p53: functions, mutations and sarcomas

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The p53 gene is the most commonly altered gene in a multitude of human cancers. The alterations can be acquired somatically or transmitted through the germ-line. Bone and soft tissue sarcomas are frequently found to have acquired abnormalities in the p53 and mdm-2 genes. In soft tissue sarcoma, the amplification of the mdm-2 gene and the binding of its oncogene product to wild-type p53 protein functionally inactivates normal p53-regulated growth. Inherited mutations of the p53 gene are associated with the rare Li-Fraumeni familial cancer syndrome. Various tumor types arise in these families, with sarcomas of the bone and soft tissues and carcinoma of the breast being the most frequently observed. Transgenic mice with highly expressed mutated p53 have a higher incidence of tumors, including predominantly osteosarcomas and soft tissue sarcomas. In close similarity with the Li-Fraumeni syndrome, homozygously p53-null mice (transgenic mice carrying two non-functional p53 allele) are developmentally normal however they are susceptible to spontaneous tumor formation. This article reviews briefly the structure, function, and dysfunction of the p53 tumor-suppressor gene with particular focus on its role in the development of bone and soft tissue sarcoma.

The p53 protein was discovered in 1979 as a 53 kDa cellular nuclear phosphoprotein that binds to the large T (transforming) antigen of the SV40 DNA virus (SV40 T antigen) (Lane and Crawford 1979; Linzer and Levine 1979). When initially cloned it was regarded as a dominant oncogene and a member of the same class of nuclear oncogenes as the *myc* oncogene. Murines p53 cDNA transfectants were found to be able to immortalize primary cells *in vitro*, and the *p53* gene could either alone or in cooperation with an activated *ras* gene transform primary rodent cells to tumorigenicity (Jenkins et al. 1984; Parada et al. 1984). However, it is now known that the original clones were mutated forms of p53, and that wild-type p53 actually suppresses transformation (Finlay et al. 1989; Hinds et al. 1989). In the late 1980s, several critical discoveries challenged the concept that wild type p53 acted as a dominant oncogene. When the normal *p53* gene was transfected into RAT-1 cells the tumorigenic potential of *ras* and *myc*, mutated *p53* or adenovirus E1A was decreased (Eliyahu et al. 1989; Finlay et al. 1989). Furthermore, rearrangement of one allele, with loss of other allele, resulted in a functionally eliminated *p53* gene as is often seen in cell lines. Also, structural changes and loss of heterozygosity (LOH) for chromosome 17p are detected frequently in cancers of the colon, lung, cervix, brain, adrenal cortex, bladder as well as in osteosarcomas. Knudson's model of tumor suppressor gene action predicts such allelic loss as indicative of the presence of a target-suppressor gene (Knudson 1989). Therefore, mutant p53 is dominant acting while wild-type p53 has the characteristics of a tumor suppressor gene.

The p53 *gene and structure*

The human *p53* gene is assigned to chromosome 17 at position p13.1 and encompasses a 16–20 kb pair span of DNA (McBride et al. 1986; Miller et al. 1986). The gene itself includes 11 exons, interrupted by 10 introns. The first exon is untranslated, and there is a large 8 kb-10 kb first intron before the coding exons 2 - 11. The gene transcript is spliced to produce a 2.2 - 2.5 kb mRNA that is synthesised in all cells. The thymus, spleen, testis and ovary have the highest expression of *p53* mRNA (Rogel et al. 1985).

The product of the *p53* gene is a 393-amino acid nuclear phosphoprotein of 53 kDa. The *p53* gene is conserved throughout vertebrate evolution (human and mouse sequences show greater than 90% homology). The p53 protein contains an acidic N-terminal transcription activating domain, a central DNA binding domain and a carboxy terminal oligomerization

domain. The five highly conserved regions (domains I–V) are in the centre of the molecule (amino acid residues 13–19, 117–142, 171–181, 234–258, and 270–286) (Soussi et al. 1990). Domains II–V are within exons 4–9 and have been implicated as the most common sites for tumor associated *p53* mutations.

The N-terminal domain lying within residues 1–43, interacts with many of the general transcription factors when associated with DNA through the DNA binding domain (Unger et al. 1992). This domain also binds the eukaryotic single stranded DNA-binding cellular replication protein RP-A (Dutta et al. 1993; Li and Botchan 1993). Intron 1 of the cellular oncogene *murines double minute 2* (*mdm-2*) also binds to p53 in this domain and these interactions may be important in transcriptional regulation (Momand et al. 1992). When bound to *mdm-2*, p53 transcriptional activity is abolished. The gain of function phenotype of some p53 mutant proteins also maps to this amino terminal domain (Lin et al. 1995).

The next set of highly conserved residues at amino acid residues 75–150 is hydrophobic and rich in proline. Most of the mutations reported to occur in human cancers are clustered within this central core region. This region is important in maintaining the overall structure of the protein (Levine et al. 1991) and contains the sequence specific DNA binding domain. The highly charged basic region at the carboxy terminus domain spans amino acid residues 319 - 393 and is important for the protein to form a homologous tetrameric complex (Iwabuchi et al. 1993). The dominant negative property of the p53 protein is mapped genetically to this tetramerization domain. The accumulation of p53 in the nucleus is mediated by the nuclear transport sequence at amino acid residues 316 - 325 (Shaulsky et al. 1990).

An X-ray crystallographic structure of the core domain of p53 bound to DNA has been described (Cho et al. 1994). The core structure shows that p53 has a unique b sandwich structure that acts as a scaffold to support the DNA-binding element. The sandwich consists of two anti-parallel b-sheets containing four to five b-strands respectively sandwiching the DNA. The four conserved domains within the core provide the DNA-binding element that is responsible for contact with the major and minor grooves of the p53-binding site. The structure data suggest that many of the p53 mutations directly affect protein-DNA interactions (Vogelstein and Kinzler 1994).

Interaction of wild-type p53 protein with viral oncoproteins

The wild type p53 protein binds to the transforming proteins of many small DNA tumor viruses. It complexes to the SV40 large T antigen, the 55 kd E1B antigen of adenovirus and the E6 oncoprotein of human papillomavirus type 16 and 18. The binding of the large T antigen and E1B protein to p53 leads to the formation of stable protein complexes that likely inactivate the normal function of p53, while the E6 oncoprotein triggers the degradation of p53. The interaction of these viral oncoproteins with wild-type p53 proteins functionally inactivates the growth-suppressive properties of p53. Interestingly, the adenovirus E1A protein, the SV40 large T antigen, and the oncogenic papillomavirus E7 protein also bind to the retinoblastoma (Rb) gene product, resulting in functional inactivation of the Rb protein as well (review by Chang et al. 1993 and references therein).

Transcriptional target genes

Specific DNA binding and the transcriptional activity of p53 are undoubtedly the major components of its biological effects. It is now known that p53 proteins induce the transcription of several genes that control cell-cycle arrest or apoptosis. A considerable number of transcriptional target genes which are up regulated by p53 have been identified (review by Ko and Prives 1996, and references therein).

mdm-2 itself is a transcriptional target of p53, and may be involved in the autoregulatory feedback loop between p53 and *mdm-2* (Wu et al. 1993). The *mdm-2* gene product forms a stable complex with normal p53 protein and inhibits p53-mediated transactivation (Oliner et al. 1993). The GADD45 gene contains a p53 responsive element in its third intron. p53 protein isolated from irradiated cells binds to this DNA element. GADD45 expression induced in response to DNA damage following irradiation leads to arrest in the G_1 phase of the cell-cycle (Kastan et al. 1992). Activation of p21/WAF1/Cip1/CDKN1 is required in part for p53 mediated G_1 arrest in response to irradiation. However, deletion of the p21 gene does not affect radiation-induced apoptosis. Also, in p53 null mice, p21 is expressed in a variety of tissues suggesting that it can be regulated by a mechanism independent of p53 (Brugarolos et al. 1995).

Other p53 responsive genes with functions that are relevant to the biological functions of p53 are the bax gene, HIC-1 (hypermethylated in cancer), a new zinc finger transcription factor gene, insulin-like growth factor binding protein 3 gene (IGF-BP3) which is induced in cells after DNA damage, thrombospondin-1, muscle creatine kinase gene, and Rb.

Function of wild-type p53

Although several known specific cellular functions of p53 are known, the way it acts in tumorigenesis is still unclear. While the normal p53 protein acts as a transcription factor by binding to specific DNA sequences adjacent to p53 responsive genes, the mutant forms lack this DNA binding activity. The wild-type p53 gene product is expressed at low levels in all normal cells with a rapid turnover rate of less than 10 minutes, whilst transformed cells in culture and in human tumors often have much higher levels of p53 (5–100 fold) due to an increase in half-life to several hours (Reich et al. 1983). The normal function of p53 appears to be as a checkpoint factor, playing an important growth-controlling role in stressed cells. In response to genotoxic stress, p53 induces either cell-cycle arrest or apoptosis. If DNA is damaged in cells with normal p53 protein, p53 accumulates and arrests cells at the G_1-S phase in the cell-cycle to allow time for damaged DNA to be repaired. If repair fails, then p53 can trigger programmed cell death by apoptosis, thus protecting the genome from accumulating mutations (review by Kastan et al. 1995 Lane 1992; Lane 1994a; Lane 1994b; Vogelstein and Kinzler 1992 and references therein). p53 may also function in G_2 arrest as well, by acting as a mitotic checkpoint factor (Cross et al. 1995; Guillouf et al. 1995).

Mutations in *p53*—consequences for tumorigenesis

Eighty-six percent of the mutations in *p53* in human cancers are missense mutations, deletions/insertions account for 8%, 5% are nonsense mutations, and the remaining 1% are silent. The missense mutations reported so far are found throughout the opening reading frame, though most are clustered in exons 5 through 8 with mutational "hot spots" at amino acid residues 175, 248, 273 and 282 (Hollstein et al. 1994).

Mutations of *p53* affect its function in one of three ways. These result in either a dominant negative effect, gain of function or loss of function of the *p53* gene. Some mutant allele have a trans-dominant effect through oligomerization with wild-type p53 thus blocking its ability to bind DNA and activate transcription (dominant-negative effect). Transgenic mice with a dominant-negative *p53* transgene have a higher incidence of tumor formation than non-transgenic mice (Hann and Lane 1995; Harvey et al. 1995).

If LOH results in loss of one wild-type *p53* allele, then there may be a selective pressure to retain the mutant allele. Investigation suggests that some p53 mutants are capable of inducing a gain of function phenotype (Ditter et al. 1993; Lin et al. 1995). Mutant p53 alleles have been shown to preferentially activate the transcription of several genes, for example, that of the multi-drug resistant gene, which is normally not regulated by wild-type p53 (Ditter et al. 1993). The gain of function mutations of *p53* gene have also been reported to enhance metastatic potential, increase tumorigenicity, and activate protein kinase C to induce expression of vascular endothelial growth factor (VEGF) (Kieser et al. 1994).

Missense mutations with concurrent loss of the remaining allele, lead to a loss of wild-type function. Inherited mutations (transmitted through the germline) are associated with Li-Fraumeni familial cancer syndrome (Birch et al. 1994; Malkin et al. 1990; Srivastava et al. 1990). LOH is frequently seen in human cancers, suggesting that the loss of the p53 "guardian of the genome" role (Lane 1992) may contribute to the characteristic genetic instability of tumors.

Recently, a model for the involvement of mutant p53 in tumor progression and evolution has been proposed (Kinzler and Vogelstein 1996). Hypoxia found in those parts of tumors with inadequate blood supply was shown to induce p53-dependent programmed cell death (Graeber et al. 1996). Such hypoxic conditions confer selective survival advantage for cells carrying mutations in *p53* over cells with wild-type *p53*. Angiogenesis, the formation of new blood vessels is required for efficient growth of tumors (Folkman 1992). Inhibitors of angiogenesis are secreted in cells expressing normal wild-type p53. Hence, the clonal expansion of mutant p53 containing cells that are resistant to hypoxia and no longer express anti-angiogenic factors can contribute to tumor growth and progression.

Germ-line mutations of *p53*

Germ-line *p53* mutations in the general population are very rare, however the presence of such mutations confers an extremely high risk of developing cancers characteristic of Li-Fraumeni syndrome in affected individuals. About 50% of Li-Fraumeni syndrome families carry germ-line *p53* mutations in affected members. Germ-line *p53* mutations are also found in cancer-prone individuals in families which are not indicative of the Li-Fraumeni syndrome. The tumor types that arise in these families are quite variable, with breast carcinoma, soft tissue sarcoma, osteosarcoma, brain carcinoma, leukemia and adrenocortical carcinoma being most frequently observed. The age of onset of these tumor types is unusually early. Affected individuals carrying the mutant germ-line *p53* allele have a 50% incidence of cancer by age 30. Most germ-line mutations reported in families with Li-Fraumeni syndrome involve missense point muta-

tions and cluster within the highly conserved coding regions, primarily at codons 245–258 in exon 7 of the *p53* gene. Most of the reported mutations have been unique. No haplotype information yet exists to determine the origin of these mutations (Birch et al. 1994; Eeles 1995; Fraumeni 1996; Law et al. 1991; Li et al. 1992; Li 1996; Malkin et al. 1992; Santibanez-Koref et al. 1991).

Tumor development in Li-Fraumeni familial cancer syndrome is assumed to require *p53* point mutations and the loss of the wild-type allele. Tumor tissues from affected individuals frequently show a loss of the wild-type allele but retain the mutant allele, consistent with the model put forward by Knudson in 1971 for familial retinoblastoma (Knudson 1971; 1993). It is not known whether loss of the wild-type allele takes place as an early or late event in tumorigenesis.

Li-Fraumeni syndrome was first described by Li and Fraumeni in 1969. They described the first anecdotal observation of four families with an autosome dominant pattern of breast cancers in the young women and their offspring and other blood relatives having a diverse form of childhood cancers particularly soft-tissue sarcomas (Fraumeni 1996; Li 1996). The clinical definition of Li-Fraumeni syndrome requires the proband with a sarcoma diagnosed before age 45; a first degree relative with cancer before age 45; and another first or second degree relative with a sarcoma diagnosed at any age or any cancer before age 45 (review by Eeles 1995 and references therein).

p53 *and* mdm-2 *in soft tissue sarcoma and osteosarcoma*

Inactivation of *p53* may be an important step in the development of bone and soft-tissue sarcomas (Mulligan et al. 1990). The evidence supporting this view is as follows: 1) in a high percentage of osteogenic sarcomas, the *p53* gene has been deleted and rearranged resulting in loss of its expression (Miller et al. 1990; Yamaguchi et al. 1992); 2) the development of osteosarcoma and soft tissue sarcomas is common in *p53* transgenic mice (Donehower et al. 1992; Harvey 1993; Lavigueur et al. 1989); 3) the *mdm-2* gene is amplified and overexpressed in about 30% of human soft-tissue sarcomas (Leach et al. 1993; Oliner et al. 1992); 4) bone and soft tissue sarcomas occur as part of the tumor spectrum in the Li-Fraumeni family syndrome, which involves germ-line *p53* mutations (Malkin et al. 1990; Srivastava et al. 1990). Sarcoma in the proband is a prerequisite for Li-Fraumeni syndrome; and 5) evidence that alterations in p53 facilitate sarcoma growth and progression comes from a study in which it was found that metastatic tumors arising from a primary soft tissue sarcoma with one wild-type p53 allele and one allelic deletion, had a mutation in the remaining allele, leading to the production of mutant p53 protein (Pollock et al. 1996).

Conclusions

Over the past several years, genetic, biochemical and physiological studies have brought significant advances in our understanding of the effects of p53 expression in normal cells and tumors. Clinical applications of this knowledge, eg, gene therapy, immunotherapy, strategies to restore normal p53 function, development of drugs that target mutant p53 function, detection of residual disease, predicting prognosis, and early diagnosis are now emerging. The recent discovery of germ-line mutations in Li-Fraumeni familial cancer syndrome and in families with multiple cancers have opened the possibilities of predictive testing in high risk affected individuals. However, many key issues on the risks and benefits of p53 predictive testing remain to be clarified.

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