

## Review

# Mouse models with modified p53 sequences to study cancer and ageing

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## Abstract

Experiments with p53 transgenic and p53 gene-targeted mouse strains have substantiated, and in some cases challenged, a number of hypotheses on the biology of the p53 protein. New questions have emerged regarding similarities and differences between murine and human genetic networks in various tissues. Mouse models with targeted p53 alleles are now applied not only to investigate tumour susceptibility, but also to address questions pertinent to molecular epidemiology, chemoprevention, development of anticancer p53-specific pharmaceuticals, and ageing.

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**Keywords:** transgenic; p53; Cre-Lox; ageing; neoplasia; murine

**Abbreviations:** DMBA, dimethyl-benzanthracene; TPA, 12-O-tetradecanoylphorbol-13-acetate; Apc, adenomatous polyposis coli; DBD, DNA binding domain; Hupki, human p53 knock-in

## Introduction

In contrast to several other genes known to be of central importance in human cancers, for example, the ras oncogenes, or the BRCA1 and 2 tumour suppressor genes, mutant p53 alleles resulting in the absence or dysfunction of p53 protein arise both in somatic cells during sporadic human tumorigenesis and in the germline (Li-Fraumeni cancer syndrome). Modelling of these two human conditions experimentally involves two distinct aims: one in which expression of mutations is delayed and confined to certain tissues to parallel sporadic cancer, and one in which the mutation is expressed in the germline. Different approaches (transgenic, knockout, knockin, conditional gene-targeting) have been used or are being considered, providing an illuminating exercise in stepwise improvements in mouse models of human cancer. The conditional gene-targeting field is still comparatively young, and conditional missense p53 mutant mouse strains are not available yet. Mice with conditionally targeted

missense p53 mutations would model, for example, the typical mutation events induced by tobacco exposure in the development of human bronchial carcinomas of smokers.<sup>1</sup> It is of particular interest to generate variants that express protein from the targeted allele (rather than to introduce deletion or stop mutants that produce no protein) because, unlike tumour mutations in other tumour suppressor genes, most human tumour p53 mutations are missense mutations in the DNA binding domain (DBD) rather than stop mutations,<sup>2,3</sup> resulting in the accumulation of a defective protein rather than absence of p53. Furthermore, many overexpressed mutants acquire new functions that are deleterious to genome and tissue homeostasis, thus are not biologically equivalent to p53 null mutations.<sup>4,5</sup>

In the meantime, mutant p53 transgenes have permitted a first examination of the biological effects of aberrant p53 protein in mice and the phenotypic differences between null and missense mutants.

## P53 transgenic mice encoding mutant p53 protein (Table 1)

Injection of DNA fragments encompassing mutant p53 gene sequences into mouse oocytes to produce a transgenic strain in which the randomly inserted exogenous sequences are stably transmitted through the germline has been relatively straightforward, permitting an initial look at the effects of mutant p53 protein on tumour susceptibility. Alan Bernstein and co-workers<sup>6</sup> were the first to show that mice with a V135A transgene were tumour prone: spontaneously arising adenocarcinomas of the lung, osteosarcomas and lymphomas appeared on average at 11 months of age. In these strains, the transgene is present in multiple copies and overexpresses mutant murine p53 protein. The model has the appealing feature that transgene expression remains under control of endogenous murine p53 promoter sequences originally present in the injected DNA fragment. By breeding these mice with p53 gene-targeted heterozygous and homozygous mice that express no p53 protein from the mutant alleles (p53 null alleles), investigators later showed that the expressed transgene has a dominant-negative character, and predisposes to lung cancer only if the wild-type allele is also present. Susceptibility to lymphomas and sarcomas, the most common tumours in p53 null mice, remains high in mice deficient in endogenous p53, whether or not the mutant transgene is present.<sup>7</sup> Present conjecture from a variety of experimental observations is that different p53 mutations will have different effects on overall tumour susceptibility and on spectrum, and will thus fall into different phenotypic classes that may or may not in a consistent fashion correspond to a given biochemical/physicochemical class of p53 mutant protein (reviewed

**Table 1** Mice with Transgenic p53 Sequences

Designation	Promoter	Transgene tissue expression	Coding sequence (cgs)	Species of cds	cgs mutation	Tumour-prone phenotype ?	Tumour type	Notes	Reference
pL53	Mouse p53	Ubiquitous	p53 gene (all exons) (genome-derived, >10 kb)	Mouse	Codon A135V	Yes <sup>a</sup>	LADC, O, L	p53 transgene in tandem copies	Laviguer <i>et al.</i> <sup>6</sup>
[TrP53/172L]	WAP	Mammary gland	p53 gene, exon 2–exon 11 (genome-derived)	Mouse	Codon R172L	No <sup>a</sup>		hum 175	Li <i>et al.</i> <sup>9</sup>
[TrP53/172H]	WAP	Mammary gland	p53 gene, exon 2–exon 11 (genome-derived)	Mouse	Codon R172H	Yes <sup>b</sup>	M	hum 175	Li <i>et al.</i> <sup>10</sup>
HK1.p53m	Keratin 1	Epidermis	p53 cds	Mouse	Codon R172H	Yes <sup>b</sup>	Sp, Sca	hum 175	Wang <i>et al.</i> <sup>12</sup>
AT3-p53	Anti-thrombin III (human)	Liver: p53 wt overexpression	5'cgs and genomic p53 intron 2–intron 11	Mouse	Wild type	No <sup>a</sup>			Gillet <i>et al.</i> <sup>78</sup>

Abbreviations: WAP=whey acid protein; wt=wild type; LADC=lung adenocarcinoma; O=osteosarcoma; L=lymphoma; M=mammary tumour; Sp; Sca=skin papilloma, carcinoma<sup>a</sup>Spontaneous tumours<sup>b</sup>Chemically induced tumours.

recently by Bullock and Fersht).<sup>8</sup> The human mutant analogous to murine A135V (V138A) is not found often in human cancers<sup>3</sup>, in contrast to a more recently generated p53 missense mutation transgenic that harbours a base substitution at codon 172, equivalent (by sequence alignment) to human codon 175, which is the most common site of base substitutions in sporadic human cancers. In this strain, the mutant p53 transgene was put under control of whey acid protein regulatory sequences, allowing for high expression in mammary tissue. Two codon 172 mutant strains were generated, one replacing the 172<sup>Arg</sup> residue with leucine, and one in which histidine is substituted.<sup>9,10</sup> In human cancers, mutants with R175H are exceptionally common, whereas R175L is rare. While neither strain was susceptible to spontaneous tumours, the mutant strain with the common mutation decreased latency and increased multiplicity of tumours induced by DMBA<sup>10</sup> and other carcinogenic agents.<sup>11</sup> Comparable findings were reported with a transgenic model harbouring the same p53 mutation (R172H), but in which expression is under control of the keratin 1 promoter, directing high expression to the epidermis. Again, the mice do not reveal a tumour-prone phenotype until challenged with carcinogen. Transgenic mice treated topically with DMBA and TPA had a greater burden of skin papillomas, and of highly malignant carcinomas in particular than nontransgenic mice.<sup>12</sup>

As with transgenics modelling of other diseases, however, the p53 strains shown in Table 1 are, at best, approximate as genetic parallels. The exogenous DNA fragment integrates as a multiple copy concatemer, the integration site is not controlled and can affect phenotype, and in most p53 transgenics the coding sequence is under the control of a strong heterologous promoter, overriding endogenous cellular controls of gene transcript levels. Use of a heterologous, tissue-specific promoter to achieve temporally or spatially

restricted expression of transgenic constructs is nevertheless a rapid and accessible technique, and has provided the first tools to examine the tumorigenic role of mutant p53 in specific tissues.

A major quandary in evaluating the tumour suppressor role of p53 in mouse models stems from rapid progression and lethality of the tumour type with the shortest latency, which can preclude assessment of susceptibility to other malignancies, a difficulty that strains with tissue-restricted expression of modified p53 can circumvent. A second problem, one that plagues comparisons of tumour susceptibility among the p53-modified strains by whatever means they are generated, is the influence of genetic background on tumorigenesis.<sup>13</sup>

## P53 mice generated by gene-targeting technology (Table 2)

### Knockout mice

Constitutive inactivation of p53 through gene targeting has allowed the *in vivo* analysis of loss of p53 activity, and has circumvented some of the difficulties associated with conventional transgenic approaches. P53 null mice have been generated by several different groups, and, despite subtle differences in the targeting strategies adopted, all strains exhibit very similar phenotypes.<sup>14–16</sup> Perhaps, the first major surprise from these analyses was that p53 was largely dispensable for normal embryonic development, with all the deficient strains being initially reported as viable. A closer look, however, has made it clear that p53 does play a role in normal embryonic development. Both Armstrong *et al.*<sup>17</sup> and Sah *et al.*<sup>18</sup> have reported significant *in vivo* female lethality, which is heavily influenced by background such that on a 129 background almost all female embryos develop neural tube defects and die *in utero*. The precise reason underlying this

**Table 2** Mouse Strains With Modified p53 Alleles

	Publication year	allele designation <sup>a</sup>	Ct, Cn, Ch	Novel sequence:	Effects	Tumour prone	Primary reference
(A) Knockout strains	1992	p53 null	Ct	(Neomycin); deletion in p53 exon 5	No p53 protein	Yes (L,S)	Donehower <i>et al.</i> <sup>15</sup>
	1993	(p53-) p53 null	Ct	(Neomycin); deletion of p53 exons 2-6	No p53 protein	Yes (L)	Clarke <i>et al.</i> <sup>14</sup>
	1994	(p53-) p53 null	Ct	(Neomycin); deletion of p53 exons 2-6	No p53 protein	Yes	Jacks <i>et al.</i> <sup>24</sup>
	2001	(p53-) Trp53F2-10	Cn	Deletion of p53 exons 2-10 in epithelial cells	No p53 protein	(Yes)	Jonkers <i>et al.</i> <sup>60</sup>
	2002	p53+/m	Ct	Deletion of p53 exons 1-6, and mutation at c.245	Nontargeted p53 is overexpressed	Tumour resistant; accelerated ageing	Marino <i>et al.</i> <sup>59</sup> Tyner <i>et al.</i> <sup>61</sup>
(B) Knockin strains	2000	Trp53QS	Ct	Mutation at c.25,26 in TA domain	Loss of trans-activation by p53	(Yes)	Jimenez <i>et al.</i> <sup>65</sup>
	2000	p53R172H	Ct	Mutation at c.172, and at intron splice site	Low mutant protein expression	Yes (L,C)	Liu <i>et al.</i> <sup>64</sup>
	2001	Hupki (p53KI)	Ct	Human wild-type sequences from introns 3 to 9	Functional chimeric hum/mus p53	(No)	Luo <i>et al.</i> <sup>67</sup>
	2001	p53+/R270H p53+/P275S	Ch	Missense mutation at c.270 Missense mutation at c.275	Reduced apoptosis	NG	De Vries <i>et al.</i> <sup>66</sup>

Abbreviations: Ct: constitutional; Cn: conditional; Ch: chimeric mice (germline transmission not reported) c., codon; cds, coding sequence; hum, human; mus, mouse; TA, transactivation domain; NG, not given;<sup>a</sup>As given by authors

phenotype remains somewhat unclear, although a role for p53 in suppressing radiation-induced teratogenesis has been demonstrated suggesting that p53 may play a role as 'guardian' of the embryo through the deletion of aberrant cells.<sup>19,20</sup> An even clearer demonstration of the relevance of p53 to normal development comes from analyses of mice bearing inactivating mutations of the p53 regulators Mdm2 and Mdm4. These proteins both regulate p53 activity, either by targeting p53 for degradation or by specifically inhibiting p53 transcriptional activity. Deficiency of both of these genes results in early embryonic lethality, nonoverlapping phenotypes both rescued by the absence of p53.<sup>21,22</sup> This role for p53 is further underlined by the partial rescue of the lethal phenotype associated with Brca1 mutation, normally characterized by decreased cellular proliferation and reduced levels of Mdm2.<sup>23</sup>

It rapidly became evident that p53-deficient mice were strongly predisposed to neoplasia, with spontaneous development of tumours being widely reported,<sup>15,24,25</sup> although the spectrum of lesions differed from that predicted by the Li-Fraumeni syndrome with a notable absence of tumours of epithelial origin. As might have been predicted, challenge of these mice by radiation or carcinogen (e.g. dimethylnitrosamine) accelerated neoplasia development.<sup>26,27</sup> However, apparently paradoxical results were obtained from some systems, highlighting an emerging complex reliance upon p53 in tumour suppression. For example, in a well-characterised model of skin tumorigenesis, p53 deficiency was shown to protect against formation of the initial lesion (papilloma),

although greatly accelerated progression to malignancy.<sup>28</sup> Similar results were obtained from other models of skin tumorigenesis, reliant upon the keratin K1 promoter to drive expression of either v-rasHa, v-fos or TGF alpha to the epidermis. Here again p53 deficiency failed to simply predispose to neoplasia.<sup>29</sup> These studies suggest that, in certain systems, p53 deficiency may actually cause the deletion of cells bearing excessive DNA damage by failing to permit normal attempts at repair.

While some studies have yielded apparently paradoxical results, the majority of intercrosses have underpinned our understanding of p53 as a potent tumour suppressor. For example, in direct contrast to the epithelial model, overexpression of TGF alpha in the pancreas leads to ductal tumorigenesis, which is dramatically accelerated by the absence of p53.<sup>30</sup> Similar synergy in pancreatic acinar cell neoplasia has been demonstrated between p53 deficiency and the Min mutation of the Apc (adenomatous polyposis coli) gene.<sup>31</sup> Indeed, p53-deficient mice are now frequently viewed as a baseline upon which to test other potential tumour predisposing events, with the result that the list of genetic changes for which *in vivo* synergy can be shown with p53 is extensive, ranging from overexpression to misexpression to gene deficiency. For example, excess E2F1 has been shown to lead to increased skin carcinoma in the absence of p53;<sup>32</sup> misexpression of casein kinase II leads to accelerated lymphoma development;<sup>33</sup> and combined inactivation of genes (such as the decorin gene) can reveal tumour predisposition otherwise undetectable.<sup>34</sup> In very occasional

instances this type of analysis has failed to clearly determine interactions with p53. For example, apparently contradictory results have been generated for PARP-1 deficiency, whereby inactivation of PARP-1 has been shown to both increase<sup>35</sup> or decrease tumour latency<sup>36</sup> in the absence of p53. Such diametrically opposed results must reflect stark differences in the gene dependency within specific cell systems, and strongly warn against drawing broad, global conclusions concerning the consequences of p53 loss.

PARP-1 plays a role in the immediate response to DNA damage, and analysis such as those described above naturally arose from the pursuit of mechanisms by which p53 mediates tumour suppression. The use of p53 null alleles has proven particularly potent here, with roles now demonstrated in mediating damage signals, modulating transcription, genomic stability, cell cycle arrest, and the induction of cell death. A series of intercrosses have also begun to address where p53 lies within the damage response hierarchy. For example, deficiency of the recombinational repair protein rad51 results in embryonic lethality, which can at least be partially rescued by p53 deficiency.<sup>37</sup> Similarly, neoplasia is dramatically accelerated in mice deficient in both p53 and the damage sensor protein atm, indicating clear synergy in tumour suppression here, although also a degree of redundancy to other responses such as the apoptotic response to ionising radiation.<sup>38</sup> One point that should be noted here is the possibility that p53 deficiency may be able to 'rescue' some of the DNA damage mutants that would normally invoke p53-dependent death, so raising a note of caution about the interpretation of these crosses.

The null mice have given significant insights into the mechanics of tumour suppression, for example, acceleration genomic instability in many systems such as mammary tumorigenesis driven by Wnt-1 transgene expression.<sup>39</sup> One of the most significant discoveries has been the requirement for functional p53 to mediate apoptosis following DNA damage.<sup>14,16,40</sup> This gave rise to the notion of p53 as 'guardian' as it would normally function to delete cells (through apoptosis) bearing excess DNA damage. This hypothesis yields several predictions for a p53 null environment: there should be increased clonogenic survival following DNA damage, and surviving clones would be characterised by higher levels of mutation and a greater predisposition to neoplasia. This hypothesis also predicts that p53 status will be a critical determinant of clinical outcome, where apoptosis is an important mediator of chemosensitivity.

Many of these outcomes have been tested directly using the null mice, with extremely varied results. Thus, although p53 may be shown to confer increased clonogenic survival in many *in vitro* assays,<sup>41</sup> this has been difficult to establish *in vivo* with ionising radiation. Thus, despite clear loss of the apoptotic programme, p53 deficiency has relatively little effect upon clonogenic survival in the small intestine as measured by the microcolony assay.<sup>42,43</sup> Part of the reason for this appears to be that survival of the crypt following ionising radiation is primarily defined by survival of the endothelial cells, rather than of the enterocytes.<sup>44</sup> This explanation fits well with more recent observations using agents other than ionising radiation (cisplatin), where increased clonogenic

survival can be observed in this system in the absence of p53.<sup>45</sup>

Even more surprisingly, it has been extremely difficult to show that p53 deficiency confers an increased mutation burden. Studies scoring spontaneous mutation in either a lacZ transgene or at the endogenous *Dib-1* locus failed to show increased mutation frequencies in a range of tissues.<sup>46,47</sup> Even following exposure to the powerful mutagen ENU, p53 status made little impact upon mutation frequency.<sup>48</sup> It is however clear that within certain systems, p53 deficiency will confer increased mutation burden, as has been reported in pre-B cells.<sup>41</sup> Significantly, the authors of this study also showed that this increase probably derived from increased clonogenic survival.

Despite these differences, it is clear that the p53 status can be a determinant of outcome after anticancer therapy *in vivo*<sup>63</sup>; and further, that the ability to engage apoptosis can be an important determinant of neoplasia (reviewed in Johnstone *et al.*<sup>49</sup>). This has been shown many times; for example, by the direct demonstration that p53 can induce apoptosis and suppress tumorigenesis in an SV40 T antigen fragment model;<sup>50</sup> or by the observation that p53 deficiency abrogates apoptosis mediated by hypoxia, so promoting survival within tumour masses.<sup>51</sup> Perhaps some of the best examples derive from studies of p53-dependent, c-Myc-induced apoptosis.<sup>52</sup> The importance of c-Myc-driven death to neoplasia was recently elegantly underlined by Pelengaris *et al.*,<sup>53</sup> who used an inducible c-myc transgene to show that tumorigenesis driven by c-myc is absolutely dependent upon loss or suppression of the apoptotic programme.

In essence, these studies have demonstrated that p53 status and the ability to engage apoptosis can be critical to both carcinogenesis and neoplastic progression. However, the relations are not simplistic and often system dependent. This makes generalisations very difficult or impossible to draw. Nowhere is this more evident than in the ongoing debate as to whether p53 status can be used as a good prognostic indicator of clinical outcome.

Although homozygous p53 null animals have yielded a mass of information, mice characterised by germline p53 absence (p53<sup>-/-</sup>) remain relatively limited as genetic models of human disease, particularly since a second somatic mutation of p53 usually occurs during tumorigenesis. Heterozygous (p53<sup>+/-</sup>) mice should therefore be a more appropriate model, and it is therefore encouraging that high incidence of mammary neoplasia has now been reported in p53 heterozygotes when on a BALB/c background.<sup>54</sup> One question that has persisted throughout these analyses is that of p53 gene dose dependency. Although it has been shown repeatedly that when one allele is mutated, the second hit at the remaining wild type usually follows, and reveals latent phenotype. It is becoming increasingly clear, however, that reduction to one functional p53 allele already confers a phenotype. Thus, cellular responses to DNA damage such as gene induction can be shown to be p53 dose-dependent.<sup>55</sup> Perhaps, the clearest indication of a heterozygous phenotype stems from the observation that a proportion of tumours arising in p53 (p53<sup>+/-</sup>) heterozygotes retain p53 functionality.<sup>56</sup> More recently, we have shown that heterozygosity for p53 can accelerate microsatellite instability in a mismatch

repair-deficient context.<sup>57</sup> It seems likely that the consequences of such dose-dependent effects are currently being overlooked simply because reduction in p53 activity is difficult to score and therefore usually not addressed in the majority of studies, whether in humans or in the mouse.

There are two further potentially serious caveats to the p53 null mice. First, their very high predisposition to lymphoma effectively limits their lifespan and precludes studies of tumorigenesis characterised by long latencies. Second, because the null mice develop entirely in the absence of p53 there is a strong possibility that developmental compensation has occurred such that absence of p53 in the null mouse poorly models somatic loss of p53. One approach to the first of these problems has been to generate multiply mutant strains, as discussed briefly above. A second approach has been to use transplanted material, for example, by repopulating cleared mammary fat pads of wild-type mice with p53 null mammary epithelium.<sup>58</sup> Even this strategy, however, cannot deal with the issue of developmental compensation. A more satisfying genetic approach to both issues has been the creation of conditional p53 alleles through use of the Cre-LoxP system. Essentially, this system permits tissue-specific inactivation of any gene of choice by Cre-recombinase-mediated recombination between two 34 bp loci or LoxP sequences. Insertion of the LoxP sites, usually into intronic sequences, is predicted not to perturb expression. However, following exposure to Cre recombinase, the region flanked by the LoxP sites is deleted. This strategy is usually designed to mediate gene inactivation. In the case of p53, LoxP sites were placed in introns 1 and 10, and Cre expression delivered by conventional transgenesis to either the granular layer cells of the cerebellum using the GFAP promoter,<sup>59</sup> or to the mammary epithelium using the keratin 14 promoter.<sup>60</sup> Both of these experiments resulted in tissue-specific inactivation of p53, which accelerated neoplasia in each tissue in conjunction with Cre-mediated loss of either retinoblastoma or Brca2. These examples have established the potential of this approach for analysing p53 function in any tissue, dependent upon Cre delivery. It is notable that, as yet, this strategy has not been used to address the issue of developmental compensation.

One somewhat unconventional p53 mutant was reported earlier this year.<sup>61</sup> This strain was generated as a consequence of an aberrant targeting event originally designed to introduce a mutation at codon 245 (Arg245Trp). Although the codon 245 mutation was introduced, exons 1–6 of p53 were deleted, together with an undefined upstream region extending at least 20 kb. Somewhat surprisingly, this mutant allele, which can produce a truncated C-terminal p53 protein fragment *in vitro*, confers a novel phenotype, possibly by augmenting stabilisation of wild-type p53. Mice carrying one copy of this mutant allele (p53<sup>+m</sup>) show multiple signs of premature ageing and have a greatly reduced lifespan (the phenotype of the homozygous mutants has not yet been reported). The authors also argue that the mutant allele suppresses neoplasia as fibroblasts derived from the p53<sup>+m</sup> mice show resistance to transformation and substantially reduced tumour incidence at the time of death when compared to their p53<sup>+/+</sup> and p53<sup>+/-</sup> counterparts. Although this latter phenomenon may simply reflect greatly truncated

lifespan, the observation of p53-associated early senescence is clearly of importance, with data from murine embryonic fibroblasts also supporting the idea that the ability to induce senescence is partly dependent upon p53.<sup>62</sup> The notion that cellular senescence truly occurs *in vivo* is still somewhat controversial, as indeed is the potential of senescence programmes to modify the response to chemotherapy. However, Schmitt *et al.*<sup>63</sup> have recently used p53 heterozygous mice to study the response to chemotherapy and show, in a series of experiments, both p53 and p16<sup>INK4</sup> can mediate *in vivo* senescence, or at the very least multiple cellular changes consistent with senescence.

### Knockin mice

The p53 knockout models have been of unquestionable value in revealing the importance of p53 loss of function in tumorigenesis; however, we are still faced with the fact that the great majority of human cancers, rather than showing an absence of p53 protein and function, overexpresses mutant p53 protein that has lost wild-type function and has gained new tumour-promoting properties. The human tumour missense mutations map, with rare exception, to the DBD of the gene. DBD mutant strains would provide a tool to explore the particular biological properties of the different mutant p53 proteins found in sporadic tumours. Knockin strains with p53 DBD missense mutations also would represent appropriately the inherited Li-Fraumeni p53 variants, because the mutations most frequently detected in sporadic cancers are also the most common mutations in Li-Fraumeni families. Up to the present, efforts to generate mice with germline expression of a missense mutation equivalent to a common Li-Fraumeni inherited mutation either have been unsuccessful, or have produced unexpected, spontaneously modified p53 alleles during the gene-targeting procedure.<sup>61,64</sup> Given the human experience, this is somewhat puzzling at first glance because affected individuals in Li-Fraumeni cancer syndrome families with one wild type and one mutant p53 allele transmit the mutant allele to their progeny, and germline transmission in mice of transgenes overexpressing mutant p53 has been successful also (Table 1, and the section on p53 transgenic mice encoding mutant p53 protein). Difficulties in producing an Li-Fraumeni mutant mouse model are thought to lie in the deleterious effects of an expressed gain-of function mutation on embryonic stem cells during *in vitro* manipulation. The only germline targeted p53 DBD mutant mouse reported thus far harbours not only a common tumour missense mutation (R172H), but also a fortuitous splice site mutation that severely compromises expression of the mutant allele,<sup>64</sup> which may explain why this mouse with constitutive expression of this missense mutation could be generated. Various protocol modifications are now at hand to avoid pitfalls in generating mice with germline transmission of p53 DBD missense mutations.

Whereas modelling of DBD mutants in mice with gene-targeting approaches presents special challenges, production of mutant strains with knockin missense mutations in other domains of the gene (e.g. that regulate activity of the encoded protein) has been relatively unproblematic and the mutant mice have brought clarity to a plethora of data from somewhat

contradictory *in vitro* experiments. A central question in p53 tumour biology has been whether the transcriptional transactivation function of p53 is essential for tumour suppression and p53-mediated apoptosis. Wahl and co-workers<sup>65</sup> generated a mouse harbouring missense mutations in the transactivation domain of the endogenous murine p53 gene to address this issue. The mutant protein, which bears two adjacent amino-acid substitutions at residues 25 and 26, while still able to bind to p53 response elements in DNA, is deficient in transactivation, compromises p53-dependent apoptosis in thymocytes *in vivo*, and renders embryonic fibroblasts tumorigenic. The homozygous mice are tumour prone. These *in vivo* data argue convincingly that transactivation by p53 is crucial to its tumour suppressor function, and outweigh contradictory inferences from *in vitro* experimental systems. Not surprisingly, evidence both from analysis of human tumour samples and from experimental work in cell lines indicates that most, if not all, DBD missense mutations are defective in apoptosis induction and are unable to activate transcription of downstream genes, because binding and contact with DNA at promoter response elements is faulty or absent. Thymocytes harvested from chimeric mice harbouring either of the two common mutations in exon 8 of p53 are also deficient in p53-dependent apoptosis.<sup>66</sup>

In mouse strains with altered p53 gene sequences described in the literature thus far the modified p53 sequences are murine-derived, with one exception. In the Hupki (for human p53 knockin) model, we replaced the murine p53 sequences encoding the polyproline and DNA-binding domains with homologous sequences from the human genome, and we designed the strain to retain rather than compromise p53 wild-type function, in contrast to other p53-targeted strains.<sup>67</sup> Homozygous Hupki mice indeed develop normally and show wild-type p53 responses to DNA-damaging agents.<sup>67,68</sup> Nevertheless, differences exist between the human and mouse p53 genetic network, and human p53 does not behave identically to murine p53 in mouse cells.<sup>69,70</sup> An in-depth comparison of p53 function in Hupki mice with mice harbouring unmodified murine p53 (in particular, by molecular profiling with high density arrays to examine transcriptional responses) should contribute to an evaluation of strain and species differences in this context.

There are several fields of application for the prototype Hupki humanized p53 strain and derivatives: (i) Molecular epidemiology: a mutation spectrum, whether in the mouse or the human p53 gene, can provide important information on the nature of the exogenous and endogenous mutagenic factors that gave rise to the mutations. The p53 core domains of mice and humans differ at 15% of base residues and the mutation spectrum is dependent on exact DNA base context, so spectrum comparisons become more difficult between species. (ii) *In vivo* testing of drugs targeting human p53 protein for pharmacological rescue of apoptotic function: the amino-acid sequences of mouse and human p53 core domains differ at 10% of residues, and homology is lower for the polyproline and apoptosis regulatory domains. The p53 protein is affected significantly by single amino-acid substitutions,<sup>2</sup> and single amino-acid intragenic suppressors of p53 mutants have been discovered.<sup>71,72</sup> The Hupki strain offers an opportunity to test new compounds in an *in vivo* model presenting the human

core domain, or core domain mutant, as target. (iii) Regulation of p53 activity: phosphorylation of Ser46 is important for the induction of p53-dependent apoptosis in human cells.<sup>73–76</sup> Murine p53 lacks a serine at or near this location. In Hupki mice, the human sequence-derived residues of the chimeric protein include Ser46, and phosphorylation occurs in Hupki mouse cells in response to DNA damage (J-L Luo and M Hollstein, unpublished observations).

## Implications and new directions

The availability of the p53 mutant strains reviewed here has greatly accelerated our understanding of the biological roles played by p53. These strains, however, have also shown this biology to be exceedingly complex and often highly context dependent. Thus, although it is very clear that loss of p53 can impact upon a plethora of pathways relevant to neoplasia, the precise importance of each of these pathways remains relatively unclear. Similarly, we do not yet truly understand the biological ramifications of subtle mutation to p53, such as altered phosphorylation. These are difficulties that we can now address either by exploiting existing strains or by the creation of new variants. Effectively, we can now mutate the p53 gene in any way we choose to; furthermore, we can upregulate or inactivate any of these mutants both temporally and spatially. Some of these mutant strains, such as the conditional p53 allele and the Hupki mouse already exist; others remain to be established.

The questions we can resolve with these strains should be manifold. Most pertinently, we will be able to determine the consequences of specific mutations in specified tissues, so building much better models of human tumorigenesis. We also should be able to address the therapeutic consequences of reactivating potentially therapeutic genes within these mutant contexts, using technology similar to that recently demonstrated for c-myc.<sup>53</sup> Allied to such a test of genetic modification of tumour predisposition, these p53 mutants are also available for the screening of chemopreventative agents, an area that until now has been relatively little pursued. Finally, we should be aware that p53 does not function in isolation, and that now, through the use of expression profiling,<sup>77</sup> we have an outstanding opportunity to examine the genetic networks within which p53 functions.

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