7

The Retinoblastoma Protein in Osteosarcomagenesis

Elizabeth Kong and Philip W. Hinds
Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA USA

1. Introduction

Osteosarcoma is one of the most common primary non-hematologic bone tumors present in both children and adults. The etiology of most cases of osteosarcoma is unknown although a genetic predisposition is suspected. Most osteosarcomas are diagnosed before the age of 20, where the pathology is likely distinct from osteosarcoma diagnosed over the age of 60, which is associated with bone diseases such as Paget disease or Rothmund-Thomson syndrome. Although rare, osteosarcoma is a complication in survivors of childhood cancers treated with radiation therapy. Tumors from patients diagnosed early in life are reported to have a broad range of genetic and molecular factors that are potential targets in disease formation. However, *RB1* and/or *TP53* mutations are consistently detected in the majority of osteosarcomas. This chapter will focus on understanding the potential links between disruption of genetic components in osteogenic differentiation and osteosarcoma.

Osteosarcoma is the most frequent primary bone tumor, but is relatively rare with less than 900 new cases diagnosed each year in the United States (Sandberg and Bridge, 2003). There is considerable diversity in histologic features and grade, but osteosarcoma is defined as a malignant tumor of mesodermal origin, where tumor cells produce bone or osteoid (Schajowicz et al., 1995). There are two peaks of occurrence for osteosarcoma. The first peak is during adolescence, when approximately 70% of osteosarcomas are diagnosed (Hauben et al., 2003), but the disease is rarely diagnosed before the age of five. The second peak, accounting for approximately 10% of osteosarcomas, is observed in patients over the age of 60, and is associated with individuals with underlying non-cancerous bone diseases such as Paget, Werner or Rothmund-Thomson Syndrome or in patients previously exposed to radiation therapy (McNairn et al., 2001; Wang et al., 2001; Nellissery et al., 1998; Lindor et al., 2000; Picci, 2007; Paulino and Fowler, 2005).

Although osteosarcoma can develop in any part of the skeleton, in younger patients, areas of rapid bone growth such as the lower long bones have a higher frequency of occurrence (Mirabello et al., 2009). Osteosarcoma in the mandible or skull is more frequently seen in patients diagnosed after the 6th decade of life (Caron et al., 1971) and is often a secondary lesion (Longhi et al., 2008). Irrespective of age, approximately 80% of patients have either detectable metastasis, primarily to the lung, or sub-clinical micro-metastasis (Kaste et al., 1999). The disease is seen globally and more frequently in males compared to females (Stiller et al., 2006), but is observed earlier in females correlating to growth spurts. Osteosarcoma diagnosed during the first two decades of life is often poorly differentiated, and cells from these tumors

have characteristics of early osteoprogenitor or mesenchymal progenitor cells (Dahlin, 1988; Huvos, 1993; Hopyan et al., 1999; Klein and Siegal, 2006). Correlations between early markers of osteogenesis in conventional osteosarcoma with periods of rapid cell division infer that the disease may be associated with defects in osteoblast differentiation.

Most osteosarcomas are de novo, but inherited predispositions for osteosarcoma have provided insight into possible genetic factors associated with the disease. Mouse models and *in vitro* studies have proven to be powerful tools in elucidating molecular mechanisms and the genetic pathology of osteosarcoma. Interestingly only *RB1* and/or *TP53* mutations are consistently detected in the majority of human osteosarcomas despite a broad range of reported factors associated with the disease. Presented here are some of the genetic aspects of osteosarcoma with a focus on the tumor suppressor protein, pRB, in tumor formation and metastasis.

2. The retinoblastoma tumor suppressor and osteosarcoma

Loss of *RB1* was the first genetic predisposition associated with osteosarcoma development. The predominant tumor types seen in patients with a germline mutation in the *RB1* gene are retinoblastoma, osteosarcoma and rarely small cell lung, bladder and breast carcinomas (Gurney et al., 1995). This suggests that tumors exhibiting the highest frequency of pRb loss, such as osteosarcoma, may require pRb function in multiple cell cycle exit programs and differentiation. Children with hereditary retinoblastoma have a 69-fold increased risk of osteosarcoma. Previous radiation treatment to the primary tumor site increases the risk of osteosarcoma by 406-fold (Mohney et al., 1998; Savage and Mirabello, 2011; Kitchin and Ellsworth, 1974; Gonzalez-Vasconcellos et al., 2011). Although heterozygous loss of *RB1* predisposes to tumor formation, *RB1* loss is also consistently observed in 60% of sporadic osteosarcomas (Friend et al., 1986; Hansen, 1991; Wadayama et al., 1994) and loss of *RB1* heterozygosity is associated with poor patient prognosis (Marina et al., 2004; Tang et al., 2008).

The retinoblastoma protein, pRb, is part of a regulatory pathway that is targeted in most human cancers Weinberg, 1994 Mulligan and Jacks, 1998. pRb functions in every cell type to control the exit from G1 by regulating the E2F family of transcription factors. This process is facilitated by the recruitment of histone deacetylase enzymes (HDACs), SWI/SNF and histone methyltransferases at the promoter of cell cycle genes (Alland et al., 1997; Hassig et al., 1997; Grunstein, 1997; Luo et al., 1998) to repress E2F family member activity and prevent S-phase progression. This repression promotes cell cycle exit during differentiation and senescence programs (Alexander and Hinds, 2001; Chen et al., 1996; Schneider et al., 1994; Sellers et al., 1998; Tiemann and Hinds, 1998). Regulation of pRb and thus cell cycle progression from G1 to S begins with mitogenic signals that induce activation of cell-cycle dependent kinase complexes Cdk4/Cdk6-cyclin D and Cdk2-cyclin E (Hinds et al., 1992; Kato et al., 1993; Hatakeyama et al., 1994; Sherr and Roberts, 1999; Takaki et al., 2005). These complexes phosphorylate pRb on multiple serine and threonine residues. When pRb is sufficiently hyperphosphorylated, repression of E2F is released and transcription of genes important in S-phase progression occurs (Buchkovich et al., 1989; Sherr, 1996; Dyson, 1998; Trimarchi and Lees, 2002; Bracken et al., 2004). Mechanisms that police premature entry into S phase focus on inhibition of the cyclin-Cdk complexes. These inhibitors include members of the INK4 family ($p15^{INK4b}$, $p16^{INK4a}$, $p18^{INK4c}$ and $p19^{INK4d}$), which specifically interact with Cdk4/6 and impair interaction with D-type cyclins, and CIP/KIP ($p21^{CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$) family members, that act on cyclin/CDK by forming a ternary complex (Shapiro et al., 1995; Sherr, 1996; Stiegler et al., 1998). Mutation of genes encoding members of the

retinoblastoma pathway is associated with osteosarcoma, although with far less frequency than mutation of *RB1* itself.

Studies have shown that inactivation of *CDKN2A*, the gene encoding $p16^{INK4a}$, is observed in osteosarcomas (Miller et al., 1996; Benassi et al., 1999) and loss of $p27^{KIP1}$ expression has been correlated with high-grade osteosarcomas (Thomas et al., 2004). $p15^{INK4b}$ loss has been reported in osteosarcomas at a low frequency, and animals mutant for this gene develop osteosarcoma at low penetrance (Miller et al., 1996; Sharpless et al., 2001; Krimpenfort et al., 2007). Of note, increased expression of CDK4 is associated mainly with low-grade osteosarcomas (Dujardin et al., 2011).

Early *in vivo* evidence of pRb's involvement in osteosarcoma was observed in transgenic mice that expressed SV40 large T-antigen (Knowles et al., 1990). pRb can be inactivated by viral oncoproteins such as human adenovirus E1A, SV40 large T antigen, and the E7 protein of human papillomavirus type-16 (Whyte et al., 1988; DeCaprio et al., 1988; Dyson et al., 1989). By binding to pRb, the viral proteins interfere with its normal function, thus mimicking the loss of pRb (Stabel et al., 1985; Harlow et al., 1986; Lee et al., 1987; Whyte et al., 1988; DeCaprio et al., 1988; Munger et al., 1989; Vousden and Jat, 1989; Dyson et al., 1989). These animals developed metastatic osteosarcoma at about 15 months of age.

A paradox is that targeted disruption of one allele of *RB1* in mice does not lead to cancer. In addition, studies of conditional *RB1* knockout mice have shown that loss of *RB1* in mouse photoreceptor cells does not result in retinoblastoma or any other phenotypic changes in these cells (Vooijs et al., 2002). Instead, mice that are heterozygous for *RB1* show an increased predisposition to pituitary and thyroid tumors within one year of age, which are associated with a loss of heterozygosity at the *RB1* locus (Jacks et al., 1992; Williams et al., 1994). Often tumor formation in mouse models requires the loss of p107 or p130, pRb related pocket protein family members, which may account for the phenotypes seen in $RB1^{-/-}$ animals (Dannenberg et al., 2004). Contrary to osteosarcoma seen in humans, where only the loss of *RB1* appears to be required for tumor formation. $RB1^{-/+}$; $p107^{-/-}$ or $RB1^{-/+}$;$p130^{-/-}$ animals do develop retinoblastoma and osteosarcoma mimicking the loss of *RB1* in humans (Dannenberg et al., 2004). Neither $p107^{-/-}$ nor $p130^{-/-}$ mutant mouse strains have apparent phenotypes (Cobrinik et al., 1992; Lee et al., 1996). Additional mouse models of osteosarcoma include animals with the loss of both *RB1* and *Trp53*.

A significant proportion of osteosarcomas have mutations in p53, thus identifying p53 loss as a predisposition to the disease. p53 mutations were initially observed in sporadic osteosarcoma, and then discovered in approximately 3% of patients diagnosed with Li-Fraumeni syndrome, an autosomal dominant disorder characterized by a germline mutation of *TP53* (Li and Fraumeni, 1969; Hisada et al., 1998; Porter et al., 1992; Upton et al., 2009). Mice bearing germline disruption of Trp53 (which encodes p53 in mice) develop a wide variety of tumors, including osteosarcoma, which is observed in up to 32% of heterozygous Trp53 mutant animals (Harvey et al., 1993; Olive et al., 2004). Significantly, when *Trp53* is deleted from *RB1* heterozygous mice, the animals develop retinal dysplasia (Morgenbesser et al., 1994). Work from two separate groups generated an animal model with conditional targeted mutations in the bone of both *RB1* and *Trp53.* The combined loss of both tumor suppressors greatly enhances the formation of osteosarcomas and additional neoplasms not observed to be associated with the loss of *RB1* in humans such as rhabdomyosarcoma, neuroendocrine tumors and hibernomas (Berman et al., 2008; Walkley et al., 2008). This indicates that loss of p53 could be a rate-limiting event in the initiation of this tumor in mice

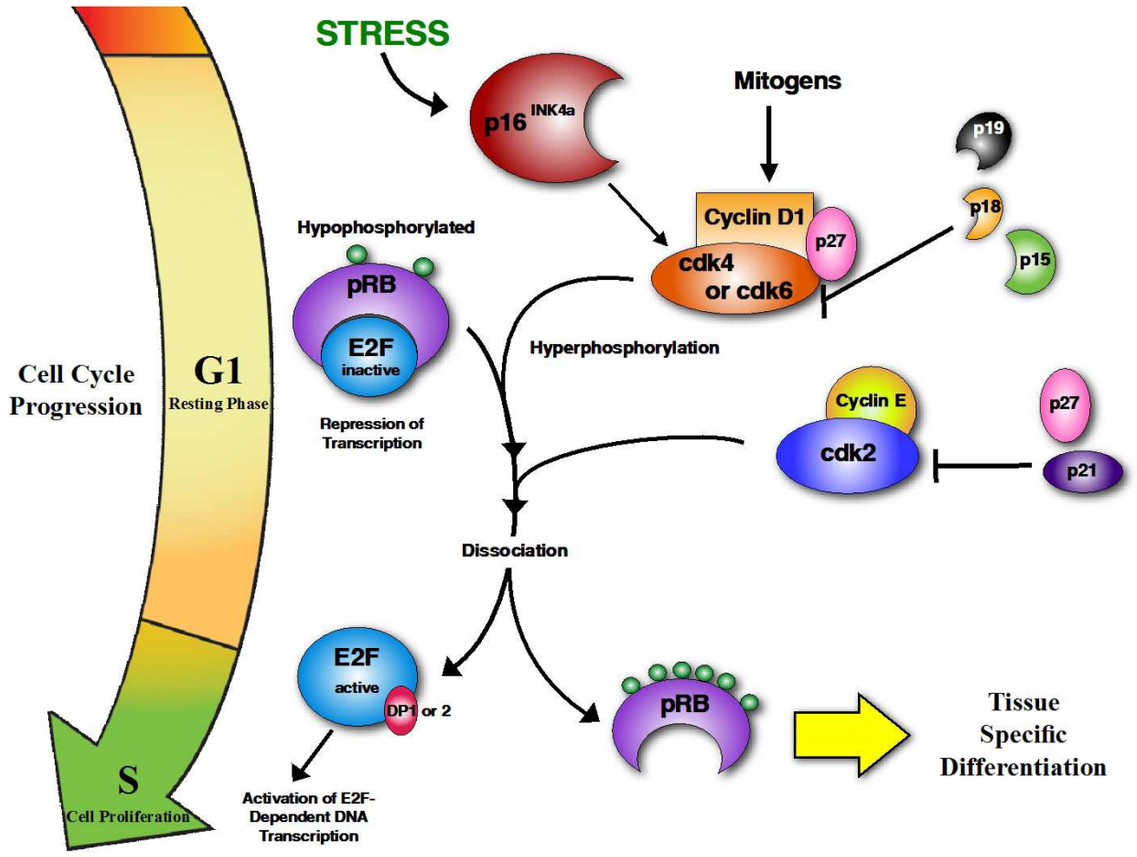
in the context of germline *RB1* mutations, but not in humans. Although the loss of p53 or variants of p53 are observed in 22% of osteosarcomas, these occurrences have yet to consistently correlate with disease stage or prognosis (Wunder et al., 2005; Ta et al., 2009). Tumor cells from both human and mouse osteosarcomas have characteristics of early osteoprogenitor and arguably mesenchymal progenitor cells. These cells are multipotent, have some self-renewal capacity, and often lack late markers of osteogenesis. This absence of differentiation and progenitor-like phenotype in conventional osteosarcoma suggests that disruption of osteoblast differentiation may be a critical event in tumor formation.

3. Osteogenesis

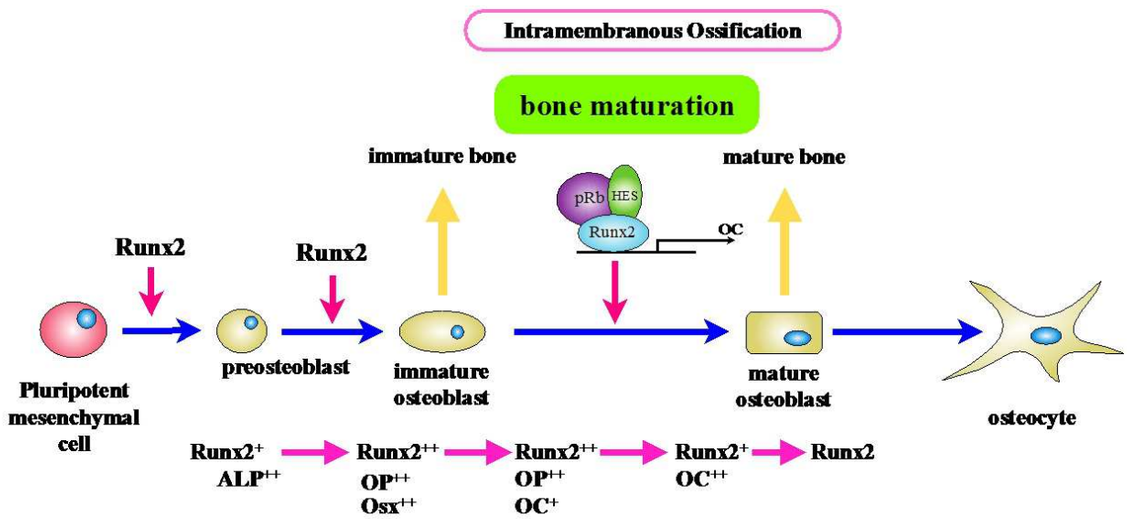
The transition from mesenchymal stem cell to osteoprogenitor and preosteoblast requires activation of the transcription factor Runx2 (AML3; Osf2; Cbfa1; PEBP2aA; Pebp2a1). Runx2 (runt-related transcription factor 2) is a transcription factor that belongs to the Runx family (Komori, 2002) and acquires DNA binding activity by heterodimerizing with Cbfβ (Kundu et al., 2002; Miller et al., 2002; Yoshida et al., 2002). *Runx2*$^{-/-}$ mice lack bone formation due to the absence of osteoblast differentiation, and die just after birth (Komori et al., 1997; Otto et al., 1997). Runx2 is found to be restricted to the mesenchymal condensations that form bone (Ducy et al., 1997). Thus, Runx2 is termed the master regulator/platform protein of osteoblast differentiation and is essential to bone formation. In rare cases, *RUNX2* amplification is observed in osteosarcoma, but Runx2 function in tumorigenesis has yet to be identified. Preliminary reports suggest that an increased level of Runx2 is associated with chemoresistance (Kurek et al., 2010; Longhi et al., 2006) and that high expression of Runx2 may be related to metastasis in osteosarcoma (Won et al., 2009). However, other studies suggest that Runx2 function may be impaired during the process of osteosarcomagenesis, despite its elevated expression in these tumors (Thomas et al., 2004).

Early evidence for a role for pRb in osteogenesis came from the discovery that osteoblast differentiation can be inhibited in cells by the viral oncoproteins SV40 large T antigen and E1A, which specifically target the pocket proteins (Feuerbach et al., 1997). These studies showed that osteoblasts immortalized by temperature sensitive SV40 large T antigen targeting pRb demonstrate reduced expression of late markers of differentiation. This process can be reversed by deactivation of the viral oncoproteins (Feuerbach et al., 1997). The reintroduction of a pRb variant (R661W), discovered in a patient tumor sample that lacks canonical E2F binding capacity, is capable of arresting and differentiating osteosarcoma cells (SAOS2) (Sellers et al., 1998). Thus, the ability of pRb to promote osteoblast differentiation may be separable from its role as a regulator of the E2F family of transcription factors. These observations established a clear connection between pRb and osteogenesis.

pRb has been shown to directly bind to Runx2 in osteoblasts. The pRb-Runx2 complex can localize to the promoter of a late bone specific marker, osteocalcin, and promote transcriptional activity. In the absence of pRb, expression of osteocalcin is significantly reduced. The pRb-Runx2 complex also involves the transcription factor HES1. This process appears to be specific to pRb and not the other pocket protein family members, p107 and p130 (Thomas et al., 2001; Lee et al., 2006). In osteosarcoma cell lines, pRb was shown to be necessary for displacement of RBP2 (KDM5A/JARID1A), a histone demethylase, from the osteocalcin promoter to relieve repression (Benevolenskaya et al., 2005). Thus, pRb is required for the transcriptional activation of the osteocalcin promoter and in removing repressive components late in differentiation. Increasing evidence suggests that pRb may also be important in early osteogenesis and osteoblast lineage commitment (Figure 1).



A)



B)

Fig. 1. Schematic of the retinoblastoma pathway and osteoblast differentiation

A) Progression through the cell cycle from G1 to S begins with mitogenic signals that induce activation of cell-cycle dependent kinase complexes Cdk4/Cdk6-cyclin D and Cdk2-cyclin E.

These complexes phosphorylate pRb and other substrates important for cell cycle progression. Cyclin D-cdk4/6 phosphorylates pRb early in G1, while cyclin E-cdk2 phosphorylates the protein near the end of G1, and cyclin A–cdk2 maintains phosphorylation of pRb during S phase. When pRb is sufficiently hyperphosphorylated, E2F is released and transcription of genes important in S-phase progression occurs. Mechanisms that police premature entry into S phase focus on inhibition of the cyclin-Cdk complexes. These inhibitors include members of the INK4 family (p15, p16, p18 and p19), which specifically interact with Cdk4/6 and impair interaction with D-type cyclins, and CIP/KIP (p21, p27 and p57) family members, that act on cyclin-Cdk complexes. p27 also stimulates the formation of cyclin D-Cdk4/6 complexes, which titrates it away from inhibiting cyclin E-Cdk2 complexes. This seemingly contradictory process permits further phosphorylation of pRb. B) During the development of intramembranous bones, Runx2 is strongly detected in preosteoblasts, immature osteoblasts, and early mature osteoblasts. Increased expression of osterix (Osx) and alkaline phosphatase (ALP) is detected in the transition between preosteoblasts to immature osteoblasts. Osteoblasts express high levels of osteocalcin (OC), which is transcriptionally regulated by the pRb-HES1-Runx2 complex.

Gene expression profiles of different stages in osteogenesis suggest that increased expression of ALP is seen early in osteoprogenitors (Narisawa et al., 1997; Komori, 2005). Recent work showed that pRb is required to recruit BRG1, an activating SWI/SNF chromatin-remodeling complex, to the alkaline phosphatase promoter (Flowers et al., 2010). Unlike the osteocalcin system, pRb does not appear to be required for removal of suppressive proteins. Thus in the absence of pRb, progression both at early and late stages of differentiation may be disrupted. Moreover, mouse embryos conditionally deleted for pRb display defects in bone ossification in part due to an increased progenitor population (Gutierrez et al., 2008). Recent work has suggested that pRb may be important in progenitor cell fate between bone and adipose tissue (Calo et al., 2010). Together, these data imply an important role for pRb in promoting tissue-specific differentiation and early lineage commitment. Recently the hedgehog and the Wnt/beta-catenin pathways have been shown to be important in osteoblast differentiation and are associated with osteosarcoma. The hedgehog signaling pathway is crucial for proliferation, cell fate and differentiation during embryonic development (McMahon et al., 2003). This pathway functions through several components including the transmembrane proteins PATCHED (PTCH1) and SMOOTHENED (SMO) to activate the GLI zinc-finger transcription factors (Ingham and McMahon, 2001; Ruiz i Altaba et al., 2002). Evidence of hedgehog pathway activation early in tumor development has been reported in basal cell carcinomas and medulloblastoma (Gupta et al., 2010), but the role of this pathway in osteosarcoma is not clear. The first association between Ihh and Runx2 came with the finding that Runx2 can directly bind to the promoter of *Ihh* and induce promoter activity (Yoshida et al., 2004). Further, Ihh expression is significantly reduced in *Runx2*$^{-/-}$ animals, which are devoid of any osteoblast formation. Work done in C3H10T1/2 mouse embryonic fibroblasts showed that Ihh regulates osteoblast differentiation of mesenchymal cells through up-regulation of the expression and function of Runx2 by Gli2 (Shimoyama et al., 2007). This suggests a possible feed-forward loop between hedgehog signaling and Runx2 expression early in lineage commitment.

Studies of conditional *Ihh*$^{-/-}$ mice showed that Ihh is required for epithelial stem cell proliferation and differentiation in the gut (Ramalho-Santos et al., 2000; Mao et al., 2010).

Additional work in the intestinal epithelium showed conditional *RB1*$^{-/-}$ animals have an increased proliferation of enteroendocrine, differentiated cells (Yang and Hinds, 2007) and elevated levels of Ihh expression. Recent *in vitro* data suggests that in some osteosarcoma cell lines the hedgehog pathway is overexpressed and that inactivation of SMO may prevent tumor cell growth (Hirotsu et al., 2010). Thus it is possible that pRb and Runx2 may complex to regulate hedgehog signaling early in osteoblast differentiation, but this has yet to be established.

Aberrations in the Wnt/Beta-catenin signaling pathway, important in osteoblast differentiation, have been associated with osteosarcoma (Haydon et al., 2002; Iwaya et al., 2003). The canonical Wnt pathway involves binding of the Wnt glycoprotein to the transmembrane Frizzled receptor and LRP5/6 co-receptors. Ligand-receptor binding prevents downstream phosphorylation of beta-catenin, allowing it to translocate to the nucleus and activate downstream genes that mediate cell proliferation and differentiation [Reviewed in Luo et al., 2007]. Members of the Wnt pathway are expressed in early osteoprogenitors (Luo et al., 2007; Glass and Karsenty, 2007). Increased nuclear localization of beta-catenin and atypical localization of adherens junction proteins, such as cadherins, are detected in osteosarcoma and correlate with metastasis (Iwaya et al., 2003; Kashima et al., 1999; Park et al., 2006; Hunter, 2004). Cadherins mediate cell-to-cell attachments, and osteoprogenitor cells express cadherins that stimulate differentiation into mature osteoblasts (Cheng et al., 2000; Hynes and Lander, 1992; Hay et al., 2000; Stains and Civitelli, 2005). An important component of adherens junction complexes includes the RhoA GTPases, Rac1 and RhoA. High Rac1 expression suppresses RhoA, which prevents merlin activation and cadherin, beta-catenin junction assembly. Recent work has shown that pRb is important in repressing Rac1, and thus regulates the assembly of adherens junctions for cell adhesion (Sosa-Garcia et al., 2010). Therefore the loss of *RB1* may not only disrupt cell cycle regulation and lineage commitment, but also cell-cell interactions that may lead to metastasis in osteosarcoma.

4. Other genetic factors in osteosarcoma

Although LOH of *RB1* is strongly associated with osteosarcoma and may be responsible for several important phenotypes as discussed above, pRB loss manifests these properties in the context of many other genetic and epigenetic alterations. Allelic amplification and/or loss at many chromosomal sites have been reported in as many as 70% of all osteosarcomas (Yamaguchi et al., 1992; Kruzelock et al., 1997; Sandberg and Bridge, 2003). This implies other potential genes involved in osteosarcoma development and progression. Overexpression of oncogenes such as c-MYC and HER2/neu (c-erbB-2) have been reported in osteosarcoma and have been associated with early pulmonary metastases (Barrios et al., 1993; Ladanyi et al., 1993; Onda et al., 1996; Gorlick et al., 1999; Zhou et al., 2003). In rare cases telomerase activity has been reported in osteosarcoma and may also be associated with pulmonary metastases (Scheel et al., 2001). In addition, amplification of MDM2, a suppressor of p53, is related to progression and metastasis of osteosarcoma (Ladanyi et al., 1993). These and other genetic mutations aside from pRb and p53 may be key to the properties of discrete forms of osteosarcoma.

Patients with bone diseases such as Rothmund-Thomson or Werner syndrome, which are associated with RECQ helicases, important for double strand break repair, are predisposed

to developing osteosarcoma (Wang et al., 2003; Hickson, 2003; Chu and Hickson, 2009). About a third of the patients diagnosed with these bone diseases will develop osteosarcoma. Recently, a possibly *RB1*-independent mouse model for spontaneous osteosarcoma was reported that involves the transcription factor Twist and the APC complex in Wnt signaling (Entz-Werle et al., 2010). Twist and APC loss have been reported in several cases of human osteosarcoma where the loss of Twist and APC are associated with a poor patient prognosis (Entz-Werle et al., 2007). Neither Twist nor APC loss alone in murine models results in development of osteosarcoma, but *in vitro* assays suggest that Twist and APC/Beta-catenin/GSK complex coordinate to activate Runx2 expression (Entz-Werle et al., 2010; Stein et al., 2004). The molecular mechanism associated with these factors remains unclear as the heterogeneity of the tumors compounded with the rare occurrence of the disease hinders a clear definition for the pathology of osteosarcoma.

5. Conclusion

Functional heterogeneity is a widely recognized trait of osteosarcoma tumor cells. Osteosarcoma is a broad terminology for a tumor that arises from bone and consists of dozens of sub-classes. While the full etiological spectrum of the disease remains unclear due to its rare occurrence, various reports suggest environmental factors, bone syndromes that predispose a patient to osteosarcoma, signaling pathways and multiple genetic factors may be involved in tumor formation. Although environmental factors such as radiation therapy and bone disorders such as Paget and Rothmund-Thomas syndrome may help explain some occurrences of osteosarcoma later in life, it does not account for patients diagnosed with the disease before the age of 25. Studies of germline mutations, such as those in *RB1*, that predispose individuals to osteosarcoma may provide clues to the molecular pathology of the disease. Emerging evidence suggests osteosarcoma is a disease of differentiation caused by genetic changes that disrupt lineage progression (Haydon et al., 2007).

Growing *in vivo* and *in vitro* data reveal an increasingly complex role for pRb both in early and late osteoblast differentiation, and possibly in cell fate and metastasis (Calo et al., 2010; Gutierrez et al., 2008; Sosa-Garcia et al., 2010). Loss of *RB1* at different stages of lineage commitment could account for the various phenotypes observed in sporadic osteosarcomas. The progenitor phenotype observed in conventional osteosarcomas raises the question of whether committed osteoblasts "back up" following the loss of *RB1* to display properties of progenitor cells and/or whether the loss of *RB1* predominantly acts to delay the progression of multipotent progenitors. An increased population of progenitor-like cells that are delayed in differentiation would then be susceptible to additional transforming events (Figure 2). Although the loss or mutation of p53 is reported in some osteosarcomas (Papachristou and Papavassiliou, 2007) the question remains if p53 is essential in initiation or strictly in tumor progression. Increased understanding of the retinoblastoma gene and protein in osteogenesis presents a more complex role of this tumor suppressor as a target in tumorigenesis. Through an understanding of the molecular mechanism underlying osteogenic differentiation, we may begin to unravel the molecular pathogenesis of human osteosarcoma and identify possible co-activators or repressors that may become key targets for treating the disease.

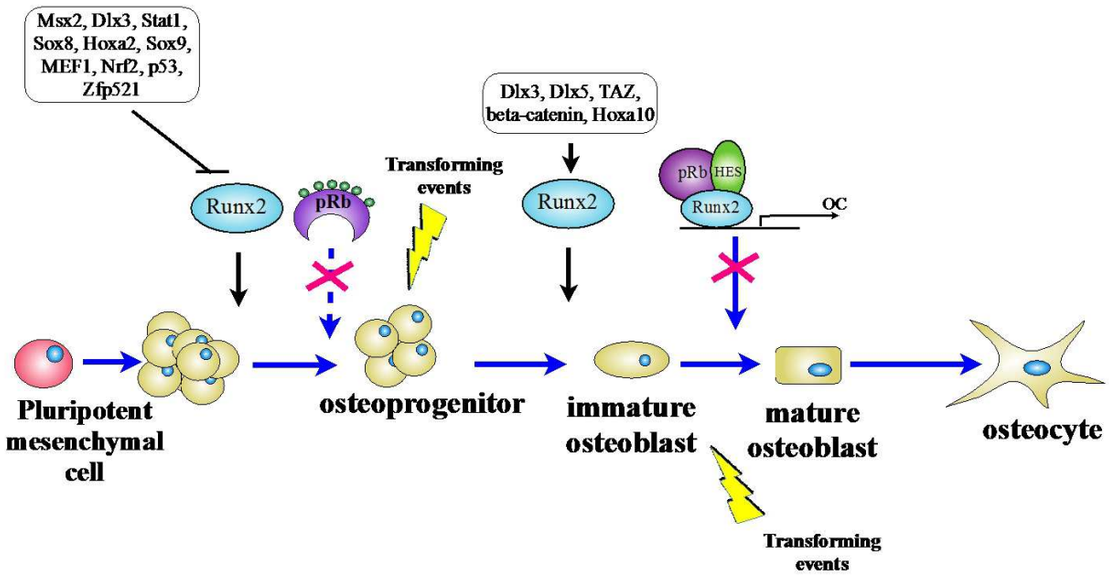


Fig. 2. Disruption of genetic components in osteoblast differentiation and osteosarcoma.

The presence of only early markers of osteoblast differentiation in osteosarcoma with periods of rapid cell division infer that the disease may be associated with genetic defects in osteoblast differentiation. Loss of *RB1* early in differentiation may result in an increased progenitor-like population that is conceivably susceptible to transforming events that may result in poorly differentiated and aggressive osteosarcoma. Genetic alterations at different stages of differentiation may be one explanation for the heterogeneity of the disease. It is unclear if genetic defects and transforming events late in differentiation generate a situation where committed osteoblasts can "back up" to display the progenitor-like phenotype observed in aggressive osteosarcomas.

6. Acknowledgements

The authors thank all members of the Hinds lab for discussion and comment. Work described in this article was supported by NIH grant AG020208 to PWH.

7. References

Alexander, K and PW Hinds (2001), 'Requirement for p27(KIP1) in retinoblastoma protein-mediated senescence.', *Mol Cell Biol*, 21 (11), 3616-31.

Alland, L, et al. (1997), 'Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression.', *Nature*, 387 (6628), 49-55.

Barrios, C, et al. (1993), 'Amplification of c-myc oncogene and absence of c-Ha-ras point mutation in human bone sarcoma.', *J Orthop Res*, 11 (4), 556-63.

Benassi, MS, et al. (1999), 'Alteration of pRb/p16/cdk4 regulation in human osteosarcoma.', *Int J Cancer*, 84 (5), 489-93.

Benevolenskaya, EV, et al. (2005), 'Binding of pRB to the PHD protein RBP2 promotes cellular differentiation.', *Mol Cell*, 18 (6), 623-35.

Berman, SD, et al. (2008), 'Metastatic osteosarcoma induced by inactivation of Rb and p53 in the osteoblast lineage.', *Proc Natl Acad Sci U S A*, 105 (33), 11851-56.

Bracken, AP, et al. (2004), 'E2F target genes: unraveling the biology.', *Trends Biochem Sci*, 29 (8), 409-17.

Buchkovich, K, LA Duffy, and E Harlow (1989), 'The retinoblastoma protein is phosphorylated during specific phases of the cell cycle.', *Cell*, 58 (6), 1097-105.

Calo, E, et al. (2010), 'Rb regulates fate choice and lineage commitment in vivo.', *Nature*, 466 (7310), 1110-14.

Caron, AS, SI Hajdu, and EW Strong (1971), 'Osteogenic sarcoma of the facial and cranial bones. A review of forty-three cases.', *Am J Surg*, 122 (6), 719-25.

Chen, PL, et al. (1996), 'Retinoblastoma protein positively regulates terminal adipocyte differentiation through direct interaction with C/EBPs.', *Genes Dev*, 10 (21), 2794-804.

Cheng, SL, et al. (2000), 'A dominant negative cadherin inhibits osteoblast differentiation.', *J Bone Miner Res*, 15 (12), 2362-70.

Chu, WK and ID Hickson (2009), 'RecQ helicases: multifunctional genome caretakers.', *Nat Rev Cancer*, 9 (9), 644-54.

Cobrinik, D, et al. (1992), 'The retinoblastoma protein and the regulation of cell cycling.', *Trends Biochem Sci*, 17 (8), 312-15.

Dahlin, DC (1988), 'Malignant bone tumors: improvement in prognosis.', *Mayo Clin Proc*, 63 (4), 414-15.

Dannenberg, JH, et al. (2004), 'Tissue-specific tumor suppressor activity of retinoblastoma gene homologs p107 and p130.', *Genes Dev*, 18 (23), 2952-62.

DeCaprio, JA, et al. (1988), 'SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene.', *Cell*, 54 (2), 275-83.

Ducy, P, et al. (1997), 'Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation.', *Cell*, 89 (5), 747-54.

Dujardin, F, et al. (2011), 'MDM2 and CDK4 immunohistochemistry is a valuable tool in the differential diagnosis of low-grade osteosarcomas and other primary fibro-osseous lesions of the bone.', *Mod Pathol*, 24 (5), 624-37.

Dyson, N, et al. (1989), 'The cellular 107K protein that binds to adenovirus E1A also associates with the large T antigens of SV40 and JC virus.', *Cell*, 58 (2), 249-55.

Dyson, N (1998), 'The regulation of E2F by pRB-family proteins.', *Genes Dev*, 12 (15), 2245-62.

Entz-Werle, N, et al. (2007), 'Involvement of MET/TWIST/APC combination or the potential role of ossification factors in pediatric high-grade osteosarcoma oncogenesis.', *Neoplasia*, 9 (8), 678-88.

Entz-Werle, N, et al. (2010), 'Targeted apc;twist double-mutant mice: a new model of spontaneous osteosarcoma that mimics the human disease.', *Transl Oncol*, 3 (6), 344-53.

Feuerbach, D, et al. (1997), 'Establishment and characterization of conditionally immortalized stromal cell lines from a temperature-sensitive T-Ag transgenic mouse.', *J Bone Miner Res*, 12 (2), 179-90.

Flowers, S, GR Jr Beck, and E Moran (2010), 'Transcriptional activation by pRB and its coordination with SWI/SNF recruitment.', *Cancer Res*, 70 (21), 8282-87.

Friend, SH, et al. (1986), 'A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma.', *Nature*, 323 (6089), 643-46.

Glass, DA 2nd and G Karsenty (2007), 'In vivo analysis of Wnt signaling in bone.', *Endocrinology*, 148 (6), 2630-34.

Gonzalez-Vasconcellos, I, et al. (2011), 'Differential effects of genes of the Rb1 signalling pathway on osteosarcoma incidence and latency in alpha-particle irradiated mice.', *Radiat Environ Biophys*, 50 (1), 135-41.

Gorlick, R, et al. (1999), 'Expression of HER2/erbB-2 correlates with survival in osteosarcoma.', *J Clin Oncol*, 17 (9), 2781-88.

Grunstein, M (1997), 'Histone acetylation in chromatin structure and transcription.', *Nature*, 389 (6649), 349-52.

Gupta, S, N Takebe, and P Lorusso (2010), 'Targeting the Hedgehog pathway in cancer.', *Ther Adv Med Oncol*, 2 (4), 237-50.

Gurney, JG, et al. (1995), 'Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type.', *Cancer*, 75 (8), 2186-95.

Gutierrez, GM, et al. (2008), 'Impaired bone development and increased mesenchymal progenitor cells in calvaria of RB1-/- mice.', *Proc Natl Acad Sci U S A*, 105 (47), 18402-07.

Hansen, MF (1991), 'Molecular genetic considerations in osteosarcoma.', *Clin Orthop Relat Res*, (270), 237-46.

Harlow, E, et al. (1986), 'Association of adenovirus early-region 1A proteins with cellular polypeptides.', *Mol Cell Biol*, 6 (5), 1579-89.

Harvey, M, et al. (1993), 'Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice.', *Nat Genet*, 5 (3), 225-29.

Hassig, CA, et al. (1997), 'Histone deacetylase activity is required for full transcriptional repression by mSin3A.', *Cell*, 89 (3), 341-47.

Hatakeyama, M, et al. (1994), 'Collaboration of G1 cyclins in the functional inactivation of the retinoblastoma protein.', *Genes Dev*, 8 (15), 1759-71.

Hauben, EI, et al. (2003), 'Multiple primary malignancies in osteosarcoma patients. Incidence and predictive value of osteosarcoma subtype for cancer syndromes related with osteosarcoma.', *Eur J Hum Genet*, 11 (8), 611-18.

Hay, E, et al. (2000), 'N- and E-cadherin mediate early human calvaria osteoblast differentiation promoted by bone morphogenetic protein-2.', *J Cell Physiol*, 183 (1), 117-28.

Haydon, RC, et al. (2002), 'Cytoplasmic and/or nuclear accumulation of the beta-catenin protein is a frequent event in human osteosarcoma.', *Int J Cancer*, 102 (4), 338-42.

Haydon, RC, HH Luu, and TC He (2007), 'Osteosarcoma and osteoblastic differentiation: a new perspective on oncogenesis.', *Clin Orthop Relat Res*, 454 237-46.

Hickson, ID (2003), 'RecQ helicases: caretakers of the genome.', *Nat Rev Cancer*, 3 (3), 169-78.

Hinds, PW, et al. (1992), 'Regulation of retinoblastoma protein functions by ectopic expression of human cyclins.', *Cell*, 70 (6), 993-1006.

Hirotsu, M, et al. (2010), 'Smoothened as a new therapeutic target for human osteosarcoma.', *Mol Cancer*, 9 5.

Hisada, M, et al. (1998), 'Multiple primary cancers in families with Li-Fraumeni syndrome.', *J Natl Cancer Inst*, 90 (8), 606-11.

Hopyan, S, et al. (1999), 'Expression of osteocalcin and its transcriptional regulators core-binding factor alpha 1 and MSX2 in osteoid-forming tumours.', *J Orthop Res*, 17 (5), 633-38.

Hunter, KW (2004), 'Ezrin, a key component in tumor metastasis.', *Trends Mol Med*, 10 (5), 201-04.

Huvos, AG (1993), 'Osteosarcoma in adolescents and young adults: new developments and controversies. Commentary on pathology.', *Cancer Treat Res*, 62 375-77.

Hynes, RO and AD Lander (1992), 'Contact and adhesive specificities in the associations, migrations, and targeting of cells and axons.', *Cell*, 68 (2), 303-22.
Ingham, PW and AP McMahon (2001), 'Hedgehog signaling in animal development: paradigms and principles.', *Genes Dev*, 15 (23), 3059-87.
Iwaya, K, et al. (2003), 'Cytoplasmic and/or nuclear staining of beta-catenin is associated with lung metastasis.', *Clin Exp Metastasis*, 20 (6), 525-29.
Jacks, T, et al. (1992), 'Effects of an Rb mutation in the mouse.', *Nature*, 359 (6393), 295-300.
Kashima, T, et al. (1999), 'Anomalous cadherin expression in osteosarcoma. Possible relationships to metastasis and morphogenesis.', *Am J Pathol*, 155 (5), 1549-55.
Kaste, SC, et al. (1999), 'Metastases detected at the time of diagnosis of primary pediatric extremity osteosarcoma at diagnosis: imaging features.', *Cancer*, 86 (8), 1602-08.
Kato, J, et al. (1993), 'Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4.', *Genes Dev*, 7 (3), 331-42.
Kitchin, FD and RM Ellsworth (1974), 'Pleiotropic effects of the gene for retinoblastoma.', *J Med Genet*, 11 (3), 244-46.
Klein, MJ and GP Siegal (2006), 'Osteosarcoma: anatomic and histologic variants.', *Am J Clin Pathol*, 125 (4), 555-81.
Knowles, BB, et al. (1990), 'Osteosarcomas in transgenic mice expressing an alpha-amylase-SV40 T-antigen hybrid gene.', *Am J Pathol*, 137 (2), 259-62.
Komori, T, et al. (1997), 'Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts.', *Cell*, 89 (5), 755-64.
Komori, T (2002), 'Runx2, a multifunctional transcription factor in skeletal development.', *J Cell Biochem*, 87 (1), 1-8.
Komori, T (2005), 'Regulation of skeletal development by the Runx family of transcription factors.', *J Cell Biochem*, 95 (3), 445-53.
Krimpenfort, P, et al. (2007), 'p15Ink4b is a critical tumour suppressor in the absence of p16Ink4a.', *Nature*, 448 (7156), 943-46.
Kruzelock, RP, et al. (1997), 'Localization of a novel tumor suppressor locus on human chromosome 3q important in osteosarcoma tumorigenesis.', *Cancer Res*, 57 (1), 106-09.
Kundu, M, et al. (2002), 'Cbfbeta interacts with Runx2 and has a critical role in bone development.', *Nat Genet*, 32 (4), 639-44.
Kurek, KC, et al. (2010), 'Frequent attenuation of the WWOX tumor suppressor in osteosarcoma is associated with increased tumorigenicity and aberrant RUNX2 expression.', *Cancer Res*, 70 (13), 5577-86.
Ladanyi, M, et al. (1993), 'MDM2 gene amplification in metastatic osteosarcoma.', *Cancer Res*, 53 (1), 16-18.
Lee, JS, et al. (2006), 'HES1 cooperates with pRb to activate RUNX2-dependent transcription.', *J Bone Miner Res*, 21 (6), 921-33.
Lee, MH, et al. (1996), 'Targeted disruption of p107: functional overlap between p107 and Rb.', *Genes Dev*, 10 (13), 1621-32.
Lee, WH, et al. (1987), 'The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity.', *Nature*, 329 (6140), 642-45.
Li, FP and JF Jr Fraumeni (1969), 'Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome?', *Ann Intern Med*, 71 (4), 747-52.

Lindor, NM, et al. (2000), 'Rothmund-Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome.', *Am J Med Genet*, 90 (3), 223-28.

Longhi, A, et al. (2006), 'Primary bone osteosarcoma in the pediatric age: state of the art.', *Cancer Treat Rev*, 32 (6), 423-36.

Longhi, A, et al. (2008), 'Osteosarcoma in patients older than 65 years.', *J Clin Oncol*, 26 (33), 5368-73.

Luo, J, et al. (2007), 'Wnt signaling and human diseases: what are the therapeutic implications?', *Lab Invest*, 87 (2), 97-103.

Luo, RX, AA Postigo, and DC Dean (1998), 'Rb interacts with histone deacetylase to repress transcription.', *Cell*, 92 (4), 463-73.

Mao, J, et al. (2010), 'Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract.', *Development*, 137 (10), 1721-29.

Marina, N, et al. (2004), 'Biology and therapeutic advances for pediatric osteosarcoma.', *Oncologist*, 9 (4), 422-41.

McMahon, AP, PW Ingham, and CJ Tabin (2003), 'Developmental roles and clinical significance of hedgehog signaling.', *Curr Top Dev Biol*, 53 1-114.

McNairn, JD, et al. (2001), 'Inheritance of osteosarcoma and Paget's disease of bone: a familial loss of heterozygosity study.', *J Mol Diagn*, 3 (4), 171-77.

Miller, CW, et al. (1996), 'Alterations of the p15, p16,and p18 genes in osteosarcoma.', *Cancer Genet Cytogenet*, 86 (2), 136-42.

Miller, J, et al. (2002), 'The core-binding factor beta subunit is required for bone formation and hematopoietic maturation.', *Nat Genet*, 32 (4), 645-49.

Mirabello, L, RJ Troisi, and SA Savage (2009), 'Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program.', *Cancer*, 115 (7), 1531-43.

Mohney, BG, et al. (1998), 'Second nonocular tumors in survivors of heritable retinoblastoma and prior radiation therapy.', *Am J Ophthalmol*, 126 (2), 269-77.

Morgenbesser, SD, et al. (1994), 'p53-dependent apoptosis produced by Rb-deficiency in the developing mouse lens.', *Nature*, 371 (6492), 72-74.

Mulligan, G and T Jacks (1998), 'The retinoblastoma gene family: cousins with overlapping interests.', *Trends Genet*, 14 (6), 223-29.

Munger, K, et al. (1989), 'Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product.', *EMBO J*, 8 (13), 4099-105.

Narisawa, S, N Frohlander, and JL Millan (1997), 'Inactivation of two mouse alkaline phosphatase genes and establishment of a model of infantile hypophosphatasia.', *Dev Dyn*, 208 (3), 432-46.

Nellissery, MJ, et al. (1998), 'Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18 region genetically linked with Paget disease of bone.', *Am J Hum Genet*, 63 (3), 817-24.

Olive, KP, et al. (2004), 'Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome.', *Cell*, 119 (6), 847-60.

Onda, M, et al. (1996), 'ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma.', *Cancer*, 77 (1), 71-78.

Otto, F, et al. (1997), 'Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development.', *Cell*, 89 (5), 765-71.

Papachristou, DJ and AG Papavassiliou (2007), 'Osteosarcoma and chondrosarcoma: new signaling pathways as targets for novel therapeutic interventions.', *Int J Biochem Cell Biol*, 39 (5), 857-62.

Park, HR, et al. (2006), 'Ezrin in osteosarcoma: comparison between conventional high-grade and central low-grade osteosarcoma.', *Pathol Res Pract*, 202 (7), 509-15.

Paulino, AC and BZ Fowler (2005), 'Secondary neoplasms after radiotherapy for a childhood solid tumor.', *Pediatr Hematol Oncol*, 22 (2), 89-101.

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

Porter, DE, et al. (1992), 'A significant proportion of patients with osteosarcoma may belong to Li-Fraumeni cancer families.', *J Bone Joint Surg Br*, 74 (6), 883-86.

Ramalho-Santos, M, DA Melton, and AP McMahon (2000), 'Hedgehog signals regulate multiple aspects of gastrointestinal development.', *Development*, 127 (12), 2763-72.

Ruiz i Altaba, A, P Sanchez, and N Dahmane (2002), 'Gli and hedgehog in cancer: tumours, embryos and stem cells.', *Nat Rev Cancer*, 2 (5), 361-72.

Sandberg, AA and JA Bridge (2003), 'Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: osteosarcoma and related tumors.', *Cancer Genet Cytogenet*, 145 (1), 1-30.

Savage, SA and L Mirabello (2011), 'Using epidemiology and genomics to understand osteosarcoma etiology.', *Sarcoma*, 2011 548151.

Schajowicz, F, HA Sissons, and LH Sobin (1995), 'The World Health Organization's histologic classification of bone tumors. A commentary on the second edition.', *Cancer*, 75 (5), 1208-14.

Scheel, C, et al. (2001), 'Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcomas.', *Oncogene*, 20 (29), 3835-44.

Schneider, JW, et al. (1994), 'Reversal of terminal differentiation mediated by p107 in Rb-/- muscle cells.', *Science*, 264 (5164), 1467-71.

Sellers, WR, et al. (1998), 'Stable binding to E2F is not required for the retinoblastoma protein to activate transcription, promote differentiation, and suppress tumor cell growth.', *Genes Dev*, 12 (1), 95-106.

Shapiro, GI, et al. (1995), 'Multiple mechanisms of p16INK4A inactivation in non-small cell lung cancer cell lines.', *Cancer Res*, 55 (24), 6200-09.

Sharpless, NE, et al. (2001), 'Loss of p16Ink4a with retention of p19Arf predisposes mice to tumorigenesis.', *Nature*, 413 (6851), 86-91.

Sherr, CJ (1996), 'Cancer cell cycles.', *Science*, 274 (5293), 1672-77.

Sherr, CJ and JM Roberts (1999), 'CDK inhibitors: positive and negative regulators of G1-phase progression.', *Genes Dev*, 13 (12), 1501-12.

Shimoyama, A, et al. (2007), 'Ihh/Gli2 signaling promotes osteoblast differentiation by regulating Runx2 expression and function.', *Mol Biol Cell*, 18 (7), 2411-18.

Sosa-Garcia, B, et al. (2010), 'A role for the retinoblastoma protein as a regulator of mouse osteoblast cell adhesion: implications for osteogenesis and osteosarcoma formation.', *PLoS One*, 5 (11), e13954.

Stabel, S, P Argos, and L Philipson (1985), 'The release of growth arrest by microinjection of adenovirus E1A DNA.', *EMBO J*, 4 (9), 2329-36.

Stains, JP and R Civitelli (2005), 'Cell-cell interactions in regulating osteogenesis and osteoblast function.', *Birth Defects Res C Embryo Today*, 75 (1), 72-80.

Stein, GS, et al. (2004), 'Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression.', *Oncogene*, 23 (24), 4315-29.

Stiegler, P, M Kasten, and A Giordano (1998), 'The RB family of cell cycle regulatory factors.', *J Cell Biochem Suppl*, 30-31 30-36.

Stiller, CA, et al. (2006), 'Bone tumours in European children and adolescents, 1978-1997. Report from the Automated Childhood Cancer Information System project.', *Eur J Cancer*, 42 (13), 2124-35.

Ta, HT, et al. (2009), 'Osteosarcoma treatment: state of the art.', *Cancer Metastasis Rev*, 28 (1-2), 247-63.

Takaki, T, et al. (2005), 'Preferences for phosphorylation sites in the retinoblastoma protein of D-type cyclin-dependent kinases, Cdk4 and Cdk6, in vitro.', *J Biochem*, 137 (3), 381-86.

Tang, N, et al. (2008), 'Osteosarcoma development and stem cell differentiation.', *Clin Orthop Relat Res*, 466 (9), 2114-30.

Thomas, DM, et al. (2001), 'The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation.', *Mol Cell*, 8 (2), 303-16.

Thomas, DM, et al. (2004), 'Terminal osteoblast differentiation, mediated by runx2 and p27KIP1, is disrupted in osteosarcoma.', *J Cell Biol*, 167 (5), 925-34.

Tiemann, F and PW Hinds (1998), 'Induction of DNA synthesis and apoptosis by regulated inactivation of a temperature-sensitive retinoblastoma protein.', *EMBO J*, 17 (4), 1040-52.

Trimarchi, JM and JA Lees (2002), 'Sibling rivalry in the E2F family.', *Nat Rev Mol Cell Biol*, 3 (1), 11-20.

Upton, B, Q Chu, and BD Li (2009), 'Li-Fraumeni syndrome: the genetics and treatment considerations for the sarcoma and associated neoplasms.', *Surg Oncol Clin N Am*, 18 (1), 145-56, ix.

Vooijs, M, et al. (2002), 'Tumor formation in mice with somatic inactivation of the retinoblastoma gene in interphotoreceptor retinol binding protein-expressing cells.', *Oncogene*, 21 (30), 4635-45.

Vousden, KH and PS Jat (1989), 'Functional similarity between HPV16E7, SV40 large T and adenovirus E1a proteins.', *Oncogene*, 4 (2), 153-58.

Wadayama, B, et al. (1994), 'Mutation spectrum of the retinoblastoma gene in osteosarcomas.', *Cancer Res*, 54 (11), 3042-48.

Walkley, CR, et al. (2008), 'Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease.', *Genes Dev*, 22 (12), 1662-76.

Wang, LL, et al. (2001), 'Clinical manifestations in a cohort of 41 Rothmund-Thomson syndrome patients.', *Am J Med Genet*, 102 (1), 11-17.

Wang, LL, et al. (2003), 'Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome.', *J Natl Cancer Inst*, 95 (9), 669-74.

Weinberg, RA (1994), 'Oncogenes and tumor suppressor genes.', *CA Cancer J Clin*, 44 (3), 160-70.

Whyte, P, et al. (1988), 'Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product.', *Nature*, 334 (6178), 124-29.

Williams, BO, et al. (1994), 'Extensive contribution of Rb-deficient cells to adult chimeric mice with limited histopathological consequences.', *EMBO J*, 13 (18), 4251-59.

Won, KY, HR Park, and YK Park (2009), 'Prognostic implication of immunohistochemical Runx2 expression in osteosarcoma.', *Tumori*, 95 (3), 311-16.

Wunder, JS, et al. (2005), 'TP53 mutations and outcome in osteosarcoma: a prospective, multicenter study.', *J Clin Oncol*, 23 (7), 1483-90.

Yamaguchi, T, et al. (1992), 'Allelotype analysis in osteosarcomas: frequent allele loss on 3q, 13q, 17p, and 18q.', *Cancer Res*, 52 (9), 2419-23.

Yang, HS and PW Hinds (2007), 'pRb-mediated control of epithelial cell proliferation and Indian hedgehog expression in mouse intestinal development.', *BMC Dev Biol*, 7 6.

Yoshida, CA, et al. (2002), 'Core-binding factor beta interacts with Runx2 and is required for skeletal development.', *Nat Genet*, 32 (4), 633-38.

Yoshida, CA, et al. (2004), 'Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog.', *Genes Dev*, 18 (8), 952-63.

Zhou, H, et al. (2003), 'Her-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification.', *J Pediatr Hematol Oncol*, 25 (1), 27-32.

Osteosarcoma

Edited by Dr. Manish Agarwal

ISBN 978-953-51-0506-0

Hard cover, 174 pages

Publisher InTech

Published online 11, April, 2012

Published in print edition April, 2012

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.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Elizabeth Kong and Philip W. Hinds (2012). The Retinoblastoma Protein in Osteosarcomagenesis, Osteosarcoma, Dr. Manish Agarwal (Ed.), ISBN: 978-953-51-0506-0, InTech, Available from: http://www.intechopen.com/books/osteosarcoma/the-retinoblastoma-protein-in-osteosarcomagenesis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen