

The presence of p53 mutations in human osteosarcomas correlates with high levels of genomic instability

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The *p53* gene is a critical tumor suppressor that is inactivated in a majority of cancers. The central role of p53 in response to stresses such as DNA damage, hypoxia, and oncogene activation underlies this high frequency of negative selection during tumorigenic transformation. Mutations in *p53* disrupt checkpoint responses to DNA damage and result in the potential for destabilization of the genome. Consistent with this, *p53* mutant cells have been shown to accumulate genomic alterations in cell culture, mouse models, and some human tumors. The relationship between p53 mutation and genomic instability in human osteosarcoma is addressed in this report. Similar to some other primary human tumors, the mutation of *p53* correlates significantly with the presence of high levels of genomic instability in osteosarcomas. Surprisingly, osteosarcomas harboring an amplification of the *HDM2* oncogene, which inhibits the tumor-suppressive properties of *p53*, do not display high levels of genomic instability. These results demonstrate that the inactivation of p53 in osteosarcomas directly by mutation versus indirectly by *HDM2* amplification may have different cellular consequences with respect to the stability of the genome.

The p53 protein is a critical tumor suppressor and central mediator of cellular responses to stress. Inactivating mutations of the *p53* gene occur in $\approx 50\%$ of all sporadic human cancers (1–3). In addition, mutations in known p53-interacting genes such as *HDM2* and *p14ARF* bring the sum total of cancers that display functional inactivation of the p53 pathway to $>80\%$ (4–6). Highlighting the central role of p53 in tumor suppression is the predisposition of individuals who inherit a mutated *p53* allele to a variety of cancers at an early age including breast carcinoma, soft-tissue sarcomas, brain tumors, osteosarcoma, acute leukemias, and adrenocortical carcinoma (7). Similarly, homozygous *p53* knockout mice have a 100% chance of developing cancer (usually thymic lymphomas) before the age of 6 months, and heterozygous mice develop a variety of cancers at a later age including osteosarcoma, soft-tissue sarcomas, and lymphomas (8–11).

The p53 protein mediates responses to a variety of stresses including DNA damage, oncogene activation or mutation, hypoxia, depleted nucleotide pools, shortened telomeres, viral infection, spindle damage, nitric oxide, etc. (12). In response to one or more of these signals, p53 is posttranslationally stabilized and activated as a transcription factor to turn on or off the expressions of different sets of downstream target genes (13). The collective functions of these p53 target genes serve to execute various cellular programs that respond to stress by growth arrest, apoptosis, senescence, or cell–cell signaling (14–16).

Consistent with a role in the response to DNA damage, *p53* has been shown to inhibit gene amplifications and deletions, which can be initiated by DNA double-strand breaks. Control over DNA copy number by p53 was first demonstrated by using antimetabolites that induce resistance by gene amplification in

cell culture (17, 18). *p53* heterozygous (*p53*^{+/-}) fibroblasts fail to show phosphonoacetyl-L-aspartate-induced amplifications of the *CAD* gene during culture until loss of heterozygosity (LOH) for *p53* (*p53*^{-/-}), after which gene-amplification frequencies increase by 500- to 1,000-fold. More recently it was demonstrated that these primary human *p53*^{+/-} cell cultures also undergo spontaneous chromosomal deletions after LOH for *p53*, as detected by the comparative genome hybridization (CGH) technique, which detects large chromosomal gains and losses in the entire genome (19). A number of reports have shown a similar correlation between the mutational inactivation of *p53* and increased genomic instability *in vivo*. Gene amplifications are common in cells from the normal (nontumor) tissues of the *p53* knockout mouse and are detectable as early as 4–6 weeks of age (20). Also, tumors arising in *p53* heterozygous mice that undergo LOH for *p53* accumulate 5-fold more chromosomal aberrations than tumors in which one wild-type *p53* allele is retained (21). In human tumors, mutations in the *p53* gene have been shown to correlate with genome-wide instability in colorectal carcinomas ($P = 0.05$) as defined by CGH (22), with an increase of karyotypic abnormalities and chromosomal amplifications detected by fluorescence *in situ* hybridization on four chromosomes in breast carcinomas ($P < 0.008$) (23), and with genome-wide allelic imbalances detected by single-nucleotide polymorphism array analysis in bladder cancer (24). Taken together, these data suggest that the loss of p53 function contributes to tumorigenesis by the destabilization of the genome.

Although the initial experiments describing a role for p53 in surveillance of genomic integrity were performed with primary human fibroblast cell cultures, human sarcomas, which also arise from mesenchymal cells, have not been assayed for genomic instability and *p53* status *in vivo*. Sarcomas, including osteosarcomas and several types of soft-tissue sarcomas, are induced by the loss of p53 function in both heterozygous *p53* knockout mice (10, 11) and Li–Fraumeni cancer syndrome patients (7). Also, the effects of mutations in direct p53-interacting genes on genomic stability, such as amplifications of the *HDM2* oncogene that occur more frequently in sarcomas than in any other cancer types, have never been examined. The Hdm2 oncoprotein, the gene of which is a downstream transcriptional target of p53, functions as a direct negative regulator of p53 protein function and stability in an autoregulatory feedback loop (25). Whether amplifications of the *HDM2* oncogene, which have been demonstrated to impair the tumor-suppressor functions of p53 (26)

Abbreviation: CGH, comparative genome hybridization.

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