p53: Regular or super?

Increased p53 expression under the endogenous promoter protects "super p53" mice from tumorigenesis without the undesirable effects of premature aging.

p53, a transcription factor expressed in all cells, is the most frequently genetically altered tumor suppressor in human cancers (Vogelstein et al., 2000). In tumors where p53 itself remains intact, p53 function can be inactivated by genetic alterations of other genes in the p53 pathway, including deletion of the alternative reading frame gene (*Arf*) (Quelle et al., 1995) or overexpression of Mdm2 (Juven-Gershon and Oren, 1999). $p19^{Arf}$ in the mouse ($p14^{ARF}$ in humans), like p53, is a potent tumor suppressor that positively regulates p53 transcriptional activity by binding to and sequestering Mdm2. Mdm2, a transcriptional target of p53, is an E3 ubiquitin ligase that negatively regulates p53 transcriptional activity by binding to it and catalyzing its ubiquitination, thereby targeting it for degradation by the proteasome (Juven-Gershon and Oren, 1999). Mdm2 is frequently found overexpressed in human tumors, leading to the rapid degradation of p53, which is equivalent to loss of p53 function.

p53's transcriptional program protects cells against the destructive effects of DNA-damaging agents, including γ irradiation or chemotherapeutic drugs, or of oncogenic stresses from hyperproliferative signals induced by activated proto-oncogenes, such as Myc or Ras. p53 suppresses tumor growth by halting cell proliferation, a process called premature senescence, or by triggering a cell suicide program, also called apoptosis. However, the genes whose expression is regulated by p53 which control each of these two outcomes have not been completely mapped.

Apoptosis, or programmed cell death, is an active process activated in response to DNA damage. Mutations in the apoptotic pathway, including p53, lead to tumorigenesis and resistance to chemotherapeutic drugs in cell lines and in vivo (Johnstone et al., 2002). Senescence was originally defined as a mechanism that limits the lifespan of fibroblasts by a finite number of divisions. Replicative senescence, the so-called "Hayflick limit," is linked to the progressive shortening of telomeres leading to the physiological process of aging. Mechanisms that protect telomere ends play a role in immortalization and carcinogenesis (Hayflick and Moorhead, 1961). However, a telomere-independent, p53-dependent type of senescence, also called premature senescence, can be triggered by acute cellular stresses that include activated oncogenes, reactive oxygen systems, anticancer agents, and γ irradiation (Serrano et al., 1997). One of the stress-induced forms of senescence, coined "culture shock" by Sherr and Depinho, also involves the induction of the tumor suppressors $p16^{Ink4a}$ and $p19^{Arf}$ as well as p53, but seems to be due mainly to culture conditions that may have no significance in vivo (Sherr and DePinho, 2000).

Therefore, apoptosis and senescence both constitute failsafe mechanisms that force cells to irreversibly exit the cell cycle, and by doing so, prevent cells from unscheduled growth and cancer. p53 and $p19^{Arf}$ have both been implicated in the regulation and control of replicative and premature senescence. Mouse embryo fibroblasts (MEFs) from *p53*-deficient and *Arf* null mice are immortal and fail to enter premature senescence, and mice lacking either of these two tumor suppressors develop a variety of tumors that are refractory to therapy (Sherr and McCormick, 2002). Similarly, human tumors lacking p53 function are resistant to chemotherapy, stimulating a number of groups to attempt to restore p53 activity in tumor cells that harbor a defective p53 protein, to sensitize them to DNA damaging agents. Small molecules that reactivate mutant p53 by restoring its DNA binding activity and transcriptional activity were reported to provide antitumor activity in vivo (Bykov et al., 2002). Tyner and collaborators constitutively expressed p53 in mice and found that p53 restoration indeed decreased the incidence of spontaneous tumors (Tyner et al., 2002) (Table 1). However, unexpectedly, this beneficial effect came at a big price: the induction of premature aging. These results supported the idea that constitutively active p53 was ultimately responsible for premature aging (Ferbeyre and Lowe, 2002).

A research group from the Spanish National Center of Biotechnology in Madrid, led by Dr. Manuel Serrano, now finds that excess levels of the wild-type tumor suppressor p53 protect mice against cancer and aging (Garcia-Cao et al., 2002). These results are surprising and exciting because they offer for the first time a ray of hope that increased levels of p53, expressed from its own endogenous promoter, may protect us against cancer without the undesirable side effects of aging. Garcia-Cao and collaborators report the generation of novel transgenic mice, called "super p53." The super p53 mice express wild-type endogenous p53, and also carry one or two extra copies of a normal p53

Table 1. p53 levels dictate tumorigenesis, aging, or apoptosis

Genotypes	p53 status	Incidence of tumor formation	Premature aging	Apoptosis
Wild-type	+	+	–	+
Null	–	++++	–	–
Transgenic (Tyner et al, 2002)	++++ (under heterologous promoter)	–	++	++
Transgenic (Garcia-Cao et al., 2002)	++ (under endogenous promoter)	–	–	++

(–), cells lacking p53, absence of premature aging, tumor formation or apoptosis; (+), normal p53 levels from endogenous p53 gene, low incidence of tumor formation, and apoptosis; (++), one or two extra copies of p53 under the control of endogenous promoter, premature aging, and apoptosis; (++++), high levels of p53 expression or increased incidence of tumor formation.

gene, inserted as transgenes in the form of large genomic fragments of 130 to 175 kilobases. Because the additional p53 gene is expressed from its own promoter, the transgenically expressed p53 seems to be regulated in the same fashion as endogenous p53. This contrasts with the previous expression of an activated mutant form of p53 from a heterologous promoter reported by Tyner and collaborators. The super p53 mice were, as expected, more sensitive to DNA damage, as higher levels of p53 induce increased apoptosis and senescence, and were significantly protected from chemically induced cancers when compared to normal mice that carry only two alleles of a wild-type p53 gene. However, contrary to the initial prediction from the data reported by Tyner and collaborators, these super p53 mice did not show any signs of aging (Table 1). One of the reasons for the absence of undesirable premature aging effects in the super p53 mice may be that increased levels of the wild-type p53 were regulated from p53's own endogenous promoter. In fact, the same large genomic fragment (or transgene) was also found to restore normal p53 functions when introduced into mice lacking p53, suggesting that the p53 expressed from the transgene was appropriately regulated, and sufficient levels of p53 protein were available to compensate for the lack of endogenous p53. Another reason for the success of super p53 mice in protection from tumorigenesis may be that an increased copy number of p53 may decrease the probability for stochastic mutations. Cells would therefore commit suicide in response to DNA damage, reducing the emergence of tumor cells.

Regardless of the reason for their potential success, the super p53 mice provide hope that protection of cells against cancer might be possible by introducing large fragments of DNA containing a tumor suppressor gene in stem cells before transplantation in patients. Even though there is still a significant gap between our ability to prevent cancer in mice and in humans, this report provides an alternative to the restoration of lost or defective protein functions by retroviral gene transfer. Although in many cases, "less is more," when talking about p53, more appears to be better.

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Selected reading

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