PROSPECT
Journal of Cellular Biochemistry 111:529–534 (2010)

Journal of Cellular Biochemistry

p53 Control of Bone Remodeling

Huijuan Liu[1] and Baojie Li[1,2]*

[1]Bio-X Center, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai, China

[2]The Institute of Molecular and Cell Biology, A*STAR (Agency for Science, Technology and Research), Singapore

ABSTRACT

Tumor suppressor p53 is a transcription regulator that displays anti-proliferation activities once activated by stress, in particular genotoxic stress. Recent mouse genetic studies revealed a critical role for p53 in bone remodeling, supported by both loss-of-function and gain-of function studies. p53 deficiency concurrently enhances proliferation and accelerates differentiation in mesenchymal stem cells and osteoprogenitor cells, resulting in an increase in bone formation and bone mass/density. Moreover, Atm, c-Abl, and Mdm2, upstream regulators of p53 in DNA damage response, regulate osteoblast differentiation and bone remodeling as well. While the molecular mechanisms await further investigation, there is evidence to suggest that p53 regulate osteoblast differentiation via transcription factors Runx2/Osterix, which are involved in osteoblast differentiation and transformation and are under the control of BMP and IGF pathways. Moreover, there are studies showing that p53 inhibits cell differentiation in many other tissues. These findings suggest that p53 acts as a safeguard of differentiation in addition to ''guardian of the genome'' and challenge the conventional wisdom that tumor suppressors usually block cell proliferation and promote differentiation. J. Cell. Biochem. 111: 529–534, 2010.

KEY WORDS: p53; BONE REMODELING; OSTEOBLAST DIFFERENTIATION

p53 IS A NEGATIVE REGULATOR OF CELL PROPAGATION

p53 was first identified in 1979 by Lionel Crawford, David P. Lane, Arnold Levine, and Lloyd Old, respectively, as an oncogene [Sherr, 1998]. Later studies demonstrated that p53 is actually a tumor suppressor [Vogelstein et al., 2000]. It is a transcription regulator that can either activate or repress transcription, depending on the target genes. For transactivation, p53 usually needs to bind to the promoter regions of the target genes as a tetramer, with a consensus DNA sequence of 5′-RRRCWWGYYY-N(0-13)-RRRCWWGYYY-3′. However, p53-mediated gene repression is less well understood. p53 can repress gene transcription in a DNA-binding dependent or independent manner [Ho and Benchimol, 2003]. It is estimated that p53 can activate thousands of genes as well as repress just as many. The best-studied p53 target genes include p21, a CDK inhibitor, Mdm2, an ubiquitin E3 ligase for p53, and Puma and Bax, apoptosis promoters [Ko and Prives, 1996].

p53 is expressed at very low levels in normal cells. This is most likely caused by the feedback regulatory loop between Mdm2 and p53. Mdm2 ubiquitinates p53 in the nucleus, which leads to p53 nuclear export and proteosome-mediated degradation. On the other hand, Mdm2 is a direct target gene of p53 transcription activity. Therefore, elevation of p53 up-regulates Mdm2, which in turn down-regulates p53 [Ko and Prives, 1996]. As a transcription regulator, p53 activity is controlled at least in two ways: the expression levels and posttranslational modifications. Its expression can be regulated at the levels of transcription, translation, and protein stability, with protein stabilization as a major mechanism. The main stimuli of p53 expression include DNA damage (caused by UV, IR, or genotoxic drugs), oxidative stress, osmotic shock, ribonucleotide depletion, and deregulated oncogene activation. Genotoxic stress activates PI3 kinase-like-kinases including DNA-PKc, Atm, and Atr at the DNA break sites, which further phosphorylate p53, c-Abl, Mdm2, and other proteins, leading to p53 stabilization/activation [Zhou and Elledge, 2000; Kastan and Bartek, 2004; Peterson and Cote, 2004; Li, 2005]. In addition, p53 has been reported to be modified by acetylation and sumoylation, which modulate the function of p53 as well. Overall, p53 activation leads to cell cycle arrest, apoptosis, and/or senescence, which helps to eliminate cells with damaged genome and subsequently prevent tumorigenesis. As such, p53 is widely recognized as the ''guardian of the genome.''

A tumor suppression role for p53 is manifested by the finding that p53 is mutated in more than 50% of the primary tumors [Vogelstein et al., 2000]. Most of these mutations cause the loss of p53 function.

Grant sponsor: Agency for Science, Technology and Research of the Republic of Singapore.
*Correspondence to: Baojie Li, Shanghai Jiao Tong Univ. E-mail: libj.bio@gmail.com
Received 3 June 2010; Accepted 9 June 2010 • DOI 10.1002/jcb.22749 • © 2010 Wiley-Liss, Inc.
Published online 29 June 2010 in Wiley Online Library (wileyonlinelibrary.com).

For example, p53 is absent in a high percentage of patients with advanced stages of prostate cancer, and is associated with metastasis and hormone independence. Moreover, prostate cancer is more likely to recur in men with p53 mutations than their counterparts without this abnormality [Altieri et al., 2009]. This is substantiated by mouse genetic studies, which shows that conditional deletion of p53 in prostate epithelium leads to prostatic intraepithelial metastatic neoplasia by 600 days of age [Zhou et al., 2006].

As a prototypical tumor suppressor, p53 is proposed not only to inhibit proliferation but also promote differentiation, for example, ES cells and myoblasts [Stiewe, 2007]. Cell differentiation and proliferation are usually mutually exclusive events, with differentiation requiring the block of proliferation in many cell types. Although differentiation does not eliminate cells with damaged DNA from the tissue, it does remove these cells from the proliferating cell pools, thus helping to maintain genome stability. Thus, induction of differentiation can be another mechanism by which tumor suppressors inhibits tumorigenesis [Stiewe, 2007].

BONE REMODELING AND BONE-RELATED DISEASES

The skeleton protects the internal organs and provides the environment for hematopoiesis and the site for calcium and phosphate storage. It goes through several phases of growth in our lifetime: (1) slow growth prior to adolescence, (2) rapid growth during adolescence, under the influence of steroid hormones and growth hormone, (3) obtaining peak mass a few years after adolescence, (4) balanced bone remodeling with constant bone mass and density in adulthood, and (5) decline in bone mass/density postmenopause [Riggs et al., 2002].

Bone growth rates are determined by net output of bone formation and resorption. Bone formation is carried out by osteoblasts that can synthesize matrix proteins and mineralize the bone matrix. They are derived from bone marrow mesenchymal stem cells (MSC), which also have the potential to differentiate into myoblasts, adipocytes, and chondrocytes [Harada and Rodan, 2003]. Differentiation of MSCs to osteoblasts is a multi-step process that requires both cell expansion and differentiation. Osteoblast differentiation is controlled by osteochondral specific transcription factors Runx2 and Osterix, as well as Dlx5 and Atf4 that are also expressed in other cell types [Yang and Karsenty, 2002; Lian et al., 2004; Stein et al., 2004]. Both Runx2 and Osterix are sufficient and essential for osteoblast differentiation and bone mineralization, as mice deficient for either of them show no mature osteoblasts or bone calcification [Komori et al., 1997; Otto et al., 1997; Nakashima et al., 2002], while overexpression of either of them in non-osteoblast cells induces the expression of osteoblast markers [Ducy et al., 1997; Wang et al., 2006]. It is through the regulation of these transcription factors, growth factors and cytokines, especially BMPs, Wnts, and IGFs, control osteoblast differentiation and bone formation [Harada and Rodan, 2003].

Bone resorption, on the other hand, is carried out by osteoclasts, which are large multinucleated cells that can grow up to 100 μm in diameter. Osteoclasts are derived from hematopoietic stem cells (HSCs)-derived progenitors for monocytes and macrophages [Teitelbaum and Ross, 2003]. The rate of bone resorption is determined by the number of osteoclasts and the resorptive activity of mature osteoclasts. Many factors including cytokines and hormones regulate osteoclastogenesis. Positive regulators include IL-1β, 6, 11, 17, GM-CSF, M-CSF, TNF-α, and RANK, while negative regulators include OPG and TGF-β, though the most extensively studied are M-CSF, RANKL, and OPG [Suda et al., 1999; Boyle et al., 2003]. M-CSF can bind to its cognate receptor c-Fms on the surface of osteoclast precursor and promotes its differentiation, activation, and survival. RANKL binds to its receptor RANK on the surface of osteoclast precursor and acts similarly as M-CSF. On the other hand, OPG is a naturally occurring decoy receptor, which functions as a paracrine inhibitor of osteoclast production and activity. The balance between RANKL and OPG determines the pace of osteoclastogenesis and bone resorption [Khosla, 2001; Boyle et al., 2003].

Bone remodeling is a process in which new bones are formed to replace the old bones. Even in adults, 3% of the cortical bones and 15% of the trabecular bones are replaced each year [Manolagas and Jilka, 1995]. During bone growth period (adolescence), bone formation outpaces resorption; whereas in adults, bone resorption and bone formation are balanced; however, bone resorption usually outstrips formation in aged population. Moreover, recent studies indicate there exists a coupling between osteoblastogenesis and osteoclastogenesis [Martin and Sims, 2005]. Markers for bone formation and resorption tend to follow the same pattern during bone remodeling. In general, osteoclastic bone resorption is always followed by osteoblastic bone formation and the two processes are well coordinated. It is proposed that IGFs and TGF-β, which are synthesized by osteoblasts and stored in bone matrix, are released during resorption, which in turn stimulate osteoblast recruitment and bone formation. In addition, osteoblasts and their progenitors can synthesize and present growth factors to osteoclast precursors to regulate their differentiation and maturation. For example, RANKL and M-CSF could be synthesized by osteoblastic precursors to stimulate osteoclastogenesis. Noteworthy is the fact that RANKL is expressed on the osteoblast/precursor surface and RANK is expressed on the surface of osteoclast precursors, which makes cell–cell contact necessary for RANKL action. Negative regulator OPG could also be synthesized and secreted by osteoblasts and their precursors.

A disruption of the balance between bone formation and resorption often leads to bone-related disorders. The most common one is osteoporosis, with features including reduced bone mass/density, deterioration of microstructure, and increased fracture risk [Rodan, 1992; Manolagas and Jilka, 1995]. It affects more than 200 million people worldwide and is a major cause of morbidity, mortality, and a huge economic burden. It can be classified into senile osteoporosis, postmenopausal osteoporosis, and other pathological types such as glucocorticoid-induced osteoporosis [Manolagas and Jilka, 1995]. Postmenopausal osteoporosis is mainly caused by steroid hormone deficiency, which results in enhanced osteoclastogenesis and increased osteoclast resorption activity, while senile osteoporosis is mainly caused by reduced bone formation due to a decrease in the number of osteoblasts, the activity of osteoblasts, or both, and

affects both aged men and women [Manolagas and Jilka, 1995]. Other bone-related diseases include osteosclerosis and osteopetrosis, featured by an increase in bone mass/density, with the former being mainly caused by an increase in bone formation and the latter being mainly caused by dysfunctional bone resorption.

p53 IN OSTEOBLAST DIFFERENTIATION AND BONE REMODELING

The physiological function of p53 has been studied using genetically engineered mice. Due to its profound effects in cell proliferation, death, and aging, p53 deficient mice were expected to show severe developmental defects. Surprisingly, the mutant mice appeared normal at birth, although most of the knockout mice developed tumors, mainly lymphomas, around the age of 5–7 months [Donehower et al., 1992; Stiewe, 2007]. This raised the possibility that p53 has functionally redundant genes such as p63 and p73, which make up the loss of p53 and mask the true function of p53 in development. Another possibility is p53 might be largely dispensable for normal cell growth, cell differentiation, and development, as it has been suggested by the study of a transgenic mouse line bearing a reporter gene fused to the p53 response element, which showed little or no postnatal p53 activity in the absence of DNA damage in vivo [Gottlieb et al., 1997]. However, subsequent detailed studies revealed that a low percentage of mice did show developmental anomalies including polydactyl and exencephaly [Stiewe, 2007]. A recent study also found that p53−/− mice showed a reduced fertility. This defect is believed to be mediated by leukemia inhibitory factor (LIF), a cytokine critical for implantation and a p53 target gene [Hu et al., 2007].

A role for p53 in bone remodeling has been thoroughly studied by a few groups. Studies from our group show that p53−/− mice exhibit an osteosclerotic phenotype compared to control littermates, with a modest increase in bone mineral density and bone volume, justified by dual X-ray absorptiometry and histomorphometry analysis [Wang et al., 2006]. Moreover, these mutant mice show an increase in both bone formation and resorption. Since the overall result is an increase in bone mass and density, the increase in bone resorption should be secondary to enhanced bone formation. p53−/− mouse is thus a model of increased remodeling with an osteosclerotic phenotype. A second study deleted Mdm2 in an osteoblasts specific manner by crossing Mdm2 conditional knockout mice and Col3.6-Cre transgenic mice [Lengner et al., 2006]. The mice die at birth and show caudal defect as well as ossification defects, due to hyperactivity of p53. A third study found that while mechanical unloading suspension led to a reduction of trabecular bone volume and bone formation rate due to defective osteoblast differentiation in wild type, p53−/− mice were resistant to this treatment [Sakai et al., 2002].

What is the mechanism by which p53 regulates bone remodeling? Our studies showed that p53−/− mice displayed increased bone formation, accompanied by an increase in the number of osteoblasts and an enhancement of osteoblast differentiation, two cellular events that are usually mutually exclusive. Calvarial osteoblasts show increased proliferation that can be restored to normal by expressing p21, the target gene of p53 and a crucial player in determining cell growth rate. However, enhanced differentiation is not significantly affected by the expression of p21 [Wang et al., 2006]. Further studies indicated that the enhanced differentiation is mediated by elevated expression of Osterix as overexpression of Osterix in normal osteoblasts could mimic the differentiation phenotype of p53−/− cells, while knock-down of Osterix in p53−/− osteoblasts with siRNA slowed down differentiation. It was also found that Osterix could be repressed by p53 in reporter assays in a DNA-binding independent manner [Wang et al., 2006]. This might be one mechanism by which p53 represses Osterix expression. In Mdm2−/− osteoblasts, surprisingly, Mdm2 deletion did not significantly alter the overall levels of p53 protein. Instead, it led to an enhancement of p53 transactivation activity and an up-regulation of Runx2. This did not induce p53-mediated apoptosis but rather blocked osteoblast differentiation [Lengner et al., 2006]. The same group also confirmed that p53−/− osteoblasts showed enhanced differentiation and p53 deficiency could rescue the differentiation defects observed in Mdm2−/− osteoblasts.

Furthermore, knock-down of p53 in primary MEFs was shown to enhance differentiation into osteoblasts, accompanied by elevation of Runx2 and Osterix [Molchadsky et al., 2008]. Furthermore, p53 knock-down has been shown to enhance MEFs differentiation into adipocytes and myofibroblasts as well. In addition, when compared to control cells, p53−/− bone marrow MSCs show accelerated bone nodule formation and alkaline phosphatase staining, as well as increased expression of the early and intermediate osteogenic markers, Runx2 and osteopontin, but not terminal osteogenic marker gene osteocalcin, suggesting that p53 deficiency, which promotes early stages of osteoblast differentiation, might inhibit terminal differentiation, a event that requires termination of proliferation [Tataria et al., 2006]. These results taken together demonstrate that p53 is an important regulator in osteoblast differentiation in addition to its traditional roles in cell proliferation and tumor suppression.

p53 was also found to play a role in the coupling between osteoblastogenesis and osteoclastogenesis. In addition to enhanced bone formation, p53−/− mice also show enhanced bone resorption, manifested by an increase in the number of osteoclasts, the bone resorption surface and the secretion of deoxypyridinoline cross-links. However, p53 seems to have no cell-autonomous effects on osteoclast differentiation from monocytes or the resorption activity of the osteoclasts [Wang et al., 2006]. A co-culture experiment of osteoblasts and osteoclasts indicated that p53−/− osteoblasts acquired enhanced activity in promoting osteoclastogenesis, which is likely mediated by up-regulation of M-CSF, as p53 deficiency led to an increase in M-CSF expression, but not RANKL or OPG. This is further supported by the fact that M-CSF expression is induced by the ectopic expression of Osterix. These findings indicate that p53 plays an important role in skeletal formation: it directly inhibits bone formation and indirectly inhibits bone resorption [Wang et al., 2006]. The osteoblast-supported osteoclastogenesis data also help to explain why osteosclerotic models like p53−/− mice only show moderate increase in bone mass while they have markedly enhanced bone formation. Hence, p53−/− mice can be used as an osteosclerotic animal model, which may allow us to comprehend

factors coupling bone formation and bone resorption during bone remodeling.

p53 UPSTREAM REGULATORS IN BONE REMODELING

The conclusion that p53 regulates bone remodeling is further supported by studies of mouse lines deficient for p53 upstream regulators. In DNA damage response, p53 is regulated by Atm and c-Abl in addition to Mdm2. We and others found that both c-Abl−/− mice and Atm−/− mice showed osteoporotic phenotypes, which are accompanied by a decrease in bone formation, a decrease in osteoblast differentiation, and a reduction in the expression of Osterix [Li et al., 2000, 2004; Rasheed et al., 2006]. Similar to the findings that p53 deficiency rescued the differentiation defects of Mdm2 null osteoblasts, we found that p53 deficiency also rescued the differentiation defect of c-Abl null osteoblasts, as well as the reduction in Osterix expression [Wang et al., 2006]. These results suggest that p53 possibly acts downstream of c-Abl and Mdm2 in osteoblast differentiation.

Although the epistatic relationships among these genes need to be further analyzed, the in vivo and in vitro studies convincingly show that c-Abl, Atm, and p53 do participate in bone remodeling by regulating osteoblast differentiation in a cell autonomous manner, without affecting osteoclast differentiation, thus providing evidence that DNA damage response proteins have novel functions in postnatal tissue homeostasis.

p53 IN OSTEOSARCOMA DEVELOPMENT

The above-mentioned studies also confirmed an anti-proliferation effect for p53 in osteoblasts. p53−/− mice display an increase in the number of osteoblasts, and ex vivo osteoblasts as well as MSC cultures show a reduced doubling time. This might facilitate development of osteosarcoma, a tumor developed from osteoblast stem cells or progenitor cells. Indeed, human studies have shown that p53 is often lost in osteosarcoma either by mutation of the p53 gene or by loss of upstream signaling molecules [Miller et al., 1990]. Moreover, germline mutation of the p53 gene in Li–Fraumeni syndrome also predisposes patients to osteosarcoma [Porter et al., 1992]. This is also validated by studies of animal models. p53 deficient mice develop osteosarcoma among other malignancies [Jacks et al., 1994; Lang et al., 2004; Olive et al., 2004]. Depending on the genetic background, 3–8% of p53−/− mice develop osteosarcomas [Harvey et al., 1993; Jones et al., 1996]. This low incidence rate could be due to premature death of the p53−/− mice (>90% p53−/− mice die before 200 days) from lymphomas [Harvey et al., 1993]. Indeed, it has been recently reported that 60% of the mice with osteoblast-specific deletion of p53 showed development of osteosarcoma by 42 weeks [Lengner et al., 2006]. Further studies show that osteosarcoma development is largely dependent on the loss of p53 and can only be potentiated by the loss of pRb, thus revealing a dominant role for p53 in suppressing osteosarcoma development.

p53 AND Runx2/OSTERIX EXPRESSION

How does p53, as well as Atm and c-Abl, regulate osteoblast differentiation and bone formation? In osteoblasts deficient for each of the three genes, there is an alteration in the expression of Osterix, which is positively related to their differentiation potential. This positive correlation is only specific to Osterix but not to other transcription factors such as Runx2, Atf4, or Dlx5 [Rasheed et al., 2006; Wang et al., 2006]. For example, osteoblasts deficient in c-Abl or Atm show defective differentiation that is accompanied by reduced levels of Osterix. On the other hand, p53 deficient osteoblasts show enhanced differentiation that is accompanied with increased levels of Osterix (Fig. 1). In addition, inhibition of p38 MAPK impeded osteoblast differentiation as well as the expression of Osterix and inhibition of Cox-2 also compromises osteoblast differentiation and reduces the expression of Osterix [Wang et al., 2007]. More importantly, we found that knocking down Osterix in p53 deficient osteoblasts slowed down differentiation while overexpression of Osterix render resistance to p38 MAPK inhibitor with regard to differentiation. However, in Mdm2−/− osteoblasts and MEFs with p53 knocked down, Runx2 was also found to be up-regulated [Lengner et al., 2006]. It is thus likely that up-regulation of Runx2/Osterix in the absence of p53 is responsible for enhanced differentiation.

Osterix is under the control of BMPs and IGFs [Lee et al., 2003; Celil and Campbell, 2005], which are the driving force of osteoblast differentiation and bone formation in vivo [Li, 2008; Chau et al., 2009]. BMPs induce Osterix transcription through the canonical Smad1/5/8 pathway and the non-canonical p38 MAPK pathway. It is possible that c-Abl, a tyrosine kinase, and Atm, a Ser/Thr kinase, and p53 regulate Osterix via altering the signaling pathways that control the expression of Osterix and Runx2, for example, BMP-Smad1/5/8, BMP-p38 MAPK, or IGF-MAPK pathway.

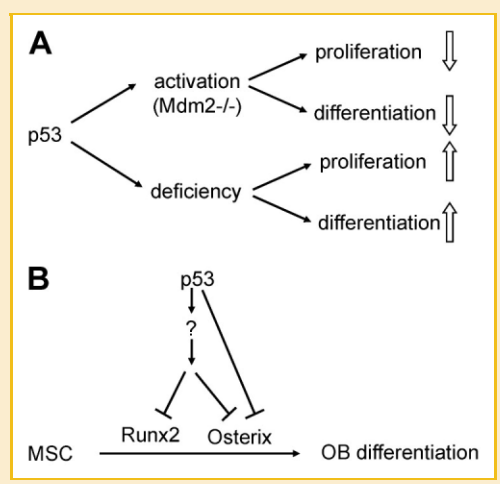

Fig. 1. A diagram showing the function of p53 in osteoblast (OB) proliferation and differentiation. A: p53 negatively regulates both osteoblast proliferation and differentiation, two cellular events that are usually mutually exclusive. B: Possible mechanisms by which p53 regulates OB differentiation.

More interestingly, both Runx2 and Osterix have been implicated in tumorigenesis, especially osteosarcoma and prostate cancer [Akech et al., 2010]. Osterix was found to be down-regulated in osteosarcoma lines and ectopic expression of Osterix inhibits proliferation of these cells [Cao et al., 2005]. The role for Runx2 in tumorigenesis is much more complicated. It can be a tumor suppressor or an oncoprotein in a cell context dependent manner. Runx2 deficiency in osteoblasts could potentiate cell immortalization and tumorigenesis [Zaidi et al., 2007]. However in other cell types, for example, in prostate cancer, Runx2 seem to have oncogenic activity. Runx2 is up-regulated in prostate cancer and this elevation correlates with their metastatic potential and expression of metastasis-related genes such as MMP9 and MMP13 [Pratap et al., 2005]. Runx2 also enhances cell growth and response to androgen and TGF-β in prostate cancer cells [Akech et al., 2010; van der Deen et al., 2010]. At least in osteoblasts and osteosarcoma cells, p53 deficiency-induced up-regulation of Runx2 and Osterix seems to help to curb cell proliferation in addition to promoting osteoblast differentiation.

PERSPECTIVES

Mouse genetic and cell-based studies revealed an unexpected role for p53 in negatively regulating the differentiation of osteoblast and many other cell types (Fig. 1). Due to p53's potent anti-proliferation activity, it has been generally believed that if p53 deficient mice have a developmental defect such as bone remodeling, it has to be caused by the alteration in the number of the cells involved and their stem cells (osteoblasts or osteoclasts in this case). Surprisingly, all the bone-related studies demonstrated a novel function for p53 in negatively regulating osteoblast differentiation from MSC. In addition, p53 has been found to inhibit the differentiation of many other cell types including pre-B cells, myoblasts, kerotinocytes, oligodendrocytes, neurons, adipocytes, and myofibroblats. These findings suggest that besides being the ''guardian of the genome,'' p53 might also act as a ''safeguard of differentiation.'' DNA damage-induced p53 activation not only stops cell proliferation or induces apoptosis, but also halts cell differentiation, in many if not all cell types. This might be another mechanism by which DNA damage and p53 activation induces premature aging in vivo, as p53 activation might block the differentiation/maturation process of the functional cells in many tissues. Together with p53-induced cell apoptosis and senescence, this would lead to a shortage of mature functional cells, which is a main feature of aging. Moreover, these findings do not support the concept that cell proliferation and differentiation are mutually exclusive events, at least at the early stage of stem cell differentiation. Somatic stem cells are usually low in numbers, and differentiation from a stem cell (e.g., MSC) to functional cells (e.g., osteoblasts) thus needs cell expansion. It seems plausible that cell proliferation and differentiation co-exist in a coordinated manner at the early stage of stem cell differentiation. Only at the very terminal differentiation stage, does cell proliferation need to be ended.

While the data demonstrate that p53 participates in bone remodeling by regulating osteoblast proliferation and differentiation, many questions remain to be answered. The most obvious one is what the molecular mechanisms are by which p53 regulates osteoblast differentiation. What connects p53 deficiency to the up-regulation of Runx2 and Osterix in osteoblasts? Is there a common pathway controlled by p53 that is behind the altered differentiation process in all cell types? Also unknown is why Atm, c-Abl and p53, which are involved in DNA damage response and tumorigenesis, also regulate osteoblast differentiation. Do DNA damage response and osteoblast differentiation share any common pathways? The answers to these questions might help us to fully understand not only how p53 controls cell differentiation and tissue homeostasis but also how p53 prevents cancer development.

ACKNOWLEDGMENTS

We thank Keng Po Lai, Wai Fook Leong, and Jenny Chau for helpful discussions. The work conducted in the authors' laboratory is supported by the Agency for Science, Technology and Research of the Republic of Singapore.

REFERENCE

Akech J, Wixted JJ, Bedard K, van der Deen M, Hussain S, Guise TA, van Wijnen AJ, Stein JL, Languino LR, Altieri DC, Pratap J, Keller E, Stein GS, Lian JB. 2010. Runx2 association with progression of prostate cancer in patients: Mechanisms mediating bone osteolysis and osteoblastic metastatic lesions. Oncogene 29:811–821.

Altieri DC, Languino LR, Lian JB, Stein JL, Leav I, van Wijnen AJ, Jiang Z, Stein GS. 2009. Prostate cancer regulatory networks. J Cell Biochem 107:845–852.

Boyle WJ, Simonet WS, Lacey DL. 2003. Osteoclast differentiation and activation. Nature 423:337–342.

Cao Y, Zhou Z, de Crombrugghe B, Nakashima K, Guan H, Duan X, Jia SF, Kleinerman ES. 2005. Osterix, a transcription factor for osteoblast differentiation, mediates antitumor activity in murine osteosarcoma. Cancer Res 65:1124–1128.

Celil AB, Campbell PG. 2005. BMP-2 and insulin-like growth factor-I mediate Osterix (Osx) expression in human mesenchymal stem cells via the MAPK and protein kinase D signaling pathways. J Biol Chem 280:31353–31359.

Chau JF, Leong WF, Li B. 2009. Signaling pathways governing osteoblast proliferation, differentiation and function. Histol Histopathol 24:1593–1606.

Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A. 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356:215–221.

Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. 1997. Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. Cell 89:747–754.

Gottlieb E, Haffner R, King A, Asher G, Gruss P, Lonai P, Oren M. 1997. Transgenic mouse model for studying the transcriptional activity of the p53 protein: Age- and tissue-dependent changes in radiation-induced activation during embryogenesis. EMBO J 16:1381–1390.

Harada S, Rodan GA. 2003. Control of osteoblast function and regulation of bone mass. Nature 423:349–355.

Harvey M, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A, Donehower LA. 1993. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. Nat Genet 5:225–229.

Ho J, Benchimol S. 2003. Transcriptional repression mediated by the p53 tumour suppressor. Cell Death Differ 10:404–408.

Hu W, Feng Z, Teresky AK, Levine AJ. 2007. p53 regulates maternal reproduction through LIF. Nature 450:721–724.

Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA. 1994. Tumor spectrum analysis in p53-mutant mice. Curr Biol 4:1–7.

Jones SN, Sands AT, Hancock AR, Vogel H, Donehower LA, Linke SP, Wahl GM, Bradley A. 1996. The tumorigenic potential and cell growth characteristics of p53-deficient cells are equivalent in the presence or absence of Mdm2. Proc Natl Acad Sci USA 93:14106–14111.

Kastan MB, Bartek J. 2004. Cell-cycle checkpoints and cancer. Nature 432: 316–323.

Khosla S. 2001. Minireview: The OPG/RANKL/RANK system. Endocrinology 142:5050–5055.

Ko LJ, Prives C. 1996. p53: Puzzle and paradigm. Genes Dev 10:1054–1072.

Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. 1997. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755–764.

Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, Valentin-Vega YA, Terzian T, Caldwell LC, Strong LC, El-Naggar AK, Lozano G. 2004. Gain of function of a p53 hot spot mutation in a mouse model of Li–Fraumeni syndrome. Cell 119:861–872.

Lee MH, Kwon TG, Park HS, Wozney JM, Ryoo HM. 2003. BMP-2-induced Osterix expression is mediated by Dlx5 but is independent of Runx2. Biochem Biophys Res Commun 309:689–694.

Lengner CJ, Steinman HA, Gagnon J, Smith TW, Henderson JE, Kream BE, Stein GS, Lian JB, Jones SN. 2006. Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling. J Cell Biol 172:909–921.

Li B. 2005. c-Abl in oxidative stress, aging and cancer. Cell Cycle 4:246–248.

Li B. 2008. Bone morphogenetic protein-Smad pathway as drug targets for osteoporosis and cancer therapy. Endocr Metab Immune Disord Drug Targets 8:208–219.

Li B, Boast S, de los Santos K, Schieren I, Quiroz M, Teitelbaum SL, Tondravi MM, Goff SP. 2000. Mice deficient in Abl are osteoporotic and have defects in osteoblast maturation. Nat Genet 24:304–308.

Li B, Wang X, Rasheed N, Hu Y, Boast S, Ishii T, Nakayama K, Nakayama KI, Goff SP. 2004. Distinct roles of c-Abl and Atm in oxidative stress response are mediated by protein kinase C delta. Genes Dev 18:1824–1837.

Lian JB, Javed A, Zaidi SK, Lengner C, Montecino M, van Wijnen AJ, Stein JL, Stein GS. 2004. Regulatory controls for osteoblast growth and differentiation: Role of Runx/Cbfa/AML factors. Crit Rev Eukaryot Gene Expr 14:1–41.

Manolagas SC, Jilka RL. 1995. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 332:305–311.

Martin TJ, Sims NA. 2005. Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol Med 11:76–81.

Miller CW, Aslo A, Tsay C, Slamon D, Ishizaki K, Toguchida J, Yamamuro T, Lampkin B, Koeffler HP. 1990. Frequency and structure of p53 rearrangements in human osteosarcoma. Cancer Res 50:7950–7954.

Molchadsky A, Shats I, Goldfinger N, Pevsner-Fischer M, Olson M, Rinon A, Tzahor E, Lozano G, Zipori D, Sarig R, Rotter V. 2008. p53 plays a role in mesenchymal differentiation programs, in a cell fate dependent manner. PLoS One 3:e3707.

Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. 2002. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29.

Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T. 2004. Mutant p53 gain of function in two mouse models of Li–Fraumeni syndrome. Cell 119:847–860.

Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ. 1997. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89:765–771.

Peterson CL, Cote J. 2004. Cellular machineries for chromosomal DNA repair. Genes Dev 18:602–616.

Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR, Reid R. 1992. A significant proportion of patients with osteosarcoma may belong to Li–Fraumeni cancer families. J Bone Joint Surg Br 74:883–886.

Pratap J, Javed A, Languino LR, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2005. The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. Mol Cell Biol 25:8581–8591.

Rasheed N, Wang X, Niu QT, Yeh J, Li B. 2006. Atm-deficient mice: An osteoporosis model with defective osteoblast differentiation and increased osteoclastogenesis. Hum Mol Genet 15:1938–1948.

Riggs BL, Khosla S, Melton LJ III. 2002. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302.

Rodan GA. 1992. Introduction to bone biology. Bone 13(Suppl 1): S3–S6.

Sakai A, Sakata T, Tanaka S, Okazaki R, Kunugita N, Norimura T, Nakamura T. 2002. Disruption of the p53 gene results in preserved trabecular bone mass and bone formation after mechanical unloading. J Bone Miner Res 17:119–127.

Sherr CJ. 1998. Tumor surveillance via the ARF-p53 pathway. Genes Dev 12:2984–2991.

Stein GS, Lian JB, van Wijnen AJ, Stein JL, Montecino M, Javed A, Zaidi SK, Young DW, Choi JY, Pockwinse SM. 2004. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene 23:4315–4329.

Stiewe T. 2007. The p53 family in differentiation and tumorigenesis. Nat Rev Cancer 7:165–168.

Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. 1999. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357.

Tataria M, Quarto N, Longaker MT, Sylvester KG. 2006. Absence of the p53 tumor suppressor gene promotes osteogenesis in mesenchymal stem cells. J Pediatr Surg 41:624–632.

Teitelbaum SL, Ross FP. 2003. Genetic regulation of osteoclast development and function. Nat Rev Genet 4:638–649.

van der Deen M, Akech J, Wang T, FitzGerald TJ, Altieri DC, Languino LR, Lian JB, van Wijnen AJ, Stein JL, Stein GS. 2010. The cancer-related Runx2 protein enhances cell growth and responses to androgen and TGFbeta in prostate cancer cells. J Cell Biochem 109:828–837.

Vogelstein B, Lane D, Levine AJ. 2000. Surfing the p53 network. Nature 408:307–310.

Wang X, Kua HY, Hu Y, Guo K, Zeng Q, Wu Q, Ng HH, Karsenty G, de Crombrugghe B, Yeh J, Li B. 2006. p53 functions as a negative regulator of osteoblastogenesis, osteoblast-dependent osteoclastogenesis, and bone remodeling. J Cell Biol 172:115–125.

Wang X, Goh CH, Li B. 2007. p38 mitogen-activated protein kinase regulates osteoblast differentiation through osterix. Endocrinology 148:1629–1637.

Yang X, Karsenty G. 2002. Transcription factors in bone: Developmental and pathological aspects. Trends Mol Med 8:340–345.

Zaidi SK, Pande S, Pratap J, Gaur T, Grigoriu S, Ali SA, Stein JL, Lian JB, van Wijnen AJ, Stein GS. 2007. Runx2 deficiency and defective subnuclear targeting bypass senescence to promote immortalization and tumorigenic potential. Proc Natl Acad Sci USA 104:19861–19866.

Zhou BB, Elledge SJ. 2000. The DNA damage response: Putting checkpoints in perspective. Nature 408:433–439.

Zhou Z, Flesken-Nikitin A, Corney DC, Wang W, Goodrich DW, Roy-Burman P, Nikitin AY. 2006. Synergy of p53 and Rb deficiency in a conditional mouse model for metastatic prostate cancer. Cancer Res 66:7889–7898.