiate into distinctive cell line types. Stem cells, broadly speaking, may fall into one of four main categories:

1. Embryonic stem cells
2. Pluripotent stem cells
3. Cancer stem cells
4. Tissue specific stem cells

Various tissue specific stem cells have been identified and mesenchymal stem cells (MSCs) are but one of these. Other tissue specific stem cells include cord blood stem cells, neural stem cells, gut stem cells, amniotic fluid stem cells and others. MSCs are multipotent cells that are able to differentiate into bone, cartilage, fat and muscle. Due to this ability they represent a promising source for tissue repair and regeneration. Research has focused on the cellular and molecular pathways that direct differentiation towards a particular cell type and aberrant differentiation of MSCs may contribute to sarcomagenesis. Prior to understanding the interactions between MSCs and osteosarcoma cells, an understanding of the biological factors that characterize MSCs is essential.

The initial work of identifying and characterising MSCs can be largely credited to the work of Friedenstein, Cohnheim and Caplan [58–61]. Cohnheim hypothesised that certain fibroblastic cells originating from bone marrow were a key factor in wound healing. In the 1970s and 1980s, Friedenstein isolated a population of plastic adherent stromal cells from bone marrow, which had the capacity to differentiate into certain colony forming units (CFU). These CFUs possessed the capacity to give rise to osteoblasts, chondrocytes, adipocytes, muscle and haematopoietic tissue. Beyond this, Kopen et al. [62] have demonstrated that not only are MSCs



able to differentiate into mesoderm-derived cells but they are also able to undergo transdifferentiation, forming endoderm-derived cells.

Since these early studies, MSCs have been identified and isolated from tissues other than bone marrow, including adipose tissue, muscle, peripheral blood, placenta, umbilical cord and amniotic fluid. Irrespective of the tissue of origin of MSCs are able to adhere to plastic and differentiate along mesenchymal cell lines. The expression of specific surface antigens has also been used to identify MSCs. The International Society for Cellular Therapy use the following characteristics to identify and standardize isolated human MSCs [63]:

1. Plastic adherence – *in vitro* under standard culture conditions (1–5 days);
2. Tri-lineage differentiation into cells of mesodermal lineage (osteoblasts, chondroblasts and adipocytes);
3. Surface antigens:
  - a. Expression of CD105, CD73, CD 90
  - b. Absence of CD45, CD 34, CD 14, CD 11b, CD79b, CD 19, HLA-DR (haematopoietic markers)

Most relevant in the setting of translational research, however, is that significant variation exists in the expression at surface antigens across species. MSCs of murine origin may be identified by the expression of CD106 and Sca-1, and the absence of CD31, CD45 and CD11b. Studies have demonstrated significant variability in surface antigen expression which changes once MSCs undergo expansion and ex-plantation [64].

### 3.1. Sources of MSCs

MSCs are found in nearly all tissues, including adult bone marrow, peripheral blood and adipose tissues. MSCs are derived from pericytes (cells surrounding blood vessels) and exist in a perivascular niche. This explains the presence of adult MSCs in a number of different tissue types [65], including:

- Bone marrow
- Synovium and synovial fluid
- Periosteum
- Peripheral blood
- Adipocytes
- Liver
- Brain
- Kidney

- Lung
- Spleen
- Blood vessels

While MSCs may be obtained from a variety of different tissue types, the concentration of MSCs in these tissues varies widely. Pittenger et al. [66] isolated MSCs from bone marrow, adipocyte and peripheral blood. 0.001-0.01% of bone marrow cells were MSCs in comparison to ~5000 cells of 1g of adipose were MSCs. Furthermore, in addition to the variable concentration of the stem cells sourced from different tissues, it has been demonstrated that there is altered capacity to form osteocytes in vivo dependent on the tissue of origin of MSCs. Cosimo De Bari showed that periosteal derived MSCs have a greater potential to form osteocytes than those derived from synovium [67].

Mesenchymal stem cells can also be obtained from birth associated tissues [65], including:

- Placenta
- Human amnion membrane
- Umbilical cord
- Cord blood
- Chorionic villi and chorion membrane
- Wharton's jelly

The major advantages of MSCs derived from birth associated tissue, over those obtained from bone marrow, are the availability of the tissue, as well as the greater proliferative and differentiation capacity of these cells. The rate of expansion varies between adult and birth associated tissue derived MSCs. The mean doubling time for umbilical cord MSCs is approximately 24 hours whilst it is 40 hours for bone marrow MSCs. Additionally, umbilical cord MSCs proliferate with multi-layering, while bone marrow MSCs demonstrate contact inhibition. Bone marrow MSCs are multipotent, while birth associated tissue MSCs are pluripotent and are able to differentiate into all three germinal layers.

### **3.2. Multi-lineage potential and transdifferentiation of MSCs**

Friedenstein et al. initially demonstrated that bone marrow derived MSCs differentiated exclusively into cells of mesodermal lineage, namely osteocytes, adipocytes and chondrocytes [59]. More recently, however, MSCs have been shown to also possess the ability to differentiate along endodermal and neuroectodermal lines. In vitro studies have shown formation of neural tissue from bone marrow derived MSCs. This has propagated multiple studies determining the factors that stimulate MSCs to differentiate into cell lineages.

Pittenger et al. [66] highlighted that in vitro mesenchymal stem cells can maintain a stable and undifferentiated state, however when exposed to certain cues or cultured in certain media

they are able to differentiate into diverse cell types. MSCs that have undergone 20 cumulative population doublings maintain this multipotent ability.

The osteogenic potential of MSCs has been observed *in vitro*, however this ability *in vivo* is still incompletely defined. Osteoblasts may stimulate the expansion of MSCs and regulate differentiation down the osteogenic pathway, however this may be secondary to the role of osteocytes in stimulating differentiation toward osteogenesis.

Huang et al. demonstrated the process of osteogenic differentiation *in vitro*, through multiple stages [68, 69]:

1. Day 1–4
  - a. Peak number of cells
2. Day 5–14
  - a. Early cell differentiation
  - b. Deposition of type 1 collagen early in this phase
  - c. Expression of alkaline phosphatase (ALP), however the level of ALP decreases at the end of the second phase
3. Day 14–28
  - a. Expression of fibroblast growth factor 2 (FGF-2) and bone morphogenetic protein 2 (BMP-2)
  - b. Expression of osteocalcin and osteopontin
  - c. Calcium and phosphate deposition

The early response growth factors were distinguished from the growth factors present in late cycle. The early response factors include transforming growth factor beta, insulin-like growth factor and vascular endothelial growth factor. The later phase growth factors include platelet derived growth factor, bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor 2 (FGF-2)

Transforming growth factor beta (TGF-beta) administration stimulates osteoblast activity as well as cell proliferation, alkaline phosphatase activity and calcium deposition. BMP-2 is a notable cytokine which is osteoinductive, and has been shown to commit cells into either a chondrogenic or osteogenic lineage depending on its culture medium. When these two factors co-exist in an environment, there is approximately five-fold greater osteogenic potential.

Other groups of factors are important for adipogenic and chondrogenic differentiation. Factors favouring adipogenic differentiation include 1-methyl-3-isobutylxanthine, dexamethasone, insulin and indomethacin, whereby the adipocytes expressed lipoprotein lipase, fatty acid-binding protein (Ap2) and peroxisome proliferation-activated receptor gamma 2 (PPAR-2) [66, 68]. Factors for chondrogenic potential include glutamine, linoleic acid, dexamethasone,

ascorbic acid, proline and sodium pyruvate. Dexamethasone is required as it promotes TGF-beta1 upregulation of type II collagen. The potent factors which were found to be important in chondrocyte formation are BMP-2 and BMP-7, with TGF-beta being a weaker factor. The effect of BMP-2 is dose-dependent, whereby it stimulates the production of mRNA for type II collagen and aggrecan [70, 71].

There are two main pathways important in differentiation. One discussed previously is through TGF-beta, involved in the formation of chondrocytes. This occurs through multiple intra-cellular cascades (mitogen activated protein, JNK, p38). The other pathway is the Wnt canonical pathway, where soluble glycoproteins stimulate and regulate cellular differentiation and expansion. Like the TGF-beta pathway, the binding of Wnt to receptors on cells trigger an intracellular cascade, however, this pathway has an osteogenic potential.

#### **4. Transformation of mesenchymal stem cells**

Transformation is the sequential accumulation of genetic changes in a cell that may lead to altered behaviour and function of the subsequent cell lineage. Transformation causes cells to both acquire new and lose certain characteristics of the original cell type. This may be reflected as changes in the morphology of the cells, altered expression of surface antigens, changes in the growth characteristics, as well as increased tumorigenicity. Differentiation of MSCs at a variety of stages may underlie sarcomagenesis. Sarcomas may arise from cells already committed to a particular differentiation pathway, or alternatively, from multipotent cells that are pushed towards a particular sarcoma subtype. Alterations in oncogenes, tumour suppressor genes, growth factors and transcription factors may underlie the transformation of MSCs.

Studies that have utilised MSCs of both murine and human origins have supported the concept of transformation of MSCs for tumorigenesis. The findings of human studies have been conflicting, however, and warrant further evaluation. Transformed murine MSCs demonstrate altered morphology and growth characteristics. Transformed murine MSCs exhibit a compact morphology, demonstrate anchorage-independent growth, lack contact inhibition and form multiple layers in culture. This is in contrast to the spindle-shaped single layer growth characteristics of MSCs [72–75]. The proliferation rates of transformed murine MSCs have been shown to be increased and genetic and molecular signalling alterations underlie these changes [72, 76, 77]. Increased chromosome number beyond the usual 40 acrocentric chromosomes have been demonstrated in transformed murine MSCs by multiple authors [72, 73, 78]. Additionally, Matushansky et al. [79] showed that inactivation of the Wnt pathway in transformed MSCs gave rise to a cell population with a similar appearance to that of malignant fibrous histiocytoma.

Human models require MSCs that are able to undergo ex vivo expansion prior to its clinical application and through this process some cells undergo spontaneous transformation. This is particularly concerning when considering the potential therapeutic use of MSCs for tissue repair and regeneration. There are also pharmacological agents that mobilise MSCs into the bloodstream. However, there has been some variability in studies using human MSCs. Some studies have shown spontaneous transformation of human MSCs in culture [80, 81]

while other research groups have demonstrated that human MSCs are not able to spontaneously transform into malignant cells and with prolonged *in vitro* culturing become senescent [82–85]. These conflicting studies have been further confounded by Torsvik et al. [86] and de la Fuente et al. [87] that demonstrated previously considered transformed MSCs were tainted by contamination. Pan et al. [88] have subsequently shown MSCs to undergo transformation and have eliminated the possibility of contamination. In this study, 46 cultures of MSCs were studied and 4 of these cultures showed characteristics of transformation, including morphological changes and increased proliferation rates. Increased tumorigenicity was demonstrated when these cells were introduced into immunodeficient mice.

In addition to the cellular, molecular and genetic changes underlying osteosarcoma pathogenesis, the transformation of MSCs have also been implicated in the tumorigenesis of osteosarcoma. Wang et al. [89] were among the first to hypothesise that a subpopulation of cancer stem cells existed in human osteosarcoma. In order to demonstrate such a subpopulation of tumorigenic cells, Wang et al. characterised cells with high aldehyde dehydrogenase (ALDH) in 4 human osteosarcoma cell lines. Of these, the OS99-1 cell line, which was derived from an aggressive primary human osteosarcoma, had significantly higher ALDH activity. When OS99-1 cells were introduced into a murine xenograft model, 3% of tumour cells demonstrated high ALDH activity and these cells demonstrated the characteristics of MSCs, namely self-renewal, tri-lineage differentiation and the expression of typical cell surface antigens.

Since then, Adhikari et al. [90] have further characterised a subpopulation of cancer stem cells in osteosarcoma using cell surface antigens. This study took the concept of tumour-initiating cells further by identifying a possible role of cancer stem cells in highly metastatic and resistant osteosarcoma. Mouse and human osteosarcoma stem cells were identified using the MSC markers CD117 and Stro-1. Expression of these markers were largely in spheres and doxorubicin-resistant cells. Cells that were positive for both CD117 and Stro-1 were serially transplantable and gave rise to more aggressive metastatic disease when applied to an orthotopic murine model. CD117 and Stro-1 positive tumours in the model were highly invasive and demonstrated drug resistance.

Alterations in oncogenes, tumour suppressor genes, growth factors and transcription factors may underlie the transformation of MSCs for osteosarcoma tumorigenesis. In one study, Mohseny et al. [74] examined the pre-malignant stages of osteosarcoma using murine mesenchymal cells. A functional and phenotypical analysis of MSCs, transformed MSCs and osteosarcoma cells was performed in parallel using. Aneuploidization, translocations, homozygous loss of the cyclin-dependent kinase inhibitor (*cdkn2*) region, and alterations in sarcoma amplified sequence (SAS), retinoblastoma 1 (*Rb1*), mouse double minute 2 homolog (*Mdm2*), *c-myc*, *p53* and *p16* have all been implicated in the transformation of MSCs for osteosarcoma formation [74, 91].

Tao et al. [92] identified the transformation of immature osteoblasts as a potential source for osteosarcoma transformation. Using a murine model of osteosarcoma with conditional overexpression of intracellular domain of Notch1 (NICD), expression of NICD in osteoblast stem cells caused the formation of bone tumours including osteosarcoma. These tumours



demonstrated histopathological, metastatic and genetic features of human osteosarcoma. Additionally, when overexpression of NICD and loss of p53 were combined in the murine model, osteosarcoma development and progression was accelerated.

## **5. Interactions between mesenchymal stem cells and osteosarcoma cells**

The interaction between MSCs and tumour cells is an evolving area of current research. MSCs have been shown to be capable of migrating to not only sites of inflammation and injury but also to tumours and sites of metastasis. Once at these tumour sites, cellular interactions may cause progression of both primary and metastatic lesions. While these interactions between MSCs and osteosarcoma cells in the tumour microenvironment have been demonstrated, some studies show that MSCs may cause increased proliferation of tumour cells while others show reduced proliferation and pro-differentiation. Khakoo et al. [93] showed that systemically injected MSCs inhibit the growth of Kaposi sarcoma using a xenotransplant model.

Yu et al. [40] characterised the interaction between MSCs and osteosarcoma cells in vitro and showed that bone marrow derived MSCs had the potential to promote osteosarcoma cell proliferation and invasion. In this study bone marrow MSCs were cultured with osteosarcoma cells. Osteosarcoma cells were also cultured with conditioned media from MSCs. Cellular proliferation was measured by cell counting kit 8 (CCK-8) assay and a matrigel assay was used to evaluate tumour cell invasion. Tumour cell proliferation and invasion were promoted under these conditions with the implication of stromal derived factor-1 (SDF-1). SDF-1 is a cytokine that controls tumour neoangiogenesis, apoptosis, migration and invasion through binding to the CXCR4 receptor.

Tsukamoto et al. [94] showed that MSCs may provide a favourable environment for osteosarcoma growth and metastasis in a rat osteosarcoma model. In this study, rat COS1NR osteosarcoma cells were injected along with rat bone marrow derived MSCs. Injections were performed subcutaneously and intravenously. Osteosarcoma tumour formation and growth was increased significantly prior to 5 weeks using the subcutaneous injection model. When injected intravenously there was increased pulmonary lesion formation in the group that received co-injections of COS1NR and MSCs. The expression of genes by MSCs involved in cellular adhesion and extracellular matrix receptors were suggested as possible explanations for this tumour behaviour.

## **6. Mesenchymal stem cell utilization for biological reconstruction**

MSCs are being portrayed in the literature as the key to biological reconstruction, however, studies are few and results are varied. There are significant challenges to be overcome if we are to utilise MSCs in biological reconstruction after tumour resection. Much of the concern relates to the yet to be fully characterised ability of MSCs to transform into sarcomas and the interactions between MSCs and tumours that cause increased tumorigenesis and disease progression. In order to apply MSCs to clinical reconstruction the cells require prior in vitro

expansion. As has been discussed above, there are concerns of chromosomal instability and malignant transformation during this process of expansion.

A number of attempts at utilizing MSCs in the reconstruction process after tumour resection have been made. Perrot et al. [95] raised concern of osteosarcoma recurrence after autologous fat grafting, reporting a case of late recurrent osteosarcoma 13 years after the use of a lipofilling procedure. Following this they utilised a pre-clinical murine model of osteosarcoma to show that injection of fat grafts and MSCs promoted tumour growth.

Since then, Centeno et al. [96, 97] has published two papers with results for 339 patients that were treated following orthopaedic procedures with *in vitro* expanded, autologous bone marrow derived MSC implantation. Follow up by general observation and MRI tracking beyond 3 years post-operatively did not demonstrate tumour formation at the sites of injection. 2 patients were diagnosed with cancer during the follow up period, however these cases were assessed not to be related to the MSC therapy and the rate of neoplasm development was comparable to that of the general population. While the results presented by Centano et al. [96, 97] appear reassuring with regards to the safety of MSCs for reconstruction, further studies, particularly in the setting of reconstruction after treatment for malignancy are required. There are hundreds of clinical trials currently underway evaluating the therapeutic safety and efficacy of MSC based treatments.

## 7. Conclusions

While the advent of multi-agent chemotherapeutic regimes dramatically improved the prognosis for patients with osteosarcoma, novel treatment agents are required in order to reduce morbidity and improve function following surgical reconstruction. The pathogenesis of osteosarcoma is complex and current research is focusing on defining the deranged cell behaviours and molecular signalling pathways that underpin tumorigenesis and disease progression. Mesenchymal stem cells have attracted great interest over recent years due to their ability to expand into mesodermal tissues including bone, cartilage, fat and muscle; however, pre-clinical studies have highlighted possible roles in the processes of sarcomogenesis through transformation and interactions with the tumour cells themselves. Further studies defining the role of MSCs in osteosarcoma pathogenesis are required prior to studies of therapeutic safety and efficacy.

## Author details

Matthew L. Broadhead<sup>1\*</sup>, Saumiyar Sivaji<sup>1</sup>, Zsolt Balogh<sup>1</sup> and Peter F.M. Choong<sup>2</sup>

\*Address all correspondence to: matthewbroadhead@me.com

<sup>1</sup> John Hunter Hospital, University of Newcastle, New Lambton Heights NSW, Australia

<sup>2</sup> St Vincent's Hospital Melbourne, University of Melbourne, Fitzroy VIC, Australia

## References

- [1] Canale ST, Beaty JH. Campbell's Operative Orthopaedics. 11th ed: Philadelphia Mosby Elsevier; 2008.
- [2] Geller DS, Gorlick R. Osteosarcoma: a review of diagnosis, management, and treatment strategies. Clin Adv Hematol Oncol. 2010;8(10):705-18.
- [3] Longhi A, Errani C, De Paolis M, Mercuri M, Bacci G. Primary bone osteosarcoma in the pediatric age: state of the art. Cancer treatment reviews. 2006;32(6):423-36.
- [4] Ta HT, Dass CR, Choong PF, Dunstan DE. Osteosarcoma treatment: state of the art. Cancer Metastasis Rev. 2009;28(1-2):247-63.
- [5] Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, et al. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(3):776-90.
- [6] Vigorita VJ, editor. Orthopaedic Pathology. 2nd ed. Philadelphia, PA: Lippincott, Williams and Wilkins; 2008.
- [7] Polednak AP. Bone cancer among female radium dial workers. Latency periods and incidence rates by time after exposure: brief communication. Journal of the National Cancer Institute. 1978;60(1):77-82.
- [8] Picci P. Osteosarcoma (osteogenic sarcoma). Orphanet J Rare Dis. 2007;2:6.
- [9] Paulino AC, Fowler BZ. Secondary neoplasms after radiotherapy for a childhood solid tumor. Pediatric hematology and oncology. 2005;22(2):89-101.
- [10] Longhi A, Barbieri E, Fabbri N, Macchiagodena M, Favale L, Lippo C, et al. Radiation-induced osteosarcoma arising 20 years after the treatment of Ewing's sarcoma. Tumori. 2003;89(5):569-72.
- [11] Rani AS, Kumar S. Transformation of non-tumorigenic osteoblast-like human osteosarcoma cells by hexavalent chromates: alteration of morphology, induction of anchorage-independence and proteolytic function. Carcinogenesis. 1992;13(11):2021-7.
- [12] Dutra FR, Largent EJ. Osteosarcoma induced by beryllium oxide. The American journal of pathology. 1950;26(2):197-209.
- [13] Mazabraud A. [Experimental production of bone sarcomas in the rabbit by a single local injection of beryllium]. Bulletin du cancer. 1975;62(1):49-58.
- [14] Romagnoli S, Fasoli E, Vaira V, Falleni M, Pellegrini C, Catania A, et al. Identification of potential therapeutic targets in malignant mesothelioma using cell-cycle gene expression analysis. Am J Pathol. 2009;174(3):762-70.

- [15] Smida J, Baumhoer D, Rosemann M, Walch A, Bielack S, Poremba C, et al. Genomic alterations and allelic imbalances are strong prognostic predictors in osteosarcoma. *Clin Cancer Res.*16(16):4256-67.
- [16] Greenspan A, Jundt G, Remagen W. Differential diagnosis in orthopaedic oncology. Philadelphia: Lippincott Williams ' Wilkins; 2007. xi, 529 p. p.
- [17] German J, Crippa LP, Bloom D. Bloom's syndrome. III. Analysis of the chromosome aberration characteristic of this disorder. *Chromosoma.* 1974;48(4):361-6.
- [18] Fukuchi K, Martin GM, Monnat RJ, Jr. Mutator phenotype of Werner syndrome is characterized by extensive deletions. *Proceedings of the National Academy of Sciences of the United States of America.* 1989;86(15):5893-7.
- [19] Hansen MF, Seton M, Merchant A. Osteosarcoma in Paget's disease of bone. *J Bone Miner Res.* 2006;21 Suppl 2:P58-63.
- [20] Hansen MF, Nellisery MJ, Bhatia P. Common mechanisms of osteosarcoma and Paget's disease. *J Bone Miner Res.* 1999;14 Suppl 2:39-44.
- [21] HUGO Gene Nomenclature Committee. TNFRSF11A TNF receptor superfamily member 11a [ Homo sapiens (human) ] [Internet]. 2016 [cited 01/08/2016]. Available from: <http://www.ncbi.nlm.nih.gov/gene/8792>.
- [22] Guo W, Wang X, Feng C. P53 gene abnormalities in osteosarcoma. *Chinese medical journal.* 1996;109(10):752-5.
- [23] Tongyuan Li NK, 1 Le Jiang,1 Minjia Tan,2 Thomas Ludwig,1 Yingming Zhao,2 Richard Baer,1 and Wei Gu1,, \*. Tumor Suppression in the Absence of p53-Mediated Cell-Cycle Arrest, Apoptosis, and Senescence. *Cell.* 2012(149):1269 - 83.
- [24] Balint EE, Vousden KH. Activation and activities of the p53 tumour suppressor protein. *British journal of cancer.* 2001;85(12):1813-23.
- [25] Shaw PH. The role of p53 in cell cycle regulation. *Pathology, research and practice.* 1996;192(7):669-75.
- [26] Kumaramanickavel G, InTech Open Access Books. Retinoblastoma An Update on Clinical, Genetic Counseling, Epidemiology and Molecular Tumor Biology. S.I.: InTech,; 2012. Available from: <http://www.intechopen.com/books/retinoblastoma-an-update-on-clinical-genetic-counseling-epidemiology-and-molecular-tumor-biology>.
- [27] Weinberg RA. The Retinoblastoma Protein and Cell Cycle Control *Cell.* 1995;81(3):320-30.
- [28] C Giacinti AG. RB and cell cycle progression. *Oncogene.* 2006(25):5220-7.
- [29] Wu JX, Carpenter PM, Gresens C, Keh R, Niman H, Morris JW, et al. The proto-oncogene c-fos is over-expressed in the majority of human osteosarcomas. *Oncogene.* 1990;5(7):989-1000.
- [30] Franchi A, Calzolari A, Zampi G. Immunohistochemical detection of c-fos and c-jun expression in osseous and cartilaginous tumours of the skeleton. *Virchows Arch.* 1998;432(6):515-9.

- [31] Gamberi G, Benassi MS, Bohling T, Ragazzini P, Molendini L, Sollazzo MR, et al. C-myc and c-fos in human osteosarcoma: prognostic value of mRNA and protein expression. *Oncology*. 1998;55(6):556-63.
- [32] Shimizu T, Ishikawa T, Sugihara E, Kuninaka S, Miyamoto T, Mabuchi Y, et al. c-MYC overexpression with loss of Ink4a/Arf transforms bone marrow stromal cells into osteosarcoma accompanied by loss of adipogenesis. *Oncogene*. 2010, 29 (42) 5687-5699
- [33] Hattinger CM, Stoico G, Michelacci F, Pasello M, Scionti I, Remondini D, et al. Mechanisms of gene amplification and evidence of coamplification in drug-resistant human osteosarcoma cell lines. *Genes Chromosomes Cancer*. 2009;48(4):289-309.
- [34] Franchi A, Arganini L, Baroni G, Calzolari A, Capanna R, Campanacci D, et al. Expression of transforming growth factor beta isoforms in osteosarcoma variants: association of TGF beta 1 with high-grade osteosarcomas. *J Pathol*. 1998;185(3):284-9.
- [35] Rikhs B, de Jong S, Suurmeijer AJ, Meijer C, van der Graaf WT. The insulin-like growth factor system and sarcomas. *J Pathol*. 2009;217(4):469-82.
- [36] Nishida T, Nakanishi T, Asano M, Shimo T, Takigawa M. Effects of CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, on the proliferation and differentiation of osteoblastic cells in vitro. *J Cell Physiol*. 2000;184(2):197-206.
- [37] Perbal B, Zuntini M, Zambelli D, Serra M, Sciandra M, Cantiani L, et al. Prognostic value of CCN3 in osteosarcoma. *Clin Cancer Res*. 2008;14(3):701-9.
- [38] Hoang BH, Kubo T, Healey JH, Sowers R, Mazza B, Yang R, et al. Expression of LDL receptor-related protein 5 (LRP5) as a novel marker for disease progression in high-grade osteosarcoma. *International journal of cancer Journal international du cancer*. 2004;109(1):106-11.
- [39] Jan A. Burger TJK. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood*. 2006;107:1751-67.
- [40] Yu FX, Hu WJ, He B, Zheng YH, Zhang QY, Chen L. Bone marrow mesenchymal stem cells promote osteosarcoma cell proliferation and invasion. *World journal of surgical oncology*. 2015;13:52.
- [41] Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem*. 2003;253(1-2):269-85.
- [42] Choong PF, Nadesapillai AP. Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. *Clin Orthop Relat Res*. 2003(415 Suppl):S46-58.
- [43] Pillay V, Dass CR, Choong PF. The urokinase plasminogen activator receptor as a gene therapy target for cancer. *Trends Biotechnol*. 2007;25(1):33-9.
- [44] Choong PF, Fernö M, Akerman M, Willén H, Långström E, Gustafson P, et al. Urokinase-plasminogen-activator levels and prognosis in 69 soft-tissue sarcomas. *Int J Cancer*. 1996;69(4):268-72.



- [45] Dass CR, Nadesapillai AP, Robin D, Howard ML, Fisher JL, Zhou H, et al. Downregulation of uPAR confirms link in growth and metastasis of osteosarcoma. *Clin Exp Metastasis*. 2005;22(8):643-52.
- [46] Guise TA, Chirgwin JM. Transforming growth factor-beta in osteolytic breast cancer bone metastases. *Clin Orthop Relat Res*. 2003(415 Suppl):S32-8.
- [47] Quinn JM, Itoh K, Udagawa N, Hausler K, Yasuda H, Shima N, et al. Transforming growth factor beta affects osteoclast differentiation via direct and indirect actions. *J Bone Miner Res*. 2001;16(10):1787-94.
- [48] Kingsley LA, Fournier PG, Chirgwin JM, Guise TA. Molecular biology of bone metastasis. *Mol Cancer Ther*. 2007;6(10):2609-17.
- [49] Chirgwin JM, Guise TA. Skeletal metastases: decreasing tumor burden by targeting the bone microenvironment. *J Cell Biochem*. 2007;102(6):1333-42.
- [50] Kinpara K, Mogi M, Kuzushima M, Togari A. Osteoclast differentiation factor in human osteosarcoma cell line. *J Immunoassay*. 2000;21(4):327-40.
- [51] Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol*. 2005;23(5):1011-27.
- [52] Ren B, Yee KO, Lawler J, Khosravi-Far R. Regulation of tumor angiogenesis by thrombospondin-1. *Biochim Biophys Acta*. 2006;1765(2):178-88.
- [53] Clark JC, Thomas DM, Choong PF, Dass CR. RECK--a newly discovered inhibitor of metastasis with prognostic significance in multiple forms of cancer. *Cancer Metastasis Rev*. 2007;26(3-4):675-83.
- [54] Cai J, Parr C, Watkins G, Jiang WG, Boulton M. Decreased pigment epithelium-derived factor expression in human breast cancer progression. *Clin Cancer Res*. 2006;12(11 Pt 1):3510-7.
- [55] Quan GM, Ojaimi J, Li Y, Kartsogiannis V, Zhou H, Choong PF. Localization of pigment epithelium-derived factor in growing mouse bone. *Calcif Tissue Int*. 2005;76(2):146-53.
- [56] Moses MA, Wiederschain D, Wu I, Fernandez CA, Ghazizadeh V, Lane WS, et al. Troponin I is present in human cartilage and inhibits angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 1999;96(6):2645-50.
- [57] Kreuter M, Bieker R, Bielack SS, Auras T, Buerger H, Gosheger G, et al. Prognostic relevance of increased angiogenesis in osteosarcoma. *Clin Cancer Res*. 2004;10(24):8531-7.
- [58] Friedenstein AJ, Piatetzky S, II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol*. 1966;16(3):381-90.
- [59] Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell*. 2008;2(4):313-9.
- [60] Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. *Cell Tissue Kinet*. 1987;20(3):263-72.

- [61] Caplan AI. Mesenchymal stem cells. *J Orthop Res*. 1991;9(5):641-50.
- [62] Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout fore-brain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proceedings of the National Academy of Sciences of the United States of America*. 1999;96(19):10711-6.
- [63] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-7.
- [64] Adam R, Williams JMH. Mesenchymal Stem Cells Biology, Pathophysiology, Translational Findings, and Therapeutic Implications for Cardiac Disease. *Circulation research*. 2011(109):923-40.
- [65] Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal*. 2011;9:12.
- [66] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284(5411):143-7.
- [67] Alan Tyndall UAW, Andrew Cope, Francesco Dazzi, Cosimo De Bari, Willem Fibbe, Serena Guiducci, Simon Jones, Christian Jorgensen, Katarina Le Blanc, Frank Luyten, Dennis McGonagle, Ivan Martin, Chiara Bocelli-Tyndall, Giuseppina Pennesi, Vito Pistoia, Constantino Pitzalis, Antonio Uccelli, Nico Wulffraat and Marc Feldmann. Immunomodulatory properties of mesenchymal stem cells: a review based on an interdisciplinary meeting held at the Kennedy Institute of Rheumatology Division, London, UK, 31 October 2005. *Arthritis Research ' Therapy*. 2007(9):301.
- [68] Huang Z1 NE, Smith RL, Goodman SB. The sequential expression profiles of growth factors from osteoprogenitors [correction of osteoprogenitors] to osteoblasts in vitro. *Tissue Engineering*. 2007;13(9):2311-20.
- [69] Hanumantha Rao Balaji Raghavendran SP, Sepehr Talebian, Malliga Raman Murali, Sangeetha Vasudevaraj Naveen, G. Krishnamurthy, Robert McKean, and Tunku Kamarul. A Comparative Study on In Vitro Osteogenic Priming Potential of Electron Spun Scaffold PLLA/HA/Col, PLLA/HA, and PLLA/Col for Tissue Engineering Application. *PLOS* 2014;9(8):e104389.
- [70] Grunder T, Gaissmaier C, Fritz J, Stoop R, Hortschansky P, Mollenhauer J, et al. Bone morphogenetic protein (BMP)-2 enhances the expression of type II collagen and aggrecan in chondrocytes embedded in alginate beads. *Osteoarthritis Cartilage*. 2004;12(7):559-67.
- [71] Kurth T, Hedbom E, Shintani N, Sugimoto M, Chen FH, Haspl M, et al. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. *Osteoarthritis Cartilage*. 2007;15(10):1178-89.

- [72] Furlani D, Li W, Pittermann E, Klopsch C, Wang L, Knopp A, et al. A transformed cell population derived from cultured mesenchymal stem cells has no functional effect after transplantation into the injured heart. *Cell Transplant*. 2009;18(3):319-31.
- [73] Ren Z, Wang J, Zhu W, Guan Y, Zou C, Chen Z, et al. Spontaneous transformation of adult mesenchymal stem cells from cynomolgus macaques in vitro. *Exp Cell Res*. 2011;317(20):2950-7.
- [74] Mohseny AB, Szuhai K, Romeo S, Buddingh EP, Briaire-de Bruijn I, de Jong D, et al. Osteosarcoma originates from mesenchymal stem cells in consequence of aneuploidization and genomic loss of Cdkn2. *J Pathol*. 2009;219(3):294-305.
- [75] Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, Bell S, et al. Sarcoma derived from cultured mesenchymal stem cells. *Stem Cells*. 2007;25(2):371-9.
- [76] Zheng Y, He L, Wan Y, Song J. H3K9me-enhanced DNA hypermethylation of the p16INK4a gene: an epigenetic signature for spontaneous transformation of rat mesenchymal stem cells. *Stem Cells Dev*. 2013;22(2):256-67.
- [77] Zhou YF, Bosch-Marce M, Okuyama H, Krishnamachary B, Kimura H, Zhang L, et al. Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. *Cancer Res*. 2006;66(22):10849-54.
- [78] Miura M, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, Patel V, et al. Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. *Stem Cells*. 2006;24(4):1095-103.
- [79] Matushansky I, Hernando E, Socci ND, Mills JE, Matos TA, Edgar MA, et al. Derivation of sarcomas from mesenchymal stem cells via inactivation of the Wnt pathway. *J Clin Invest*. 2007;117(11):3248-57.
- [80] Rosland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, et al. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res*. 2009;69(13):5331-9.
- [81] Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, et al. Spontaneous human adult stem cell transformation. *Cancer Res*. 2005;65(8):3035-9.
- [82] Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, et al. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res*. 2007;67(19):9142-9.
- [83] Choumerianou DM, Dimitriou H, Perdikogianni C, Martimianaki G, Riminucci M, Kalmanti M. Study of oncogenic transformation in ex vivo expanded mesenchymal cells, from paediatric bone marrow. *Cell Prolif*. 2008;41(6):909-22.
- [84] Wang Y, Huso DL, Harrington J, Kellner J, Jeong DK, Turney J, et al. Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. *Cytotherapy*. 2005;7(6):509-19.

- [85] Aguilar S, Nye E, Chan J, Loebinger M, Spencer-Dene B, Fisk N, et al. Murine but not human mesenchymal stem cells generate osteosarcoma-like lesions in the lung. *Stem Cells*. 2007;25(6):1586-94.
- [86] Torsvik A, Rosland GV, Svendsen A, Molven A, Immervoll H, McCormack E, et al. Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track - letter. *Cancer Res*. 2010;70(15):6393-6.
- [87] de la Fuente R, Bernad A, Garcia-Castro J, Martin MC, Cigudosa JC. Retraction: Spontaneous human adult stem cell transformation. *Cancer Res*. 2010;70(16):6682.
- [88] Pan Q, Fouraschen SM, de Ruiter PE, Dinjens WN, Kwekkeboom J, Tilanus HW, et al. Detection of spontaneous tumorigenic transformation during culture expansion of human mesenchymal stromal cells. *Exp Biol Med (Maywood)*. 2014;239(1):105-15.
- [89] Wang L, Park P, Zhang H, La Marca F, Lin CY. Prospective identification of tumorigenic osteosarcoma cancer stem cells in OS99-1 cells based on high aldehyde dehydrogenase activity. *Int J Cancer*. 2011;128(2):294-303.
- [90] Adhikari AS, Agarwal N, Wood BM, Porretta C, Ruiz B, Pochampally RR, et al. CD117 and Stro-1 identify osteosarcoma tumor-initiating cells associated with metastasis and drug resistance. *Cancer Res*. 2010;70(11):4602-12.
- [91] He JP, Hao Y, Wang XL, Yang XJ, Shao JF, Guo FJ, et al. Review of the molecular pathogenesis of osteosarcoma. *Asian Pac J Cancer Prev*. 2014;15(15):5967-76.
- [92] Tao J, Jiang MM, Jiang L, Salvo JS, Zeng HC, Dawson B, et al. Notch activation as a driver of osteogenic sarcoma. *Cancer Cell*. 2014;26(3):390-401.
- [93] Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira, II, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med*. 2006;203(5):1235-47.
- [94] Tsukamoto S, Honoki K, Fujii H, Tohma Y, Kido A, Mori T, et al. Mesenchymal stem cells promote tumor engraftment and metastatic colonization in rat osteosarcoma model. *Int J Oncol*. 2012;40(1):163-9.
- [95] Perrot P, Rousseau J, Bouffaut AL, Redini F, Cassagnau E, Deschaseaux F, et al. Safety concern between autologous fat graft, mesenchymal stem cell and osteosarcoma recurrence. *PLoS One*. 2010;5(6):e10999.
- [96] Centeno CJ, Schultz JR, Cheever M, Freeman M, Faulkner S, Robinson B, et al. Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. *Curr Stem Cell Res Ther*. 2011;6(4):368-78.
- [97] Centeno CJ, Schultz JR, Cheever M, Robinson B, Freeman M, Marasco W. Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. *Curr Stem Cell Res Ther*. 2010;5(1):81-93.

