

Skeletons in the p53 tumor suppressor closet: genetic evidence that p53 blocks bone differentiation and development

Gerard P. Zambetti,¹ Edwin M. Horwitz,² and Ernestina Schipani³

¹Department of Biochemistry and ²Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN 38105

³Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

A series of in vitro tissue culture studies indicated that the p53 tumor suppressor promotes cellular differentiation, which could explain its role in preventing cancer. Quite surprisingly, however, two new in vivo studies (Lengner et al., 2006; Wang et al., 2006) provide genetic evidence that p53 blocks osteoblast differentiation and bone development. These interesting results and their biological and clinical implications are the focus of this comment.

It has long been recognized that the p53 tumor suppressor plays a pivotal role in preventing cancer (for review see Vogelstein et al., 2000). Multiple lines of evidence support this tenet: (1) p53 is the most commonly mutated gene in human cancer; (2) mice lacking p53 invariably develop tumors by ~8 mo of age; and (3) individuals who inherit a p53 mutation are highly tumor prone, usually developing a malignancy during childhood or as a young adult. The latter situation is referred to as Li-Fraumeni syndrome, which is characterized by a predisposition to diverse tumor types, including osteosarcomas. With no uncertainty, p53 is essential for blocking tumor development. How it does so lies within its ability to positively and negatively regulate gene expression. p53 responds to various cell stresses such as DNA damage and oncogene activation by inducing downstream target genes to block cell proliferation or to induce apoptosis. Collectively, p53 transcriptional activities literally impact life and death by maintaining normal cell growth and eliminating abnormal, potentially tumorigenic cells.

The signaling pathways activated by p53 are negatively controlled by *Mdm2*, which is a p53 target gene that encodes an E3 ubiquitin ligase (for review see Michael and Oren, 2002). As p53 levels increase, *Mdm2* gene expression is concomitantly induced. Consequently, Mdm2 protein binds to and represses p53 function, in part, by interfering with its association with transcription factors and by targeting it for proteasomal degradation.

Homozygous deletion of *Mdm2* results in early embryonic lethality as a result of uncontrolled p53-mediated cell cycle arrest and apoptosis. Remarkably, crossing *Mdm2*-deficient mice onto a *p53* knockout background completely rescues the lethality, allowing the generation of *Mdm2*^{-/-}; *p53*^{-/-} double knockout animals. These findings establish a critical autoregulatory negative feedback loop between p53 and Mdm2 that keeps p53 activity in check. The balance between p53 and Mdm2 has clinical relevance as well. A substantial percentage of human soft tissue sarcomas and other tumor types harbor wild-type *p53* alleles that are inactive because of the amplification and overexpression of *MDM2*. A recurring theme in human cancer is the loss of p53 function either directly through mutation of the *p53* gene or by perturbations within the p53 signaling pathway.

Earlier work pioneered by Varda Rotter and colleagues and subsequently confirmed by others implicated p53 in the control of differentiation in a variety of cell types (for review see Almog and Rotter, 1997). For example, the enforced expression of wild-type p53 in murine L12 cells promotes B cell maturation and the production of antibodies (Shaulsky et al., 1991). The common conclusion of these studies was that p53 promotes differentiation, which makes perfect sense for a protein that suppresses cell proliferation in light of the general understanding that there exists an inverse correlation between cell growth and differentiation. As cells undergo differentiation, they exit the cell cycle and cease to proliferate. However, it is important to note that many of these studies relied on established cell lines to study the involvement of p53 in cell differentiation.

Two independent studies (see Lengner et al. on p. 909 of this issue; Wang et al., 2006) have addressed the role of p53 in bone differentiation in mouse models. In one case, Wang et al. (2006) examined skeletal structure and bone metabolism in *p53* knockout mice. Conversely, Lengner et al. (2006) analyzed the effects of hyperactive p53 on bone formation caused by the conditional deletion of *Mdm2* in osteoblasts. Surprisingly, and in contrast to the in vitro studies (for review see Almog and Rotter, 1997), both groups came to the same conclusion that p53 suppresses differentiation. Specifically, *p53*^{-/-} osteoblasts displayed a marked propensity to differentiate, which was manifested by a modest but significant increase

Correspondence to Gerard P. Zambetti: gerard.zambetti@stjude.org

Abbreviations used in this paper: MCSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor κ B ligand.

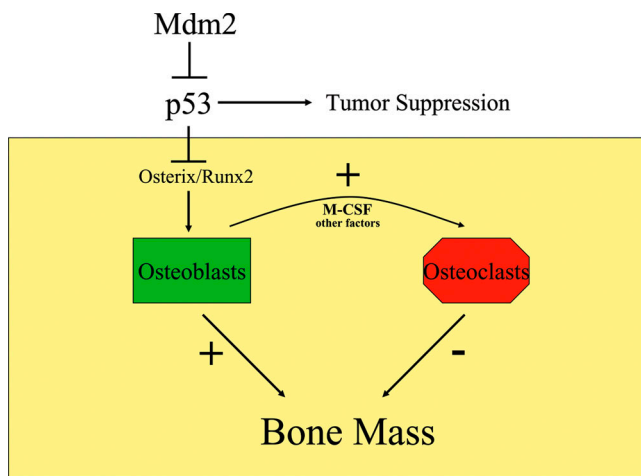


Figure 1. **The p53 signaling pathway negatively regulates tumorigenesis and bone development.** Wild-type p53 inhibits osteoblast differentiation through the repression of Osterix and Runx2. Mdm2, which is a negative regulator of p53, can override this response and promote osteoblast differentiation and bone development. Osteoblasts lacking p53 have an enhanced ability to promote osteoclast differentiation and activity. Enhanced osteoclast differentiation is associated with elevated levels of M-CSF produced by the *p53*^{-/-} osteoblasts.

in bone formation and bone density in adult *p53* knockout mice. Consistent with these results, the conditional deletion of *Mdm2* in osteoblasts interfered with terminal differentiation, leading to late stage embryonic lethality, where the embryos displayed more porous and shorter bones. These findings suggest that the interplay between p53 and Mdm2 could either positively or negatively impact bone development.

Osteoblasts originate from undifferentiated mesenchymal stem cells through the coordinated expression of transcriptional regulators that serve as “master switches” of differentiation. In particular, the transcription factors Runx2 and Osterix play a crucial role in modulating the commitment of mesenchymal stem cells toward the osteoprogenitor lineage (Ducy et al., 1999; Nakashima et al., 2002). Analysis of *Osterix*-null mice shows that Osterix is genetically downstream of Runx2. The studies of both Wang et al. (2006) and Lengner et al. (2006) provide compelling evidence that p53 suppresses osteoblast differentiation by repressing the expression of either Runx2 or Osterix. The subtle discrepancy that exists between the two studies (whether Runx2 or Osterix is the target of p53 action) may be related to how p53 activity is targeted and whether this mechanism alters the stage of cell differentiation. In either case, the concept that the absence of a tumor suppressor gene can enhance cell proliferation while favoring the differentiation of mesenchymal stem cells is intriguing but counterintuitive. It is likely that *p53*-deficient osteoprogenitors can still exit the cell cycle upon terminal differentiation, which may be enhanced as a result of the elevated expression of Runx2 and Osterix.

The activity of osteoblasts and osteoclasts must be coordinated to have a balance between bone formation and bone resorption. A coculture system of osteoblasts and hematopoietic cells has established the concept that osteoblasts are pivotally involved in osteoclast development through cell-to-cell contact. Proteins involved in this interaction have recently been identified

(for review see Boyle et al., 2003). One such example is the receptor activator of nuclear factor κ B ligand (RANKL), a membrane-bound protein of the TNF ligand family that is expressed on the osteoblast cell surface. RANKL plays a major role in osteoclast differentiation along with macrophage colony-stimulating factor (M-CSF; for review see Boyle et al., 2003). The study by Wang et al. (2006) for the first time reports the novel finding that Osterix positively regulates the level of M-CSF without affecting RANKL expression. Consequently, *p53*^{-/-} osteoblasts, which express elevated levels of Osterix, promoted the differentiation of osteoclasts in a coculture assay. Therefore, the loss of p53 stimulates both bone formation and bone resorption, with a net anabolic effect (Fig. 1). Collectively, these findings further underline the complexity of osteoblast-osteoclast cross talk and its critical importance in maintaining bone homeostasis.

Bone undergoes continuous turnover throughout life (in both mice and humans), which is essential for maintaining normal bone health. Thus, genetic modifiers of osteoblast proliferation, differentiation, and osteoblast-associated osteoclastogenesis are likely to play an important role in normal and pathologic bone biology. For example, the p53 signaling pathway identified here serves as a genetic determinant of bone mass. It follows, then, that disorders of osteoporosis or bone response to injury, such as fracture healing, may be influenced by p53 functional activity. Interestingly, an *Mdm2* promoter polymorphism has been recently identified that determines Mdm2 expression and, therefore, p53 activity and susceptibility to cancer (Bond et al., 2004). Likewise, a p53 polymorphism (Arg/Pro at amino acid 72) impacts its ability to induce apoptosis and function as a tumor suppressor (Dumont et al., 2003). Whether these naturally occurring and seemingly subtle genetic variations impact bone differentiation and development should be explored. The fact that the loss of p53 through point mutations or deletions can promote the development of osteosarcoma is consistent with this possibility. In summary, the implications of the findings reported in the Wang et al. (2006) and Lengner et al. (2006) studies identify p53 and Mdm2 as new molecular targets that could be exploited to favorably alter bone modeling and remodeling.

It is fascinating that the *p53* knockout mouse has been extensively studied for more than a decade before the aforementioned studies uncovered its osteosclerotic phenotype. These findings clearly establish p53 as a negative regulator of osteoblast differentiation both in vitro and in vivo. Whether p53 plays a role in the differentiation of other cell types, including possible positive effects, and how it may do so remains to be determined.

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