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The role of p53 in tumour suppression: lessons from mouse models

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Abstract. The use of mouse models has greatly contributed to our understanding of the role of p53 in tumour suppression. Mice homozygous for a deletion in the p53 gene develop tumours at high frequency, providing essential evidence for the importance of p53 as a tumour suppressor. Additionally, crossing these knockout mice or transgenic expression p53 dominant negative alleles with other tumour-prone mouse strains has allowed the effect of p53 loss on tumour development to be examined further. In a variety of mouse models, absence of p53 facilitates tumorigenesis, thus providing a means to study how the lack of p53 enhances tumour development and to define genetic pathways of p53 action. Depending on the particular model system, loss of p53 either results in deregulated cell-cylce entry or aberrant apoptosis (programmed cell death), confirming results found in cell culture systems and providing insight into in vitro function of p53. Finally, as p53 null mice rapidly develop tumours, they are useful for evaluating agents for either chemopreventative or therapeutic activities.

Key words. p53; mouse model; tumour suppressor gene; cancer; apoptosis; cell-cycle arrest.

Introduction

It is widely accepted that the *p53* gene plays an important role in the suppression of tumorigenesis. p53 was originally discovered, however, by virtue of being overexpressed in SV40 T antigen-expressing cell lines, and was believed for a number of years to play a role in cellular transformation [1]. This idea was supported by the isolation of the gene and the subsequent demonstration that it could cooperate with known oncogenes to transform cells. Eventually, however, it was realized that the complementary DNA (cDNA) isolated and tested in those experiments contained a point mutation. Upon isolation of the wild-type gene it was shown that p53 actually suppressed cell proliferation in culture, suggesting that it was a negative regulator of proliferation. The previously observed dominant transforming activity of p53 was then attributed to the ability of mutant p53 to act in a dominant negative fashion.

Genetic evidence bolstered the idea that p53 plays a critical role in growth suppression. The first such evidence came from the analysis of colon carcinomas, over 80% of which contained mutant *p53* alleles [2]. This finding was followed by a flurry of others demonstrating mutations in *p53* in a variety of cancers. In fact, *p53* mutations have been identified in at least 52 types of cancer, and it is estimated that over 50% of all human tumours have *p53* mutations [3, 4]. p53 is a transcription factor, and mutations are commonly found in one of four conserved regions residing in the central, DNA-binding portion of the protein [5]. These mutations effectively abolish both the DNA-binding and transcriptional activation function of p53. Typically, muta-

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tions in one allele of the *p53* gene are accompanied by loss of the other allele, a situation termed 'loss of heterozygosity' or LOH, which results in cells lacking p53 function [6]. The idea that p53 plays a critical role in tumour suppression in humans was further supported by the discovery that Li-Fraumeni patients, who develop a spectrum of tumours at an early age, inherit one defective copy of *p53* [7]. LOH is observed in the tumours of these patients, supporting the idea that p53 inactivation is an important step in tumorigenesis. While these studies identified a striking correlation between *p53* mutation and cancer, a clear cause and effect relationship between p53 inactivation and tumorigenesis was most firmly established from studies in mice. The study of p53 using mouse models has greatly contributed to our understanding of p53 as a tumour suppressor, as will be described in this review.

Initial studies of p53 in the mouse

Analysis of Friend leukaemia virus-induced erythroleukaemia provided a first hint that p53 might be involved in tumour suppression in the mouse. The initial clue came from the analysis of cell lines derived from spleens of virally infected mice, some of which expressed either no p53 protein or a smaller 44-kDa derivative [8]. Correspondingly, Southern blot analysis indicated that the *p53* locus was rearranged in most cell lines with altered p53 protein. Subsequent analysis showed that many of the cell lines with apparently normal *p53* genomic structure had sustained point mutations in parts of the *p53* gene critical for function [9]. The analysis of different cell lines from the same mouse showed the same *p53* rearrangements, indicating that these mutations were selected for during tumorigenesis in vivo rather than in cell culture. p53 inactivation thus appeared to be a central step in leukaemiagenesis induced by Friend virus.

The first attempts to use mouse models to probe p53 function directly entailed generating transgenic mice carrying genomic fragments expressing either the *p53* Pro193 or Val135 mutant alleles [10, 11]. In both transgenic mouse models, mutant p53 protein expression was high in spleen, lymph nodes, thymus and ovaries, with intermediate levels of expression in lung and testes. Both strains of mice developed mostly lung adenocarcinomas, osteosarcomas or lymphoma, at an incidence of approximately 20–30% by 18 months of age [10, 12]. These experiments demonstrated that expression of mutant p53, presumably acting as a dominant negative by hetero-oligomerizing with the wild-type protein and thereby blocking its function, could lead to tumorigenesis. As illustrated by the failure of these mice to develop ovarian tumours, however, high levels of mutant p53 protein expression were not always associated with tumour formation, indicating that only some tissues are sensitive to p53 inhibition.

Generation of *p53* knockout mice

***p53−/−* mice.** Unequivocal evidence that p53 plays a critical role in tumour suppression came from experiments in which gene-targeting technology was used to inactivate the *p53* gene in the mouse. The first reported *p53* knockout mouse strain was one in which parts of intron 4 and exon 5 of the *p53* gene were replaced with a neo cassette, such that p53 conserved region II, and consequently the DNA-binding domain, was disrupted [13]. Surprisingly, when these knockout mice were bred to homozygosity, they were found to be viable, contrary to expectations that the mutation would be lethal due to the presumed role of p53 as an essential regulator of the cell cycle. These mice, whose genetic background was 75% C57BL/6 and 25% 129/Sv, did, however, rapidly develop tumours at a high frequency. Seventy-four percent of the *p53* null mice developed tumours by 6 months of age, and all mice developed tumours or died by 10 months, with an average tumour latency of 4.5 months [13, 14]. The most common lesion, occurring in $>70\%$ of the cases, was lymphoma, either restricted to the thymus, or 'generalized', involving the thymus and other organs. Thymic lymphoma was observed in the majority of cases and was of T-cell origin, while the generalized lymphoma was usually of B-cell origin [15]. Haemangiosarcomas, testicular tumours, undifferentiated sarcomas, osteosarcomas and occasional carcinomas (i.e., mammary) or nervous system tumours were also detected [14]. These data clearly demonstrated that p53 is a critical tumour suppressor in multiple tissues of the mouse.

Importantly, similar results were observed with distinct *p53* germ line mutations generated by other groups. For example, Jacks et al. created a mutation that deleted the region between *p53* exons 2 and 6, equivalent to approximately 40% of the coding sequence [16]. In these mice, also of 75% C57BL/6, 25% 129/Sv genetic background, 71% of the homozygous mutants developed thymic lymphoma (mostly CD4, CD8 double positive), some developed various types of sarcoma (osteosarcoma, rhabdomyosarcoma, fibrosarcoma, anaplastic sarcoma, haemangiosarcoma) and some developed teratoma. Nearly all the homozygous mutants died by 6 months, and all were dead by 9 months. Analysis of an independently constructed exon 2–6 deletion mutant mouse in the 129/

Ola background that enhances tumorigenesis and predisposes to certain tumour types.

The phenotypes of the *p53* null mice provided several important take-home lessons. First, p53 may be one of the few real tumour suppressors, in the sense that it appears dispensable for normal development and general cell-cycle regulation, but is essential for suppressing tumours in multiple tissues. Secondly, loss of p53 via a deletion in the *p53* gene is sufficient to lead to a tumorigenic phenotype, indicating that it is not necessary to have a point-mutated allele acting either as a dominant negative or a dominant oncogene. Finally, both the lag time before the *p53* null animals develop tumours as well as the fact that only a small portion of the null cells progress to oncogenic transformation indicate that other genetic events are critical for the tumorigenesis in these mice.

***p53*+/− mice.** The phenotype of the *p53* heterozygous mice was also of great interest, as these mice potentially represented a model for the human Li-Fraumeni syndrome. The heterozygous mice developed a slightly different spectrum of tumours from the null mice, and did so with a longer latency time. While no clear phenotype was observed before 9 months, 50% of the mice had developed tumours by 18 months [13, 14]. 58% of the heterozygotes had osteosarcomas and soft-tissue sarcomas (haemangiosarcomas, rhabdomyosarcomas, undifferentiated sarcomas), 32% had lymphomas and 10% had carcinomas [14]. The majority of tumours in the heterozygotes [55%] showed LOH, suggesting that loss of function of p53 facilitates tumorigenesis. However, other forms of p53 inactivation, like mutations, were not ruled out in the other cases [14]. Similarly, Jacks et al. reported no tumour development before 9 months, but observed tumours in 28% of the heterozygotes by 17 months, with the majority showing LOH [16]. Sarcomas were the most commonly observed tumour type (osteosarcomas, haemangiosarcoma, anaplastic sarcomas, lyomyosarcomas, fibrosarcomas, rhabdomyosarcomas), occurring in 57% of cases, while lymphoma was the second most common, occurring in 25% of cases. In contrast, 10% of the *p53*+/− mice on the 129/Ola mouse background developed tumours by 9 months [17]. Heterozygotes most commonly developed soft-tissue sarcomas, as well as thymic lymphoma and a few rare cases of diverse types of adenocarcinomas, with approximately 50% of tumours exhibiting LOH. The fact that in all these studies some heterozygotes did not lose the wild-type *p53* allele during tumour formation may indicate that the reduced p53 dosage that occurs in the heterozygous state is sufficient to promote tumorigenesis.

The difference in tumour spectrum between the homozygous and heterozygous mutants is interesting, and is likely to relate to any of several factors, such as the rate of LOH, the developmental window of opportunity and/or the target cell size observed in different tissues in the heterozygotes. While the *p53* null animals lack p53 completely in the thymus, the heterozygotes must lose the wild-type *p53* allele in the course of thymic lymphoma development. Because there is a decrease in the number of immature thymocytes several weeks after birth due to thymic involution [16], there is a limited developmental time window for LOH to occur in this tissue. This reduced target cell population might explain the decreased frequency of lymphomagenesis observed in heterozygotes relative to null animals.

The spectrum of tumours observed in the heterozygous mice suggests that this might indeed be a suitable mouse model for the human Li-Fraumeni syndrome. Li-Fraumeni patients, who inherit one defective *p53* allele, are highly predisposed to developing a variety of tumours, including osteosarcomas, soft-tissue sarcomas, leukaemia, breast carcinomas and brain tumours [7]. The *p53* heterozygous mice also develop sarcomas and lymphomas at high frequency. Moreover, in both mice and humans half of the heterozygotes develop cancer by approximately midlife: 50% of *p53* heterozygotes develop tumours by 18 months of age, while half of Li-Fraumeni patients develop cancer by age 30 [18]. One difference, though, is that the breast and brain tumours frequently observed in Li-Fraumeni patients are rarely seen in the mice.

Influence of genetic background on tumorigenesis. It has been proposed that *p53* mutation might act by accelerating a natural tumour predisposition dictated by the background strain [13]. For example, C57BL/6 mice have a tendency to develop lymphoma, and perhaps loss of p53 simply accelerates this process. To test this hypothesis, the *p53* mutation was bred onto the 129/Sv background, which is not prone to lymphoma but is prone to testicular tumours [19]. Overall, tumorigenesis was accelerated in mice of a pure 129/Sv genetic background compared with mice of a mixed genetic background [15, 19]. The 129/Sv *p53* null animals still developed lymphoma, though, and the latency and characteristics of these tumours were similar to those on the mixed background. However, incidence of testicular tumorigenesis was increased in the homozygous mutant mice of the 129/Sv background. It appeared from these findings that the incidence of lymphoma was a consequence of losing p53 function in particular, while in the case of the testicular tumours, it is likely that loss of p53 was accentuating an inherent predisposition in 129/Sv mice [19]. This finding suggests further that there is some

genetic modifier locus either in the C57BL/6 strain or in the 129/Sv strain which affects the sensitivity to testicular cancer. In the future, crossing the *p53*-deficient mice into other genetic backgrounds might reveal additional strain-specific effects that could provide further mechanistic insight into p53 function.

Genetics of p53 action. It is clear that the complete absence of p53 function is sufficient to promote tumorigenesis in mice. However, in human tumours, *p53* is usually mutated by missense mutation, which has led to the suggestion that point-mutated p53 might actively promote tumorigenesis, possibly by displaying new functions [20]. Alternatively, point mutations in p53 could act to ablate p53 function by simultaneously resulting in inactivation of the protein produced by that allele as well as inhibiting the wild-type protein produced by the other allele via a dominant negative action. An elegant experiment to test whether the p53Val135 point mutant exerts its effect in vivo by acting as a dominant negative or as a dominant oncogene was performed by Harvey et al. [12]. The p53Val135 transgenic mice were crossed to the *p53*-deficient mice to generate mice that were transgenic and either wild-type, heterozygous or homozygous mutant for endogenous *p53*. The presence of the transgene accelerated tumorigenesis in both wild-type and *p53* heterozygous mice but failed to do so in the *p53* null mice. This finding suggests that the p53Val135 mutant can only exert its tumorigenic effect when wild-type p53 is present, and that it acts as a dominant negative protein rather than displaying any obvious gain-of-function activity. The p53Val135 protein was unable to produce a complete dominant negative effect, as *p53*+/− animals with the transgene survived longer than *p53* null animals. Consistent with this idea was the finding that wild-type mice were less affected than heterozygotes by the dominant negative protein. These observations could explain why there is still a selection for *p53* LOH in many human tumours, even in the presence of a dominant negative *p53* allele.

Response of *p53*-deficient mice to DNA-damaging agents

p53 is thought to act as a tumour suppressor in part by coordinating the cellular response to DNA damage, causing cells to undergo cell-cycle arrest or apoptosis [21]. Cells lacking p53 become resistant to DNA damage, failing to arrest or undergo apoptosis. These failures would be predicted to promote tumorigenesis by allowing inappropriate survival or cell-cycle progression following DNA damage, which could result in the fixing of oncogenic mutations. The effect of radioresistance conferred by a mutation in the *p53* gene on susceptibility to tumorigenesis has been tested directly. In one study, wild-type and transgenic mice expressing either the *p53*193Pro or the *p53*Val135 allele were irradiated and examined for tumour incidence [11]. Radiation treatment resulted in an enhanced tumour incidence in transgenic mice compared with untreated transgenic mice of the same age, while irradiated wild-type mice failed to develop tumours altogether. Measurement of DNA damage in vivo indeed showed that either irradiated *p53* transgenic mice or *p53* null mice had twice the levels of chromosome damage than did irradiated wild-type mice, suggesting a means by which tumorigenesis was enhanced.

Another study showed that 129/Ola *p53* heterozygous mice irradiated with one dose of 4 Grey of irradiation at 7–12 weeks of age developed tumours much faster than untreated heterozygous animals, and again irradiated wild-type animals failed to develop tumours [22]. Almost all the irradiated heterozygotes died before any of the untreated heterozygotes. In examining the LOH status of irradiated heterozygotes, it was noted that 96% of the tumours displayed LOH, compared with the usual 55–75% in untreated *p53* heterozygous animals. These findings indicated that *p53* is a target for inactivation by irradiation. In contrast, a single 4-Gy dose of irradiation failed to decrease tumour latency of *p53* null animals relative to untreated animals unless they were treated at a very young age (6 days).

The findings of these studies, taken together, support the idea that p53 acts to limit DNA damage accumulation, and as a consequence, inhibits tumorigenesis. Moreover, the acceleration in tumour development induced by radiation further indicates that there are other genetic events required for tumorigenesis in *p53* null mice.

Role of p53 in carcinogen-induced cancers

A number of studies have addressed the role of p53 inactivation during carcinogen-induced tumorigenesis in mice, using either chemicals or ultraviolet (UV) light. In one type of study wild-type mice are treated with carcinogens to induce tumours, and the *p53* status is examined to determine whether it is mutated and whether it may play a role in the development of those particular tumour types. The observed genetic changes in *p53* in these tumours might reveal the signatures of particular carcinogens, supporting studies aimed at understanding the aetiological agents underlying the development of specific human cancers. In a complementary type of

study, *p53* heterozygous and null mice are used to assess the effect of p53 absence during carcinogen-induced tumour development.

Skin cancer. The best-studied carcinogen model system with regard to *p53* status is that of skin cancer induction. In the chemically induced skin cancer model for multistage carcinogenesis, the discrete steps of initiation, promotion and progression can be analysed. Initiation of papillomas is performed by treatment of mice with the carcinogen dimethyl-benzanthracene (DMBA), followed by promotion using 12-O-tetradecanoyl-phorbol-13-acetate (TPA) treatment. After some time, a small fraction of these benign papillomas undergo conversion to malignant skin carcinomas. In one study, skin tumours initiated with DMBA and promoted by TPA were examined at both the papilloma and carcinoma stages for signs of p53 inactivation [23, 24]. Absence of p53 protein or *p53* gene mutations was generally only observed in carcinoma samples, suggesting that inactivation of p53 is an important step in progression but not initiation of these tumours. A similar study examining the effects of benzo[a]pyrene on *p53* status during skin cancer induction supported the idea that *p53* mutation is a late event in chemically induced skin cancer [24].

In one elegant study, which complemented those mentioned above, the step at which loss of p53 acts during chemically induced skin cancer was addressed by comparing this pathway in wild-type, *p53* heterozygous and *p53*-deficient animals [25]. Reduced p53 dosage did not accelerate the appearance of papillomas or increase the number or size of papillomas. However, the rate of progression to carcinoma was enhanced in *p53* heterozygotes, and even more so in *p53* null mice. It was not only the rate of conversion that was faster, but also the frequency of conversion: 43% of papillomas became carcinomas in *p53* null mice, in contrast to 8% in heterozygotes and 3% in wild-type mice. The conversion in heterozygotes generally correlated with LOH at the *p53* locus, further supporting the hypothesis that in chemically induced skin cancer, loss of p53 plays a role in tumour progression.

A vast body of work has examined the effect of UV light on p53, as UV light is clearly associated with the development of skin cancer in humans. In these studies, wild-type mice were treated with UV-B (280–320 nm), and the resulting squamous cell carcinomas (SCCs) were analysed for *p53* mutations [26–29]. SCCs frequently exhibited an incidence of *p53* mutation of 20–30%, although the incidence ranged from 0% (Senkar) to 100% (C3H), depending on the study and the background strain of the mice. The mutations observed were generally the hallmark ones induced by UV-B, occurring at dipyrimidine sites, indicating that *p53* is the direct target of UV light and suggesting an early role for *p53* mutation in UV-induced skin cancer.

Experiments with the *p53* mutant mice further supported a role for p53 loss in skin cancer development. When UV-treated *p53*193Pro transgenic mice were compared with UV-treated wild-type mice, it was observed that although SCC tumour latency was similar, the transgenic mice had a higher incidence of tumours and more mice had multiple tumours, consistent with a role for *p53* mutation in tumour initiation [30]. Mice completely deficient for p53, however, developed UV-induced SCCs with shorter latency than did wild-type mice [31].

The idea of an early role for p53 loss is bolstered by a study in which *p53* mutations were detected in UV-treated skin in the mouse [32]. It was also shown that early UV-induced skin cancer lesions, actinic keratosis, commonly displayed *p53* mutations in the mouse [28]. The data of Ziegler et al. suggested not only that *p53* mutation can act in tumour initiation, but also that *p53* mutation can act in tumour promotion [33]. Specifically, in the mouse, skin cells containing functional p53 normally become 'sunburn' cells after exposure to UV, undergoing apoptosis in response to UV light. The number of sunburn cells was shown to be much higher in wild-type mice than in *p53*-deficient mice, indicating that p53 is required for the efficient execution of this programme. The absence of this apoptotic pathway in *p53*-deficient cells could provide a selective advantage for those cells during repeated exposure to UV light, as the surrounding *p53* wild-type cells underwent apoptosis. As a result, the population of *p53*-deficient cells would expand, raising the number of tumour-prone target cells. Hence, p53 loss could also play a role in tumour promotion.

Other cancers. Examination of *p53* status in a variety of chemically induced tumours has suggested that p53 loss is not always important for tumour development. For example, in certain mouse carcinogen models for liver, lung and breast cancers – tumours in which *p53* is commonly mutated in humans – *p53* mutations were not detected [34–36]. Furthermore, hepatocellular carcinoma (HCC) induced by the liver carcinogen diethylnitrosamine (DEN) was examined in *p53* heterozygous mice, and no difference in number, size, growth rate or histological grade of tumours was observed between wild-type and heterozygous mutants [37]. In contrast, *p53* heterozygotes developed hepatic haemangiosarcomas faster than wild-type mice after treatment with the carcinogen dimethylnitrosamine (DMN) [14]. Thus,

while p53 loss may not contribute to HCC, it may contribute to hepatic haemangiosarcoma development.

Additional uses for *p53*-deficient mice

p53 heterozygous mice, in particular, can be useful models to identify certain carcinogenic compounds. This is due to the fact that they develop tumours at an intermediate latency, which can easily be accelerated by some carcinogens. Several studies have used *p53* heterozygotes to this end [38].

In contrast, their dramatic propensity to develop tumours rapidly makes *p53* null mice a good model system to test the effects of so-called chemopreventive compounds thought to inhibit tumorigenesis. Traditionally, chemically induced tumour models have been used for this purpose but such treatment likely leads to DNA damage which can confound the interpretations of the results. The use of a spontaneous tumour model for this purpose is therefore optimal.

For example, it was known that calorie restriction had a general effect of slowing tumorigenesis in rodents. This observation was made in the *p53* null mice as well, indicating that the effect of calorie restriction is not dependent on p53 function [39]. Calorie restriction increased the survival time of both wild-type and *p53*-deficient animals, although they still eventually developed tumours [40]. There was, however, a slight shift towards more haematopoeitic tumours relative to nonhaematopoeitic types in calorie-restricted mice of both genotypes.

Chemopreventive drugs such as dehydroepiandrosterone (DHEA), *d*-limonene, all-trans retinoic acid (ATRA) and quercetin have since been tested for their effects on tumorigenesis in *p53* null mice [41]. Only DHEA was able to retard tumorigenesis in the *p53* null mice. The mean time of death from tumours without treatment was 108 days, compared with 174 days with treatment. The tumour spectrum was also somewhat affected by DHEA treatment. Specifically, there were fewer lymphomas observed in the treated *p53* null mice. This may account for the delay in mortality; as lymphoma is the most common tumour in *p53*-deficient mice, specific inhibition of this tumour type would prolong the life of the animal and thereby indirectly promote the development of other tumour types at a later age.

Crosses with other strains

The utility of *p53* knockout animals has been extended through crosses to other cancer-prone mouse strains. These crosses have helped to establish the mechanism of action of p53 by revealing genetic interactions between *p53* and other genes important in tumour development. In particular, crosses to mice expressing oncogenes or lacking tumour suppressor genes have revealed cooperation or redundancy in genetic pathways leading to tumorigenesis, helping to define these pathways more completely.

Why are *p53*-deficient mice lymphoma prone?

Several studies have sought to understand what it is about lymphoid cells that makes them especially sensitive to p53 loss. One hint as to p53's function in T lymphocytes comes from the analysis of cell death in thymocytes. Wild-type thymocytes undergo apoptosis in response to DNA-damaging agents, while those derived from *p53* null mice fail to do so [42, 43]. As DNA double strand breaks trigger the p53 response, it is reasonable to suppose that the double strand breaks arising during the process of V[D]J recombination might activate p53. The p53 checkpoint could monitor V[D]J recombination to eliminate cells with illegitimate, potentially oncogenic rearrangements through apoptosis. Failure to undergo apoptosis could lead to the survival of defective cells, thus facilitating lymphomagenesis. That abnormal recombination can contribute to tumorigenesis is known from studies of human cancers which showed that lymphoid malignancies are associated with the translocation of antigen receptor genes and oncogenes [44]. If the critical role of p53 as a tumour suppressor in lymphoid cells is to eliminate cells undergoing defective DNA rearrangements, it might be predicted that cells with excess DNA ends might be more prone to tumorigenesis in the absence of p53. Conversely, mutations that suppress rearrangement overall might inhibit tumorigenesis caused by p53 deficiency. To test these ideas, *p53*-deficient mice were crossed with mice defective in different steps of the V[D]J recombination pathway.

Scid. *Scid* (severe combined immunodeficient) animals have a deficiency in the DNA-dependent protein kinase (DNA-PK), which results in inefficient recombination of DNA ends that are generated during the course of antigen receptor recombination [45]. The inability to join broken DNA ends during antigen receptor recombination in *Scid* mice blocks lymphopoiesis at very early stages of T- and B-cell development. Interestingly, in the absence of p53, T-cell, but not B-cell development, was partially rescued in the *Scid*$-/-$ background [46–48]. As for the tumorigenic phenotype, *Scid*$-/-$, p53$-/-$ double mutant mice showed decreased tumour latency in lymphomagenesis

relative to single mutants. Most of these lymphomas were of B-cell origin, but a few were of T-cell origin and were composed of CD4, CD8 double-positive (DP) cells, indicating again that absence of p53 allowed the T-cells to progress beyond the usual block at the double-negative (DN) stage observed in *Scid*−/− cells. While it is clear that these mutations cooperate and that tumour latency is decreased in the presence of both mutations, it may only be that they cooperate to induce B-cell rather than T-cell lymphoma. This idea is supported by data from another model system, in which the T-antigen mutant TgTΔN, which can bind and inactivate p53, is expressed in thymocytes, leading to thymic lymphoma. In the presence of TgTΔN, there was no acceleration of thymic lymphoma development in *Scid*−/− mice relative to scid heterozygotes [49]. These findings suggest that *Scid* and *p53* loss cooperate in B- but not in T-cell tumour development.

***Rag-1* and *Rag-2*.** To test the requirement for V[D]J recombination in tumorigenesis resulting from p53 absence, *p53* null mice were bred with mice deficient for either the recombination activating gene *Rag-1* or *Rag-2* [50]. *Rag-1* and *Rag-2* mice are defective in proteins that normally generate the double strand breaks required to initiate the antigen recombination process, and thus T lymphocytes arrest at the DN stage. As in the *p53*−/−, *scid*−/− mice, *p53*, *Rag* double mutant animals display some developmental rescue of thymocytes to DP cells. Tumours, which were also composed of DP cells, arose with similar latency in the *p53*-deficient and the *p53*, *Rag-2*-deficient animals, and faster in the *p53*, *Rag-1* deficient animals. As expected, there was no evidence of recombination in these animals, strongly suggesting that V(D)J recombination is not essential for lymphomagenesis in *p53*-deficient mice. This does not rule out, however, the possibility that some unusual form of recombination not detectable by typical assays could occur. Again, these results are supported by similar experiments performed using the transgenic mice in which TgTΔN is expressed specifically in thymocytes [49]. These mice show a 100% incidence of thymic lymphoma within 5 months of birth, and when crossed with *Rag-1* and *Rag-2* mutants, still developed thymic lymphoma at high frequency, indicating that antigen receptor recombination is indeed dispensable for tumorigenesis.

The fact that V[D]J recombination is not necessary for tumorigenesis suggests that aberrant rearrangements are not the trigger for tumorigenesis. It might then be proposed that the role p53 plays is in some kind of checkpoint monitoring the proper development of T cells. In the absence of p53, cells that should arrest can progress and proliferate. Both the *Scid/p53* and *Rag/p53* crosses suggest that p53 normally contributes to an arrest at the DN stage, and that even in the absence of functional recombination, cells lacking p53 can progress to the more mature DP stage. This progression may somehow be the basis of the tumour phenotype in *p53* null animals.

Crosses with mice expressing oncogenes

***neu*.** In human breast cancer, common genetic events include activation of the neu receptor tyrosine kinase and inactivation of p53 [51]. The most commonly observed p53 alteration in breast cancer is mutation of amino acid 175 from arginine to histidine. In an effort to construct a mouse model for human breast cancer, MMTV-*neu* transgenic mice, which express high levels of neu in the mammary gland, were generated [52]. These mice developed mammary tumours, 37% of which had sustained mutations in the *p53* gene, suggesting that p53 inactivation was important for tumorigenesis [53]. To explore the ability of neu and mutant p53 to cooperate in greater detail, a transgenic strain was constructed expressing the mouse equivalent of the human 175 mutant (*p53*-172H) in the mammary gland under the control of the whey acidic protein (WAP) promoter. The *p53*-172H mice generally failed to spontaneously develop mammary tumours, unless treated with the carcinogen DMBA. In the *p53*-172H, *neu* bitransgenic animals, however, mean tumour onset was ∼5 months, before any tumours were observed in the neu transgenic animals. Thus, in the context of *neu*, mutant p53 overexpression can clearly accelerate tumorigenesis, potentially providing a relevant model system in which to study breast cancer. However, the mechanism of action of *p53*-172H mutation in this model remains to be determined.

***Wnt-1*.** Another important model for breast cancer development is that of the MMTV-*Wnt-1* transgenic mice, which express the Wnt-1 growth factor in the mammary gland and develop mammary adenocarcinoma at high frequency [54]. To test the role of p53 in tumour development in this system, MMTV-*Wnt-1* transgenic mice were bred to *p53*-deficient mice [55]. An acceleration in mammary tumorigenesis was observed in transgenic, *p53* null mice, but not in transgenic, *p53* heterozygotes. Surprisingly, despite the lack of effect of the heterozygous state on tumour latency, 50% of the tumours from *Wnt-1*, *p53* heterozygotes displayed LOH, perhaps reflecting a late LOH event. Genomic instability was examined in *Wnt-1* tumours of all three *p53* genotypes by standard karyotypic analysis as well as comparative genomic hybridization (CGH). In

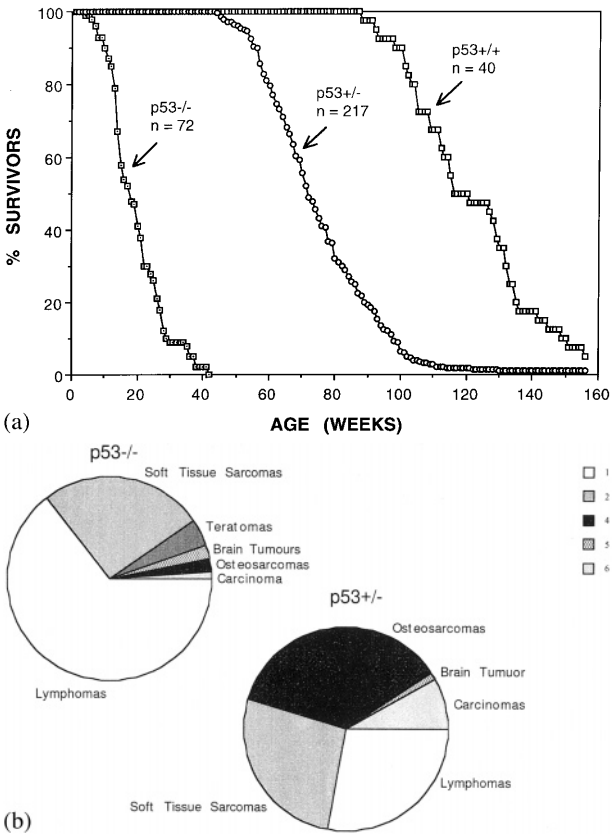

Figure 1. Tumour incidence in *p53*-deficient mice (data of L. Donehower). (*a*) The percentages of surviving *p53*+/+, *p53*+/−, and *p53*−/− animals on a mixed genetic background (C57BL/6X129/Sv) are graphed as a function of age. (*b*) Tumour spectra of *p53*+/− and *p53*−/− mice are depicted.

tumours from *p53* null or *p53*+/− animals with LOH, gross aneuploidy was evident, and CGH analysis identified regions of amplification and deletion arising in the absence of p53. Curiously, tumours from *p53* heterozygous mice showing LOH had higher levels of genomic instability (an average of 4.2 chromosomal abnormalities per tumour) than those from *p53* null animals [1.7]. These results support a role for p53 in maintaining genome integrity and suggest that loss of p53 may promote tumour progression in this model by lowering the threshold to further mutation.

In a follow-up study, growth rates of mammary tumours were compared in *Wnt-1* transgenic animals that were wild-type, heterozygous or null for p53. Growth rates were found to be faster in tumours lacking p53 (either null or *p53*+/− with LOH) [56]. Both cell proliferation and levels of apoptosis were examined to find a basis for the increased tumour growth. The number of mitotic figures and the percentage of cells in S phase measured by fluorescence activated cell sorting (FACS) analysis were both higher in the *p53*-deficient tumours than in wild-type tumours, suggesting an increased degree of proliferation in the null tumours. In contrast, levels of apoptosis were found to be generally low in both tumours and hyperplastic glands of *Wnt-1* transgenics of all *p53* genotypes, with no obvious correlation with *p53* status. These data, taken together, suggest that deregulation of the cell-cycle and genomic instability caused by *p53* mutation may explain the cooperativity with *Wnt-1*, while reduced frequency of apoptosis does not appear to influence the development of tumours in these mice.

Ras. Transgenic mice expressing an activated allele of *Ha-ras* under the control of the MMTV promoter in the salivary and mammary glands develop tumours in both tissues [57]. Crossing this transgene onto a *p53* null background accelerated tumorigenesis specifically in the salivary gland. These tumours were analyzed in detail in an attempt to determine the mechanism of p53 action. Tumors in *ras*, *p53*−/− mice appeared earlier and grew faster than in their *p53* wild-type counterparts. Cell-cycle analysis suggested that *ras*, *p53*−/− cells had a smaller fraction in G1 and a higher fraction in S and G2/M than *ras*, *p53*+/+ cells, and that perhaps the capacity to cell-cycle arrest was perturbed in the *ras*, *p53*−/− cells. Moreover, the DNA-damaging agent-induced G1 checkpoint was perturbed. Levels of apoptosis were low and constant in all tumours regardless of *p53* status, suggesting again that p53 inactivation did not appear to promote tumorigenesis by disrupting apoptosis. These data suggest that *ras* and *p53* loss cooperate in tumour formation, and the mechanism is likely to involve the disruption of a p53 cell-cycle checkpoint function.

c-myc. Multiple studies have examined the ability of the *c-myc* oncogene to cooperate with p53 loss in tumorigenesis. *c-myc* expression results in both increased cellular proliferation and apoptosis, and inactivation of p53 might provide a means to specifically inactivate the apoptotic pathway, thus suggesting a rationale for cooperation between *c-myc* expression and p53 loss. In one study, CD2-*c-myc* transgenic mice, which express c-myc in T cells and develop T-cell lymphomas at a low rate, were crossed with *p53*-deficient mice [58]. CD2-*c-myc* transgenic animals that were *p53* null, but not *p53*+/−, exhibited increased T-cell lymphoma incidence and decreased latency relative

to CD2-*c-myc* wild-type mice. In contrast, a *c-myc* transgene expressed under the control of the Eμ enhancer was able to cooperate with a *p53* heterozygous mutation in the development of aggressive B-cell lymphomas [59]. Comparison of tumours from Eμ-*c-myc*, *p53*+/+ mice and Eμ-*c-myc*, *p53*+/− mice with LOH indicated that apoptotic indices were very similar in the two cases, while proliferation seemed to be enhanced in the tumours lacking p53 function; these tumours had a higher fraction of cells in S/G2-M (50–60%) than did the wild-type tumours (30%). In this model, therefore, enhanced proliferation rather than changes in apoptosis is implicated in tumorigenesis upon p53 loss.

In another study involving *c-myc*, MMTV-*c-myc* transgenic mice were crossed to *p53*-deficient animals [60]. In this model the oncogene is expressed in the mammary gland, salivary gland and some lymphoid cells, and the mice develop mammary tumours and lymphoma. Although *p53* status did not affect mammary tumorigenesis, MMTV-*c-myc* *p53*+/− animals developed lymphoma with increased penetrance and decreased latency relative to MMTV-*c-myc* or *p53*+/− mice. These crosses indicate that synergism between two oncogenic agents can be tissue-specific, as shown by the genetic interaction in lymphoid cells but not in the mammary gland.

SCL. Ectopic expression of the *Scl/Tal-1* member of the bHLH class of transcription factors is associated with the development of greater than 60% of human T-ALL (T-cell acute lymphoblastic leukaemia) cases. Transgenic mice expressing *Scl/Tal-1* under the control of the LCK promoter develop T-cell lymphomas, often with a minor B-cell component [61], whereas transgenic animals expressing *Scl* under the control of the T-cell-specific CD2 enhancer do not [62]. Upon crossing *p53*-deficient mice to the *Scl* transgenic mice, cooperativity in thymic lymphoma development was indeed observed in the LCK-*Scl* transgenic, *p53*+/− animals, relative to single heterozygotes. The presence of the CD2-*Scl* transgene also accelerated lymphomagenesis in *p53* heterozygous mice. The presence of the CD2-Scl transgene did not affect the lifespan of *p53*-deficient mice, but the transgene did alter the site of presentation of lymphoma, being peripheral rather than thymic.

bcl-2. Not every cross has revealed cooperativity between p53 deficiency and an oncogene. *Bcl-2-Ig* transgenic mice develop B-cell lymphomas, which rarely exhibit *p53* mutations [63]. Consistent with this finding, the average tumour latency in *p53* null mice or *p53* heterozygous mice with or without the *bcl-2* transgene was approximately the same. These data indicate that p53 loss and bcl-2 expression do not cooperate, suggesting that they might act in a common cell death pathway in this cell type. This experiment also provides a good example of how such crosses might indicate that two genes are in the same pathway.

Crosses with tumour suppressor gene knockout mice

***Rb* (retinoblastoma).** The fact that mutations in the retinoblastoma (*RB1*) and *p53* genes are commonly found together in human tumours (including sarcomas, lung, breast, cervical and pancreatic tumours), suggests that coordinate loss of these tumour suppressors enhances tumour development [64 and references within]. Furthermore, DNA tumour viruses such as SV40, adenovirus and papillomavirus have evolved such that they produce proteins that inactivate both Rb and p53 function, which facilitates cellular transformation [65]. To examine this potential cooperativity directly, *Rb* and *p53* mutant mice were crossed [64, 66]. As *Rb*-deficient mice are not viable, the effect of *p53* mutation on *Rb* heterozygous mice was examined. One analysis compared *Rb*+/−, *p53*+/− and *Rb*+/−, *p53*−/− to *Rb*+/−, *p53*+/+ mice, which have a mean survival time of 11 months, developing intermediate lobe pituitary tumours at 100% frequency and thyroid carcinomas at 70% frequency [64]. *Rb*+/−, *p53*+/− mice had a mean survival time of 9 months, with approximately the same frequency of pituitary and thyroid tumours as *Rb*+/− mice, but also with the appearance of a few additional tumours: leiomyosarcoma, pinealoblastoma, islet cell carcinoma and anaplastic sarcoma. While essentially all tumours showed LOH at the *Rb* locus, primarily only the novel ones not observed in the single mutant mice displayed LOH at both the *Rb* and *p53* loci. *Rb*+/−, *p53*−/− mice had a more dramatic phenotype; the average survival time was 4 months, 1 month shorter than *p53*-deficient mice, and all mice were dead by 6 months. In addition to the usual lesions independently associated with *Rb* or *p53* mutations, islet cell tumours were seen in 20% of the mice, pinealoblastomas in 40%, bronchial hyperplasia in 40% and retinal dysplasia in 40%. These findings were supported by a comparable study, in which *Rb*+/− mice developed pituitary adenoma at 100% frequency in a *p53* wild-type background, but they also developed a host of novel endocrine tumours (medullary thyroid carcinomas, pancreatic islet cell carcinomas, parathyroid carcinomas) in a *p53* heterozygous background [66]. *Rb*+/−, *p53*−/− animals in this experiment also showed accelerated tumorigenesis relative to the single

mutants. These results support the idea that *Rb* and *p53* loss are cooperating events in tumorigenesis.

***APC* (adenomatous polyposis coli).** In human colorectal cancer, a series of discrete morphological and corresponding genetic steps, including mutations in the *APC* and *p53* genes, have defined a pathway from hyperplasia to full malignancy [67]. Mutations in *APC* predispose an individual to colon adenomas, while *p53* loss is associated with the conversion from adenoma to adenocarcinoma. The ability of mutations in both the *APC* and *p53* tumour suppressor loci to cooperatively induce intestinal tumours in the mouse was thus examined [68]. Mice heterozygous for *APC* develop adenomas of both the small and large intestine, with a small percentage of cases progressing to adenocarcinomas. *APC*+/−, *p53*−/− mice have a shorter survival time than *APC*+/−, *p53*+/+ mice, although comparable to that of *APC*+/+, *p53*−/− animals. Surprisingly, the number, severity, and latency of intestinal tumours was the same in the *APC*+/−, *p53*+/+ mice and the *APC*+/−, *p53*−/− mice. In other words, no cooperativity was observed between mutations at these two loci in the mouse intestine. The majority (>80%) of *APC*+/−, *p53*−/− mice did, however, develop a predisposition to pancreatic abnormality, either preneoplastic foci/dysplasia or pancreatic acinar cell adenocarcinoma accompanied by LOH at the APC locus. These lesions were not observed in either *APC*+/− or *p53*−/− animals. Thus, mutations in *APC* and *p53* can cooperate in tumour formation, but the effect appears to be specific for the acinar cells of the pancreas.

***Nf1* (neurofibromatosis) type 1.** A dramatic example of tumour suppressor gene cooperativity comes from crosses performed between *Nf1* and *p53* mutant mice (K. Cichowski, S. Shih and T. Jacks, unpublished observations). This example represents an interesting case as these two loci map within 5 centimorgan (cM) on the same chromosome and provide a way to test cooperativity by coordinated loss of wild-type alleles of both genes. In *Nf1*+/−, *p53*+/− 'trans' mice, which carry the wild-type alleles of *Nf1* and *p53* on opposite chromosomes 11, there is no obvious cooperativity. However, when the two mutations were recombined onto the same chromosome and paired with a wild-type chromosome, these 'cis' mutant mice had a dramatic predisposition to malignant peripheral nerve sheath tumours, a tumour not seen in either heterozygous parental strain. As expected, LOH was observed for both *Nf1* and *p53* in these tumours, most likely via loss of the whole wild-type chromosome. This striking finding shows that loss of *Nf1* and *p53* can cooperate in tumour formation affecting the peripheral nervous system and exemplifies how genetic linkage could exacerbate a tumour predis-position.

***Nf2* (neurofibromatosis) type 2.** A result similar to that for *Nf1* is found with the *Nf2* tumour suppressor gene, which also resides on chromosome 11, 40 cM from the *p53* locus [69]. *Nf2* heterozygous mice develop mostly osteosarcomas of the spine and facial bones, with an average tumour latency of ~22 months. *Nf2* mutant mice were crossed with the *p53*-deficient strain to construct cis and trans heterozygotes, as described above. *Nf2*, *p53* cis heterozygotes died by 5 months of age, much more rapidly than single heterozygotes or than 'trans' heterozygotes. As in *Nf2*+/− mice, the cis mice developed primarily osteosarcomas, but the site of tumour formation was most often in the bones of the nasal passage, rather than in the spine or facial bones. As expected, LOH was observed at both loci, again probably due to loss of the entire wild-type chromosome. *Nf2*+/−, *p53* null mice showed similar survival to the cis mice. Interestingly, *Nf2*, *p53* trans heterozygous mice also showed increased mortality relative to single heterozygotes and developed mostly osteosarcomas that exhibited LOH at both loci. This study provides another example of the dramatic cooperativity between mutations of two tumour suppressor loci.

Crosses with DNA repair-deficient mice

Several studies have investigated the compound effect of mutations in DNA repair pathways and *p53*. For example, the Ataxia-telangiectasia mutated (ATM) protein is a member of the phosphatidylinositol-3′-kinase (PI-3) kinase family involved in maintaining genome stability, at least in part through mediating the signal of DNA-damaging agents to p53. Mice deficient in *Atm* and the *Atm*−/−, *p53*+/− and *Atm*−/−, *p53*−/− mice each exhibit progressively earlier lymphomagenesis [70]. This cooperativity indicates that ATM has functions other than acting through p53. In other studies it has been shown that lymphomagenesis in mismatch repair protein *Msh-2*-deficient mice and UVB-induced skin cancer in nucleotide excision repair protein *Xpc*-deficient mice are both accelerated by *p53* mutation [71, 72]. The data from these crosses of *p53*-deficient mice with DNA repair mutant mice, taken together, indicate that the absence of diverse proteins involved in the maintenance of genomic integrity can synergize to increase the incidence of tumorigenesis.

Lessons from DNA tumour viruses

Much has been learned about p53 by studying members of the DNA tumour virus family, including adenovirus, papillomavirus and simian virus 40, all of which have evolved intricate mechanisms to transform cells [65]. In particular, these viruses all produce proteins that bind and inactivate both pRb and p53. Transgenic models in which these proteins are expressed have been illuminating for understanding p53 function in tumour suppression.

In one model, SV40 large T-antigen was expressed in the choroid plexus epithelium of the mouse brain, and as a consequence these mice rapidly developed tumours; by 6 weeks of age half developed tumours [73]. The Rb-binding function of T-antigen was sufficient for the induction of tumours, but p53 binding was critical for the rapid progression of these tumours. Specifically, expression of a truncated form of T-antigen, TgT121, which could bind and sequester Rb, but could not bind and inactivate p53, induced tumours with an average tumour latency of 26 weeks (although penetrance was still 100%). These findings suggested that p53, when active, could limit tumorigenesis. Crossing the TgT121 mutant strain into a *p53*-deficient background restored rapid tumorigenesis, indicating that p53 was indeed limiting tumour formation. Examination of tissue sections from the mice of different *p53* status provided a molecular explanation for these effects. Choroid plexus tissue from TgT121, *p53*+/+ mice showed high levels of apoptosis, while that from TgT121, *p53*−/− mice showed virtually no apoptosis, suggesting that p53 limited tumour progression by inducing apoptosis. The most solid support for this idea came from TgT121, *p53*+/− mice, which showed high levels of apoptosis in hyperplastic choroid plexus epithelium, but little apoptosis in small tumour nodules, which also displayed LOH at the *p53* locus. This suggested that upon *p53* LOH, the apoptotic pathway was inactivated and tumour development ensued. This study provided a strong in vivo correlation between the presence of p53 and apoptosis in an early tumour, suggesting a mode of p53-associated tumour suppression.

The importance of p53 in this system was further demonstrated by making a transgenic strain expressing a p53 dominant negative protein in the choroid plexus epithelium [74]. This 'p53DD' protein, which consisted of the p53 oligomerization domain and was known to behave as a dominant negative protein in cell culture, was targeted to the choroid plexus by expression under the control of the transthyretin (TTR) promoter. The survival of TgT121 mice expressing the p53DD was intermediate to *p53* heterozygous and null mice. In essence, it appeared that the p53DD behaved similarly to a *p53* null allele by reducing levels of apoptosis and accelerating tumorigenesis, with the difference in the ability to enhance tumour development probably resulting from the small fraction of cells expressing p53DD. This experiment further illustrated the possibility of inactivating p53 in a tissue-specific manner.

The mechanism of p53 action can be further probed by crossing strains such as the TgT121 strain to strains carrying mutations in putative transcriptional targets of p53. For example, bax has been proposed to be downstream of p53 in the apoptotic pathway, based on the facts that bax expression is reduced in *p53* null mice and that there is a p53 DNA-binding site in the bax promoter [75, 76]. Indeed, bax was induced in the TgT121 mice, but not in nontransgenic mice or in transgenic mice lacking p53 [77]. The TgT121 mice were crossed with bax-deficient mice, and interestingly both apoptosis levels and tumour latency were intermediate to wild-type and *p53* null mice, suggesting that bax mediates part of the p53-induced cell death effect. This is a particularly good example of how crosses between mutant strains can provide an understanding of p53's mode of action.

The use of the papillomavirus proteins E6 and E7 has also been informative in elucidating the role of p53 in tumour suppression. E7 acts by sequestering the pRb family of proteins, while E6 targets p53 for degradation [65]. Transgenic mice in which E7 or E6 is expressed either in the retina or the lens have been constructed. Retinal expression of E7 under the control of the interstitial retinol-binding protein (IRBP) promoter induced high levels of apoptosis [78], but upon crossing these transgenic mice to *p53*-deficient animals, apoptosis was reduced and retinal tumours developed. Similarly, E7 expression in the lens under the control of the αA-crystallin promoter led to inhibition of differentiation, aberrant cell-cycle entry and apoptosis [79]. Expression of both E7 and E6 in the lens abolished apoptosis and ultimately resulted in the formation of lens tumours. Crossing these E7 transgenic mice into a *p53* null background confirmed that at least some of the apoptosis observed in this system was p53-dependent [80]. These findings supported the idea that p53 can act in tumour suppression by stimulating apoptosis in abnormally cycling cells.

How does p53 function?

p53 function is complex, and its role as a tumour suppressor in vivo is likely to be multifaceted. In general, two mechanisms for p53 action have been proposed, based primarily on work in cell culture systems. The mechanism that is most relevant may vary according to tissue type. Crosses such as those described above can help to determine which function of p53 is used in different contexts.

Cell-cycle regulation. It is clear that in some tissues p53 can act as a regulator of cell-cycle progression. This notion originates from cell culture experiments, which have shown that p53 participates in a critical G1 checkpoint in response to DNA-damaging agents [21]. Specifically, cells exposed to genotoxic agents undergo a p53-dependent arrest at the G1 phase of the cell cycle. This pause in G1 is envisioned to allow the cell a chance to repair its DNA before replication and cell division. Alternatively, cells may undergo *p53*-dependent apoptosis in response to genotoxic agents. In the absence of p53, cells would either fail to arrest or to die in response to stresses such as DNA damage, and potentially oncogenic mutations could be passed on to daughter cells. Inactivation of this checkpoint function is one explanation for the genomic instability that has been observed in numerous contexts, and is attributed to loss of p53. A possibly related explanation for genomic instability is based on the finding that *p53*-deficient cells have abnormal centrosome duplication [81]. With an inappropriate number of centrosomes, segregation of chromosomes during mitosis is impaired, and the hallmarks of genetic instability, like aneuploidy, are observed. These functions have earned p53 the name 'guardian of the genome'. That p53's function in tumour suppression is due to its capacity as guardian of the genome is consistent with cases in which *p53* is mutated early in tumorigenesis, with its loss predisposing cells to the accumulation of mutations and hence increased malignancy.

In addition, some evidence exists that in the absence of p53, cells may simply proliferate too rapidly [82]. This may reflect loss of a cell-cycle regulatory function distinct from the DNA damage checkpoint. Enhanced proliferation would result in increased target cell populations and thus a greater chance for subsequent oncogenic mutations, which in turn would facilitate tumorigenesis. In the *Wnt-1* or *ras* mammary tumour models or the Eμ-*myc* lymphoma model described above, it appears clear that loss of p53 results in increased proliferation of tumour cells. In addition, p53 loss could enhance the rate of tumorigenesis in these models via genomic instability, that is, increasing rates of mutation at other loci, such as the rate of LOH at tumour suppressor loci. This could explain enhanced tumorigenesis in the $p53-/-$, $Rb+/-$ or $p53-/-$, $APC+/-$ mice, for example, where LOH is observed. Thus it appears that in some mouse models, loss of p53 may accelerate tumorigenesis because of loss of its cell-cycle regulatory function.

Apoptosis. An alternate hypothesis for p53 function is that it acts in cells that have already sustained some genetic change to commit these cells to a fate of apoptosis. It is known that cells that have become growth-deregulated are highly susceptible to p53-dependent apoptosis. For example, cells that express the oncogenes E1A or c-myc are very sensitive to p53-mediated cell death [83–86]. If such cells lose the *p53* gene, however, they can escape from apoptosis and proliferate uncontrollably. A role for p53 in apoptosis would be consistent with cases in which p53 loss occurs late in tumorigenesis, with its loss allowing the survival of malignant mutant cells.

The strongest support for the idea that p53 acts as a tumour suppressor by inducing apoptosis comes from transgenic mice expressing the DNA tumour virus oncoproteins. For example, in the T-antigen model in which the TgT121 protein lacking the p53 binding domain is expressed in the choroid plexus epithelium, tumorigenesis proceeds slowly and is accompanied by high levels of apoptosis in the tumour [73]. When the apoptotic pathway is compromised due to p53 inactivation, rapid tumour formation is restored. This appears to be the scenario in E6/E7 transgenics as well [78, 79]. Experiments with *Rb*-deficient embryos have demonstrated a similar apoptotic phenotype in the absence of Rb, which in specific tissues is dependent on p53 [87]. It is thus easily conceivable that escape from p53-dependent apoptosis could play a role in the development of tumours in *Rb*-deficient mice. In addition, it appears from the mouse models that loss of p53-induced apoptosis is an important step in UV-induced skin cancer [33]. In contrast, many of the crosses to oncogene mice seem to demonstrate that loss of the apoptotic pathway does not contribute to tumorigenesis.

Interestingly, *p53* mutations can be detected both early and late in the tumorigenic process, depending on the tumour type, suggesting that p53 function as guardian of the genome and inducer of apoptosis both play roles in tumour suppression, depending on the context.

Morphological changes. Finally, it has frequently been noted that *p53*-deficient tumours exhibit some features of higher-grade tumours including large nuclei, irregular cell sizes (pleomorphy), high mitotic indices and undifferentiated cells (ana-plasia). Although it is not known what accounts for the more aggressive nature of *p53*-deficient tumours, it may be that cells whose cell cycle is deregulated and continue to grow tend to accrue more changes. Alternatively, cells escaping apoptosis by p53 loss may have already sustained many changes that alter their character. In addition, it is possible that these changes represent loss of a role of p53 in differentiation, which has been reported in some contexts [88].

Table 1. Effects of p*53* mutation on tumour development in a variety of mouse model systems.

Model system	Test strain	Tumours developed	p53 genotype	Cooperativity?	Type of cooperativity		Reference
					tumour type showing reduced latency	novel tumours	
Oncogene-expressing mice	MMTV-*neu*	mammary tumours	WAP-*p53*172H	yes	mammary tumours		54
	MMTV-*Wnt1*	mammary tumours	*p53*+/−	no			18
		mammary tumours	*p53*−/−	yes	mammary tumours		18
	MMTV-*Ha-ras*	salivary tumours	*p53*−/−	yes	salivary tumours		38
		mammary tumours	*p53*−/−	no			38
	CD2-*c-myc*	T-cell lymphoma	*p53*+/−	no			5
		T-cell lymphoma	*p53*−/−	yes	T-cell lymphoma		5
	Em-*c-myc*	B-cell lymphoma	*p53*+/−	yes	B-cell lymphoma		37
	MMTV-*c-myc*	T-cell lymphoma	*p53*+/−	yes	T-cell lymphoma		22
		mammary tumours	*p53*+/−	no			22
	CD2-*Sc1*	none	*p53*+/−	yes		T-cell lymphoma	14
	LCK-*Sc1*	T-cell lymphoma	*p53*+/−	yes	T-cell lymphoma		12
	bc1-2-Ig	B-cell lymphoma	*p53*−/−	no			62
Tumour suppressor mutant mice	*Rb*+/−	pituitary tumours and thyroid tumours	*p53*+/−	yes		pineaoblastoma, etc.	31, 85
			p53−/−	yes		pinealoblastoma, etc.	31, 85
	Apc+/−	intestinal tumours	*p53*−/−	yes		pancreatic tumours	10
	Nf1+/−	pheochromocytomas and myeloid leukaemia	*p53*+/− cis	yes		MPNST	pers. comm.
			p53+/− trans	no?			pers. comm.
	Nf2+/−	osteosarcomas	*p53*+/− cis	yes		osteosarcomas (nasal)	pers. comm.
		osteosarcomas	*p53*+/− trans	yes	osteosarcomas		pers. comm.
DNA repair mutant mice	*ATM*−/−	T-cell lymphoma	*p53*+/−, *p53*−/−	yes	T-cell lymphoma	B-cell lymphoma	84
	Msh2−/−	T-cell lymphoma	*p53*−/−	yes	T-cell lymphoma		13
	XPC−/−	SCC	*p53*+/−	yes	SCC		9
DNA tumour virus protein-expressing mice	T-antigen 121	choroid plexus tumours	*p53*+/−, *p53*−/−	yes	choroid plexus tumours		78
		choroid plexus tumours	TTR-p53DD	yes	choroid plexus tumours		8
	IRBP-E7	none	*p53*−/−	yes		retinoblastoma	36
	αA-crystallin-E7	none	αA-crystallin-E6	yes		lens tumours	70

'Test strain' indicates the mouse model in which the role of p53 in tumorigenesis was tested. 'Tumours developed' refers to the tumour type(s) which develop in these test strains in a wild-type p53 background. 'p53 genotype' is the specific p53 mutant background tested in combination with the test strain mutations. The term 'cooperativity' is used here to indicate a reduction in tumour latency or the development of a novel tumour type in the compound mutant background.

Conclusions and future prospects

Mouse studies were originally critical for definitively showing that p53 is a tumour suppressor and have since continued to be crucial for mechanistic analyses of how p53 regulates tumour development in vivo. As mouse technology becomes more developed, it will be possible to do more and more sophisticated experiments, such as conditional inactivation of *p53* in particular tissues and at particular stages of development. The potential of such approaches has already been demonstrated using the p53 dominant negative expressed with T-antigen specifically in the choroid plexus epithelium. The use of the Cre-lox system to generate conditional alleles of *p53* will facilitate tissue-specific analysis of p53 function. More subtle analyses of p53 using point mutations rather than gene deletion, to test for gain of function effects or to assess the role of p53 mutants compromised for particular activities, will also be important. Additional mouse crosses will allow genetic pathways by which p53 acts to be further dissected, as described for the bax cross. Finally, *p53*-deficient mice can continue to serve as models for identifying agents that prevent tumours and for testing novel therapies for tumours lacking p53. Mice will continue to be useful for answering many questions about p53 and its role in suppressing tumorigenesis for many years to come.

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