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The p53-deficient mouse: a model for basic and applied cancer studies

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Inactivation of the p53 gene in the germline of mice by gene targeting has provided researchers with a model similar in many respects to the analogous human inherited cancer predisposition Li-Fraumeni syndrome. The viability of p53 null mice has allowed unexpected opportunities to study the role of p53 in many different in-vivo and in-vitro contexts. Null (p53–/–) mice have an average time to tumor development of 4.5 months, while half of the heterozygous (p53 + /–) mice develop tumors by 18 months. The p53-deficient mice have been particularly valuable in examining the effects of p53 loss on tumor progression. In addition, the mice hold significant promise as tools to assess carcinogens, teratogens, chemopreventative agents, and cancer therapeutic regimens.

Key words: gene targeting / knockout / Li-Fraumeni syndrome / mouse / p53 / tumor model / tumor suppressor

THE RECENT DEVELOPMENT of gene targeting in embryonic stem cells has provided researchers with a powerful tool to inactivate genes in the germ line of the mouse.[1,2] This approach has been particularly useful in the targeting of tumor suppressor genes, whose inactivation in germ cells and somatic cells of humans is a recurring theme in human cancer development. Currently, the most intensively studied tumor suppressor gene is the p53 gene. Close to half of all human cancers have p53 mutations, indicating that loss of its function is a critical event in tumor progression.[3] Given the attention paid to p53, it was not surprising that the p53-deficient mouse was the first reported mouse with a tumor suppressor gene knockout.[4] Since the first report of this mouse in 1992, scores of papers have been published which utilize p53-deficient mice or cells derived from them. The aim of this short review will be to describe the characteristics of the p53-deficient mice as well as to outline some of the conceptual insights derived from experiments using them. Finally, I will summarize some of the cancer and non-cancer-related applications for which these mice may be useful.

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The role of p53 in cancer prevention

In 1989, the p53 gene was conclusively demonstrated to be a tumor suppressor gene,[5,6] though mutant forms of the gene can exhibit oncogenic activity, perhaps due to the ability of mutant p53 to complex with wild type p53 and functionally inactivate it.[7,8] Cellular p53 levels are greatly increased by radiation and other DNA damaging agents and this increase in p53 is accompanied by an arrest in late G1 of the cell cycle.[9,10] Wild type p53 can also mediate apoptosis in some contexts when overexpressed.[11] It was hypothesized that a critical role for wild type p53 is to respond to various forms of DNA damage by either arresting the cell to allow adequate DNA repair or to kill the cell via apoptosis.[12] In either case, the effect would be to prevent propagation of DNA-damage induced mutations, some of which were likely to be oncogenic. In the absence of p53, apoptotic and cell cycle arrest functions would be lost and genomic instability and cell survival would be increased, thereby accelerating oncogenesis.

Such a model was consistent with the very high percentage of spontaneously arising human tumors which exhibited mutation and loss of p53. A wide variety of human tumor types incur p53 loss, sometimes at late stages of tumor progression and sometimes at earlier stages.[3] In addition, germ line p53 mutations have been identified in affected individuals of Li-Fraumeni syndrome families.[13,14] Li-Fraumeni syndrome is a familial cancer predisposition whose affected members have a 50% likelihood of developing cancer by age 30 in contrast to a 1% incidence by age 30 in the general population. The frequent association of p53 mutation with both spontaneous and inherited human tumors provided a powerful

impetus to develop a representative animal model for p53-associated cancers.

Generation of p53-deficient mice

At least five different groups have reported the development of p53-deficient or 'knockout' mice.[4,15-18] In four of the studies,[4,15-17] similar gene targeting approaches were utilized, with the primary differences relating to how much of the endogenous p53 gene was deleted during the targeting process. Since the different targeted mice showed remarkably similar phenotypes, it is likely that all have p53 null alleles. This was confirmed by the apparent absence of intact p53 protein in cells derived from p53 null animals in three of the models.[4,15,17] All targeting approaches resulted in germ line p53 null mice which were developmentally viable, yet susceptible to early spontaneous tumors. As will be seen, the ability to generate apparently normal p53 null animals has provided a number of opportunities to study various cellular processes in the complete absence of wild type p53.

Developmental abnormalities in p53 null mice

We reported that the crossing of the heterozygous p53-deficient mice yielded 23% null offspring, roughly in line with the expected Mendelian ratio of 25%.[4] However, Jacks *et al*[15] reported 16.6% null offspring following heterozygous crosses, suggesting the possibility that a fraction of null animals may have died during embryogenesis. In fact, subsequent studies by Sah *et al*,[19] and Armstrong *et al*[20] have shown that a fraction of female null embryos die during embryogenesis due to a neural tube closure defect called exencephaly. In these embryos there was an overgrowth of neural tissue, usually confined to the region of the forebrain and midbrain, which precluded normal formation of the cranium and resulting in protrusion of the brain above the skull.[19] Sah *et al*[19] reported that 8–16% of total p53–/– midgestation embryos exhibited this exencephalic phenotype, and differences in frequency were apparently strain-dependent. Armstrong *et al*[20] reported that 23% of female p53–/– embryos displayed exencephaly in an outbred genetic background. We have also observed exencephaly in our p53–/– mice though at levels below that reported by Sah *et al* and Armstrong *et al* (roughly 5% of p53–/– embryos) (M. Harvey and L. Donehower, unpublished data). This exencephaly is rarely observed in null males or in p53+/– and p53+/+ littermates of the p53–/– exencephalic embryos. These results suggest either that p53 does play a role in development, particularly during neural tube closure, or that increased mutation rates possibly associated with chromosomol events may occur more frequently in the p53 null gametes and embryos.[20]

Interestingly, treatment of males prior to mating with whole body ionizing radiation resulted in a much increased incidence of exencephaly in p53–/– female embryos.[20] We have shown that reduction of folic acid in the diet of mating females increases the incidence of exencephaly in the p53–/– embryos (A. Sands, L. Donehower and A. Bradley, unpublished data), suggesting that the environmental impact of DNA damaging agents or altered diet may interact with the genetics of these animals to affect the incidence of developmental abnormalities.

Aside from these developmental abnormalities, we have observed that fertility is reduced in the p53–/– mice, particularly the females (L. Donehower, unpublished data). Null females have significantly smaller litter sizes than their p53+/– and p53+/+ littermates. These reduced litter sizes are not only due to exencephaly in the embryos, since even p53–/– females bearing only p53+/– embryos have reduced litter sizes. In addition, the pregnant females sometimes fail to deliver their full term embryos and subsequently sicken and die (L. Donehower, unpublished observations).

Tumor susceptibility

Tumor susceptibility in p53-deficient mice is greatly enhanced. In our colony, all p53–/– mice develop tumors by 10 months of age (mean tumor time is 4.5 months), while p53+/– mice have a comparative delay in the appearance of tumors.[4,21,22] Roughly 50% of heterozygous mice develop tumors by 18 months (Figure 1),[21,22] with over 90% incidence by two years of age (L. Donehower, unpublished observations). Wild type littermate controls of the p53-deficient mice do not develop tumors before 18 months and less than 25% succumb to tumors by the age of two years (L. Donehower, unpublished data). Interestingly, while tumors in null mice have been observed as early as six weeks of age, the heterozygotes have a relatively long tumor-free period. Less than 8% of p53+/– animals develop tumors before nine months of age.[22] The tumor incidences reported by the Jacks[15] and

Clarke[16] groups are very similar to ours, though survival times for the Clarke null mice are even shorter (all p53–/– mice die by six months of age),[16] probably due to the fact that their mice were of a different genetic background (129/Ola) than the Jacks mice and our mice (mixed C57BL/6 and 129/Sv).

The types of tumors observed in the p53-deficient mice are quite varied, though certain types tend to predominate (Figure 2). For example, the p53–/– mice have a very high incidence of lymphomas, usually of the T-cell type.[4,15,16,21,22] Soft tissue sarcomas and osteosarcomas appear at a lower frequency. Malignant carcinomas are rarely observed in the null mice. In contrast, the heterozygous mice develop osteosarcomas, soft tissue sarcomas, and lymphomas in roughly equal proportion (Figure 2).[15,21,22] Carcinomas and other types of tumors remain rare, though less so than in the null mice.

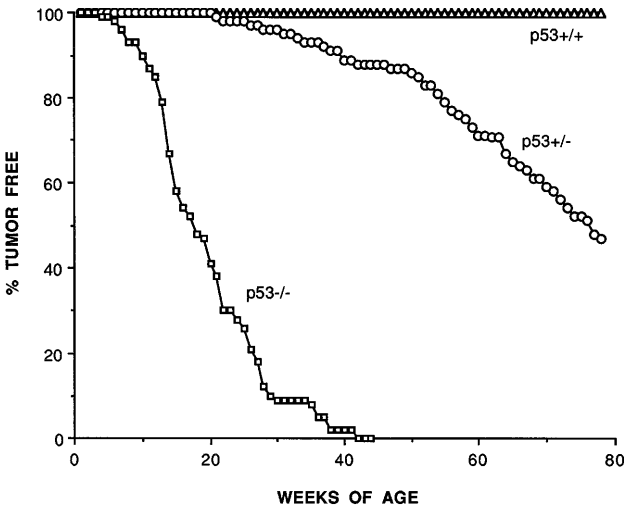

Figure 1. Tumor incidence in p53-deficient mice (p53–/– and p53+/–) and wild type (p53+/+) mice of mixed inbred C57BL/6 × 129/Sv genetic background. 72 p53–/–, 150 p53+/–, and 91 p53+/+ mice were monitored for tumor formation. Tumor-free survival is plotted up to 78 weeks (18 months) for each group.

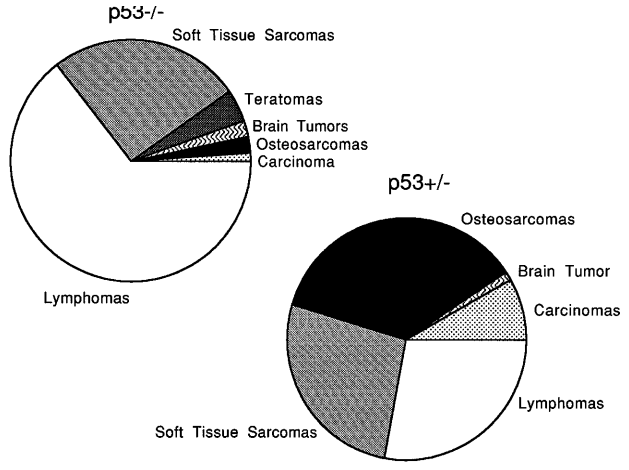

Figure 2. Tumor spectra of p53–/– and p53+/– mice. Relative frequency of each major tumor type is indicated by the size of the pie slice.

The issue of genetic background modulating tumor incidence and spectrum in the p53-deficient mice has been addressed by us in a preliminary way.[22,23] Our first studies were done with mice with an average of 75% C57BL/6 and 25% 129/Sv genetic background. We have also placed the null p53 allele into a pure 129/Sv background and have found some interesting differences in tumor susceptibility between mice with different genetic backgrounds. The 129/Sv p53+/– and p53–/– mice develop tumors significantly sooner than their counterparts of mixed background. Moreover, about half of the 129/Sv p53–/– males develop testicular teratomas, a tumor type infrequently observed in the C57BL/6 × 129/Sv null mice. Interestingly, the 129 strains of mice have previously been shown to be predisposed to testicular tumors, though at a much more modest rate than that observed in the p53–/– males.[24] The absence of p53 in the 129/Sv mice clearly exacerbates this prior cancer predisposition.

Given the tumor incidences and spectra cited above, one important issue is whether the p53-deficient mice generated thus far are a reasonable model for the Li-Fraumeni syndrome. The similarities in some respects between p53+/– mice and affected Li-Fraumeni families are quite striking (Table 1). For example, many of the tumor types frequently observed in Li-Fraumeni families (e.g. osteosarcomas, soft tissue sarcomas, leukemias, and lymphomas) are also observed in p53+/– mice. An exception is breast cancer, frequently seen in Li-Fraumeni females but rarely in p53+/– mice. In addition, the tumor incidence is roughly comparable on a lifespan basis, with 50% of Li-Fraumeni individuals developing cancer by age 30, while 50% of p53+/– mice become cancerous by age 18 months, about half their normal lifespan.[13,21,22] The p53 genetic lesions (deletions) introduced into the mice do not resemble the point mutations usually observed in humans, but a fraction of the human mutations do introduce frameshifts or premature truncations which would prevent expression of a full length protein[25,26] and these mutations

Table 1. Comparison of Li-Fraumeni syndrome families and p53+/– mice

Characteristic	Li-Fraumeni syndrome	p53+/– mice
p53 Germ line lesion	Point mutations. Primarily missense Also premature stop codons and frameshifts leading to truncated protein	Deletions leading to no protein or severely truncated protein
Tumor incidence	50% by age 30 90% by age 70	50% by 18 months 90% by 24 months (average mouse life span is 30–36 months)
Common tumors	Breast cancer Osteosarcomas Soft tissue sarcomas Brain tumors Leukemia/lymphomas	Mammary tumors (rare) Osteosarcomas Soft tissue sarcomas Brain tumors (rare) Lymphomas
Loss of heterozygosity in tumor?	Roughly half lose wild type allele*	Roughly half lose wild type allele

*Jenny Varley, personal communication.

would have results functionally equivalent to the knockout allele in mice. Thus, while the p53 + /– mice cannot be said to perfectly mimic the cognate human syndrome, they have a number of similarities which should make them useful as a cancer predisposition model.

Genetic interactions and tumorigenic co-operativity

In an attempt to assess the cooperativity of the p53 gene with other cancer-associated genes, we and others have crossed the p53-deficient mice to other strains of tumor-susceptible transgenic and knockout mice. In such crosses, if the offspring containing both parental oncogenic alleles develop cancer faster than either tumor-susceptible parent, then cooperativity is said to have occurred. Presumably, the identification of cooperating oncogenes and tumor suppressor genes which synergize with p53 loss can provide important new mechanistic insights into p53-associated tumorigenesis pathways. Table 2 describes the results from some representative crosses performed to date. Note that a number of the crosses do result in accelerated tumorigenesis in the bitransgenic offspring compared to either parent. For example, mice deficient in both Rb and p53 not only show accelerated tumorigenesis, but also exhibit novel tumor types (e.g. pancreatic islet cell carcinomas and pineaoblastomas) not seen in the parental singly deficient mice.[62, 63]

In another cross between p53-deficient mice and mutant p53 transgenic mice (carrying a codon 135 point mutation which is oncogenic),[27] we were able to show that mice inheriting the mutant p53 transgene showed accelerated tumorigenesis over their nontransgenic counterparts, but only in the presence of one or both wild type p53 alleles.[28] Null p53 transgenic mice showed no increased tumor incidence compared to p53 null nontransgenic mice, suggesting that the primary role of this mutant p53 was to accelerate tumorigenesis through inactivation of endogenous wild type p53.

As a final example of the unexpected insights which can be obtained through such crosses, mdm2 null mice generated by us[29] and Montes de Oca Luna *et al*[30] were found to result in early embryonic lethality. Surprisingly, when mice heterozygous for both mdm-2 and p53 are crossed, viable and normal double null mdm2/p53 offspring result, suggesting that the lethality characteristic of mdm2 null embryos can be rescued in the absence of p53.[29,30] This result led us to hypothesize that the primary role of mdm2 in development is to interact with p53 and inactivate it to allow proper cell cycle progression at critical points in embryogenesis.

Insights into tumor progression

Our understanding of how p53 loss contributes to tumor initiation and progression remains incomplete. However, in the last few years, the use of p53-deficient mice has significantly complemented the insights gained from human tumor studies. We and others have characterized the p53-deficient mouse tumors in an attempt to identify relevant biological mechanisms and molecular pathways affected by p53 loss. A number of exciting and novel approaches have been taken with these mice and some of these will be described.

Table 2. Oncogenic cooperativity between p53-deficiency and other genes *in vivo*

Cooperating gene	Nature of transgene	Site of expression	Tumor type(s)	Synergy with p53–/–?*	Synergy with p53+/–?*	Novel tumors?†	Ref
Rb	Knockout	Global	Pituitary adenomas	Yes	Yes	Yes Pancreatic, pinealo-blastomas	62,63
Truncated large T antigen	Transgene	Choroid plexus	Choroid plexus tumors	Yes	Yes	No	39
E7	Transgene	Eye	Retinal tumors	Yes	No	No	38
Mutant p53	Transgene	Global	Lymphomas Lung tumors Osteosarcomas	No	Yes	No	28
myc (1)	Transgene	Mammary gland	Mammary tumors	ND	No	No	64
myc (2)	Transgene	T-cells	Lymphomas	Yes	Yes	No	65
bcl-2	Transgene	B-cells	Lymphomas	No	ND	No	66
mdm-2	Knockout	Global	None	No	In progress	No	29,30 S. Jones (unpublished)
SCID	Natural mutation	Lymphoid cells	Lymphomas	Yes	ND	No	67,68
APC	Chemically induced mutation	Global	Intestinal tumors	No	No	Yes Pancreatic	69
Wnt-1	Transgene	Mammary gland	Mammary tumors	Yes	No	No	40
NF-1	Knockout	Global	Pheochromo-cytomas Myeloid leukemia		Yes	Yes Rhabdomyo-sarcomas	T. Jacks (personal comm.)

*Indicates that bitransgenic offspring develop tumors sooner on average than either transgenic parent.
†Indicates that bitransgenic offspring develop tumor types not seen in either transgenic parent.

One initial question has been with regard to the fate of the remaining wild type p53 allele in the p53+/– tumors. According to Knudson's 'two hit' hypothesis, inactivation of both relevant tumor suppressor alleles is generally considered to be an important prerequisite for tumor formation.[31] However, in our laboratory, Southern blot hybridization has revealed that roughly half of the p53+/– tumors retain their wild type allele while the other half delete the wild type allele. We have found that tumors which appear to retain the wild type p53 allele (i) appear at a later time (on average), (ii) have five-fold less genomic instability, (iii) retain wild type sequence and expression levels, and (iv) exhibit much higher levels of apoptosis in response to ionizing radiation than p53+/– tumors which lose their wild type allele (L. Donehower, A. Bradley and D. Pinkel, manuscript in preparation). Such results raise the possibility that mere reduction of p53 gene dosage is sufficient to predispose the animal to accelerated cancer formation. Loss of the second allele may be optional rather than necessary, but if it does occur may accelerate tumor progression. This result contrasts with those observed for other tumor suppressors such as the retinoblastoma gene. Rb+/– mice and Rb+/– humans invariably show loss of both alleles in their tumors.[32-34]

While the analysis of tumorigenesis in the p53-deficient mice has yielded a number of interesting insights, the study of spontaneous tumor formation in these animals has certain limitations if one wants to examine mechanistic questions in an efficient, well controlled manner. For example, the p53-deficient mice develop a wide array of tumors with a relatively long latency (particularly the p53+/– mice). Moreover, since p53+/+ mice rarely develop tumors, control tumors which develop in a p53-independent manner are difficult to obtain. To circumvent these disadvantages, investigators have taken two general approaches, either treating the p53-deficient mice with a tissue-specific carcinogen or crossing the p53-deficient mice to a tumor-susceptible transgenic mouse genetically programmed to develop a single tumor type. The resulting models usually develop a single tumor type in a relatively short amount of time and control p53+/+ tumors are available to compare

to the p53-deficient tumors. Representative examples of these approaches and the novel insights obtained from them will be discussed below.

The first carcinogen-based study on the p53-deficient mice was performed by Kemp *et al*[35] in a classic two-stage skin carcinogenesis assay. They found that DMBA initiation and TPA promotion accelerated carcinoma formation in the absence of p53. Not only did the carcinogen-induced p53–/– skin carcinomas form earlier, but they showed higher indices of malignancy as measured by histopathology, confirming the importance of p53 loss in acceleration of tumor progression. In another interesting skin-related study, Ziegler *et al*[36] showed that UV-induced sunburn cells in humans often had p53 mutations. They exposed the skin of p53 null and wild type animals to UV irradiation and found that UV light induced more sunburn cells in wild type animals than p53–/– animals. These sunburn cells were associated with increased apoptosis, suggesting that loss of p53 provides a survival advantage to UV damaged cells, clones of which may ultimately develop to skin carcinomas.

Several models utilizing p53-deficient mice have now shown the critical importance of p53-mediated apoptosis in tumor progression.[37-39] In one typical study, Symonds *et al*[39] crossed transgenic mice expressing a fragment of T antigen which inactivates Rb (but not p53) with the p53-deficient mice. In the presence of p53 the offspring developed slow growing choroid plexus tumors. In its absence, the choroid plexus tumors appeared much more rapidly. Most interesting was the rapid outgrowth of tumor nodules in the truncated T antigen mice that were p53+/–. These tumor nodules were found to have lost their remaining wild type p53 and also displayed much lower levels of apoptosis than the surrounding slow growing regions of the tumor with intact p53. Thus, loss of p53 was correlated with attenuated apoptosis and accelerated tumor growth, supporting the idea that loss of p53-mediated apoptosis is the rate limiting step in tumor progression.

Is apoptotic function the primary mechanism by which p53 regulates tumor progression or are there other mechanisms? Other models employing p53-deficient mice suggest that p53 loss may promote tumor progression through non-apoptotic means. One such model is the *Wnt-1* transgenic/p53-deficient model developed by us.[40] These mice, because of ectopic expression of the *Wnt-1* oncogene in the mammary gland develop early onset mammary adenocarcinomas.[41] In the absence of the p53 the mammary tumors developed sooner and grew more rapidly than tumors which had wild type p53. However, apoptosis in the preneoplastic mammary glands and mammary tumors was invariably low and not appreciably different between both $p53^+$ and $p53^-$ tissues and tumors (T. Jacks and J. Jones, unpublished data). However, cell proliferation in the tumors missing p53 was consistently higher than in tumors containing wild type p53 (J. Jones and L. Donehower, manuscript in preparation). In addition, tumors without p53 showed significantly higher levels of genomic instability.[40] Thus, increased cell proliferation rates (perhaps driven in part by increased genomic instability) may provide an alternative mechanism by which p53 loss may promote accelerated growth. The above results suggest that the roles of p53 in cancer prevention may be relatively complex and utilize multiple pathways. Further studies of these and other models are certainly warranted.

Cells derived from p53-deficient mice

Another useful feature of the p53-deficient mice is that primary cells and established cell lines can be readily derived from their tissues. Prior to the availability of these mice, cells missing p53 were often derived from immortalized lines or tumors and during this lengthy process they were likely to have acquired other genetic lesions which could complicate the interpretation of experimental results. Primary cells obtained from p53 null mice would presumably differ from wild type cells only in the absence of p53. Aside from their experimental usefulness, p53-deficient cells have been found to have their own intrinsically interesting properties. We have found in short-term culture assays that early passage p53 null embryo fibroblasts (i) divide more rapidly, (ii) have a shorter average cell cycle time, and (iii) grow more readily in unfavorable conditions.[42] Under long term culture conditions, p53–/– embryo fibroblasts (i) more consistently and rapidly immortalize, (ii) spontaneously transform more often, and (iii) show dramatically higher levels of genomic instability than wild type embryo fibroblasts.[17,42-44] Others have shown that primary cells from p53–/– animals show lack of G1 and G2/M checkpoint control,[9,10,44-51] increased capacity to amplify genes in the presence of cell cycle inhibitors,[44,45] increased levels of centrosome instability compared to their wild type counterparts,[49] and decreased ability to undergo apoptosis following exposure to DNA damaging

Table 3. Comparative effects of genotoxic carcinogens on p53-deficient mice

Agent	Target	Tumor type	Accelerated tumors in p53-deficient mice?	Ref
DMBA / TPA	Skin	Skin carcinoma	Yes (p53–/–), no (p53+/–)*	35
X rays	Global	Lymphoma	Yes (p53–/–), yes (p53+/–)	70
Dimethyl nitrosamine	Liver	Liver hemangiosarcoma	Yes (p53+/–)	21
p-Cresidine	Bladder, liver	Bladder carcinoma	Yes (p53+/–)	53
Benzene	Multiple sites	Histiocytic sarcomas	Yes (p53+/–)	J. French (personal comm.)
4-Vinyl-1-cyclohexene diepoxide	Skin, ovary	Skin carcinomas	Yes (p53+/–)	J. French (personal comm.)
N-Methyl-N-nitrosourea	Lymphoid	Lymphoma	Yes (p53+/–)	S. Hursting (personal comm.)
Diethyl nitrosamine	Liver	Liver adenoma	No (p53+/–)	71
DMBA	Mammary gland	Mammary adenocarcinoma	No (p53+/–)	72
NNK	Lung	Lung adenoma	No (p53+/–)	73

*Papilloma number was three-fold greater in p53+/– mice compared to p53+/+ mice.

agents,[50,51] oncogene-induced cell proliferation[52,53] and hypoxia.[54] Cell types normally refractile to significant growth in culture, such as neonatal astrocytes, have also been shown not only to grow quite successfully in the absence of p53, but to spontaneously immortalize and transform in a stepwise fashion after continued culturing.[55] Thus, the potential to develop in vitro transformation models from virtually any tissue of the null p53 mouse is a particularly exciting prospect.

Other applications for p53-deficient mice

Aside from basic studies into the mechanisms of cancer formation and p53 function, it soon became clear that the p53-deficient mice might be suitable for other applications. One such application is as a more sensitive rodent model for testing known or suspected carcinogens.[56] The high expense and long time-frame of current rodent bioassays are prohibitive for many companies developing new pharmaceuticals. Theoretically, the p53-deficient mice may be more sensitive to carcinogens than normal mice. Thus, rodent bioassays for carcinogens might require fewer animals and less time to perform. Currently, the p53+/– are envisioned as useful animals for testing primarily because they have a low incidence of tumors until nine months of age, thus allowing sufficient time for carcinogen testing without background spontaneous cancers. In addition, the p53-deficient mice may be preferable to other transgenic models in that they mimic a known human cancer predisposition syndrome and develop tumors in a variety of tissues.

Investigators have treated the p53-deficient mice with various physical and chemical carcinogens and compared tumor formation in these mice with wild type control mice (Table 3). In most instances treatment of p53+/– with carcinogens resulted in significant accelerated tumorigenesis over that seen in carcinogen-treated normal mice and untreated p53+/– mice. In three studies acceleration of tumor formation in carcinogen-treated p53+/– animals did not occur, but two of these studies only assessed early stage tumor formation (adenomas). The p53-deficient mice may be more likely to accelerate tumorigenesis in carcinogen models which develop late stage malignant tumors. Several groups have now undertaken multiple carcinogen studies to determine the efficacy and sensitivity of the p53+/– mice in carcinogen assessment (J. French and R. Tice, personal communications).

Another interesting application of the p53-deficient mice has been in cancer prevention studies. The first study of this type was performed by Hursting *et al*[57] who subjected p53 null mice to caloric restriction. Caloric restriction has historically been an inhibitor of tumors in several species. The calorie restricted p53–/– mice demonstrated significantly longer tumor-free survival time (25 weeks median) than control p53–/– mice not restricted in their caloric intake (16 weeks median). In a follow-up study, Hursting and colleagues[58] have shown that the treatment of p53–/– with the chemopreventative

agent dehydroepiandrosterone (DHEA) significantly delayed tumor formation compared to untreated p53–/– mice.

Finally, the susceptibility of p53-deficient embryos to teratogenic agents has been investigated by at least four groups.[20,59-61] Nicol *et al*[59] demonstrated that treatment of p53-deficient embryos with the environmental teratogen benzo[a]pyrene resulted in 2–4 fold higher levels of teratogenicity than similarly treated p53+/+ embryos. The authors argued that p53, in addition to its tumor suppressor function, may also serve as a teratological suppressor gene. More support for this idea has come from a study by Norimura *et al*[60] who showed that the incidence of malformations in radiation-treated p53–/– embryos was 70% compared with 20% malformations in radiated p53+/+ embryos. Interestingly, the percentage of in-utero deaths was actually much higher in the p53+/+ embryos, suggesting that the presence of p53 may in some instances induce abortion in order to prevent malformations. However, a study by Wubah *et al*[61] demonstrated dramatically *decreased* 2-chloro-2'-deoxyadenosine-induced eye defects in the absence of p53, suggesting that in some instances teratogens may cause malformations directly through p53-mediated apoptosis. The potential of the p53-deficient mouse as a model for the testing of suspected teratogens should clearly be explored further.

Future prospects

The p53-deficient mice have been useful for a variety of basic and applied research purposes. Both the mice and cells derived from their various tissues should provide valuable tools in the future for new insights into the role of p53 in cell cycle checkpoint control, apoptosis, DNA damage response and cancer prevention. In addition, new p53 mouse models (e.g. mice with germ line p53 point mutations or tissue specific p53 gene inactivations) should further supplement the first generation knockout models. The application of the p53-deficient mice in testing of teratogens, carcinogens, cancer preventative agents and cancer therapeutic agents should occur with increasing frequency as the recognition of their usefulness spreads.

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