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News and Commentary

Dissecting p53 tumor suppressor function *in vivo* through the analysis of genetically modified mice

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The p53 protein is a frequent target of mutation or functional inactivation in a wide range of human cancers. While a significant number of studies on p53 have been performed in cell culture-based systems utilizing a multitude of different cell lines, more recently, there has been a significant effort to generate models to evaluate p53 action *in vivo*. Current work described both in the literature and among members of the field during the recent 12th International p53 Workshop (reviewed in Braithwaite *et al.*[1]) has provided an exciting glimpse into the genetics of *Trp53* mutation, p53's biological functions important for tumor suppression, and the signaling pathways crucial to p53 activation. In this review, we will attempt to cover the most recent discoveries involving the study of p53 in the context of the mouse.

Examining p53 function in the mouse provides several advantages when compared to standard analyses performed in cell culture. A primary benefit of studying mice is that p53 activity is being evaluated in a complex tissue environment. Whereas cultured cells represent a relatively homogenous population, cells in an organism exist in a much more diverse setting, in that they are surrounded by a number of different cell types which are likely modulating their behavior in some manner. Likewise, the artificial nature of oxygen tensions, growth factors, and nutrient concentrations in typical cell culture conditions likely has an impact on the activities of cells *in vitro* when compared to the same population of cells *in vivo*. Another significant advantage of studying p53 in the context of the mouse is that a variety of cell types can be analyzed, thereby revealing any tissue specific differences in p53 function. However, perhaps the most compelling reason to study p53 in the mouse is that it provides an ideal setting to assess the means through which p53 suppresses neoplasia.

A significant advance in the analysis of p53 has been the generation of knock-in mice, in which altered forms of *Trp53* are introduced into its endogenous genomic locus. Due to the presence of endogenous *cis*-regulatory elements, *Trp53* mRNA is produced at physiologically relevant levels and is controlled in both a temporally- and spatially correct manner. Knock-in mice have been integral to elucidating p53 function *in vivo* without many of the caveats associated with over-expression assays commonly employed with either standard transgenic mice or cell culture based studies.

Dissecting the Genetics of Common *Trp53* Mutations

The genetic knockout of the *Trp53* gene in the mouse, originally described by Donehower *et al.*[2] and then later by Jacks *et al.*,[3] revealed p53's central role in tumor suppression. Mice lacking *Trp53* show a dramatic, and completely penetrant, predisposition to cancer development; that is, each mouse born lacking both copies of *Trp53* is assured of developing some form of malignant growth within two to 10 months after birth. Most commonly, these animals die from thymic lymphomas, although a significant number of animals develop a range of sarcomas.[2] In the analysis of *Trp53* heterozygous mice,[3] a tumor predisposition is equally evident, although the tumor spectrum and latency is different from that seen in the *Trp53* null animals. Whereas *Trp53* null mice are highly predisposed to lymphoma development, heterozygotes develop a greater number of mesenchymal cancers, including fibrosarcomas, osteosarcomas, and hemangiosarcomas, and these tumors appear at later timepoints than the malignancies observed in the null mice. Quite intriguingly, among both *Trp53* null and *Trp53* heterozygous mice, only a very small percentage develop carcinomas, or cancers of the epithelia. In contrast, carcinomas are by far the most common group of malignancies diagnosed in humans worldwide each year, with sarcomas and lymphomas occurring much less frequently. Moreover, in many human cancers, *TP53* mutation is often associated with the development of carcinomas. For example, in colorectal cancer, *TP53* loss is often associated with the transition from a benign, late-stage adenoma to a fully malignant carcinoma.[4] Carcinomas are also a common feature of Li–Fraumeni syndrome (LFS), a familial cancer disorder in which individuals inherit a mutant allele of *TP53* and are highly predisposed to a variety of cancers at a young age.[5] What can explain this striking difference between the spectrum of cancers seen in the *Trp53* knockout mice and from those seen in sporadic human cancers and LFS patients[6]?

Recent experiments have led to potential explanations for this phenomenon. For example, when the *Trp53* null allele is bred onto a Balb/C genetic mouse background to generate *Trp53* heterozygous animals, the mice now develop a significant number of breast cancers.[7] This result suggests that genetic modifiers present in individual mouse strains may exert a strong influence on the cancer spectrum seen in these mice, and may be a significant factor contributing to the large number of lymphomas and sarcomas seen in the original studies, which were performed on a mixed 129/Sv and

C57BL/6 genetic background. Another potential explanation for this tumor spectrum disparity centers on the inherent genetic differences between humans and mice, and prominent among these are the dramatically longer telomeres in *Mus musculus* compared to those in humans. Support for this theory came from the analysis of *Trp53* heterozygous mice in the context of shortened telomeres. As telomere length is reduced to critically short levels, chromosomal ends become more susceptible to chromosomal fusion–bridge–breakage cycles, generating significant genomic instability.[8] To achieve a reduced telomere length, *Trp53* knockout mice were bred to mice lacking the RNA component of telomerase, the reverse transcriptase involved in telomere lengthening and maintenance.[9] These mice now develop a range of carcinomas, including breast adenocarcinoma, gastrointestinal adenocarcinoma, and squamous cell carcinoma. This suggests that the lack of telomere attrition, and the absence of the genomic instability characteristic of telomere dysfunction, may be part of the reason why *Trp53* heterozygous mice do not fully recapitulate the disease spectrum of human LFS patients.

In addition, a third explanation for the tumor spectrum dichotomy between mice and humans was recently described at the 12th International p53 Workshop. Previous *in vitro* experiments examining mutant forms of p53 observed in LFS patients suggested that many of these mutants not only had dominant-negative activity towards the wild-type p53 protein, but also possessed an oncogenic, 'gain-of-function' activity, such as the ability to transactivate novel target genes or to interact inappropriately with other cellular proteins. Unlike the original *Trp53* knockout mice, LFS patients do not inherit a *TP53* allele that has undergone a dramatic deletion or frameshift mutation that eliminates p53 protein expression. Instead, they are much more likely to possess a *TP53* gene containing a point mutation in which protein is still produced, but is not functional in its normal tumor suppressor capacity. Thus, it may be that these mutant forms of p53 contribute some function that is necessary for the development of epithelial cancers. To evaluate the tumorigenic effects of these mutant forms of p53 in an *in vivo* setting, Jacks and co-workers[10] and Lozano and co-workers[11] produced mice in which *Trp53* point mutants commonly found in human cancers were introduced into the endogenous *Trp53* locus. In the work described by Lozano and co-workers, the R172H (corresponding to human R175H) mouse was generated, whereas in the work described Jacks and co-workers, both the R270H (corresponding to human R273H) and the R172H mice were developed. Analysis of heterozygous knock-in mice expressing these p53 point mutants, both of which are commonly found in LFS patients, led to an intriguing observation. Whereas the original *Trp53* heterozygous mice develop an extremely high incidence of sarcomas and lymphomas, the *Trp53* point mutant heterozygotes succumb to a range of carcinomas, including lung adenocarcinomas and squamous cell carcinomas. In addition, in both the R172H and R270H mouse models, a significant number of metastases are also observed, whereas in *Trp53* heterozygous mice, metastatic disease is rarely seen. Further analysis of these mice, and cells derived from them, suggested that this alteration in tumor spectrum may be due to the fact that these oncogenic p53 point mutants are able to act not only as dominant negatives in certain contexts, but also by complexing with the p53 family members p63 and p73 to potentially inhibit their function. This gain of function phenotype was further confirmed by breeding the mutant alleles of *Trp53* into a *Trp53* null background.[10] In this setting, the mice displayed a tumor spectrum distinct from that seen in the $Trp53^{+/-}$ or $Trp53^{-/-}$ mice, with a significant increase in endothelial tumors, as well as carcinomas. Likewise, in cellular based transformation assays, the oncogenic point mutant p53 had a stronger transforming effect than the simple loss of p53 protein expression.[11] Thus, it may be that altering the activity of the entire p53 family is a significant factor in the development of carcinomas, a striking finding that not only contributes to a better understanding of cancer at the molecular level, but also may have significant clinical and therapeutic implications, in terms of the development of small molecules that inhibit the interaction of mutant forms of p53 with its family members.[6]

p53 Effector Functions in Tumor Suppression

The p53 protein is stabilized and activated in response to a variety of cellular stresses, including DNA damage, hypoxia, and growth factor deprivation.[12] After receiving a stress signal, p53 initiates any of a number of different cell signaling pathways, including those leading to apoptosis, growth arrest, and DNA repair. However, the contribution of these different effector functions to p53's role as a tumor suppressor have been difficult to define *in vitro*, and thus, it has become increasingly important to evaluate this in an *in vivo* setting. The mouse models described in the following section have provided significant insight into which downstream activity of p53 is relevant for tumor suppression in specific models of cancer.

Inducing apoptosis

One of the primary means through which p53 has been shown to suppress transformation in cell culture-based studies is through initiation of the cell death cascade. Utilizing a modified version of the classic *Eμ-myc* model of Burkitt's lymphoma,[13] Lowe and co-workers have contributed significantly to understanding the relative role of p53-dependent apoptosis to tumor suppression in this setting. In this model, hematopoetic stem cells (HSCs) are isolated from *Eμ-myc* mouse fetal livers; after harvest, the HSCs then can be either directly injected into recipient mice, or genetically modified in culture via retroviral transduction, circumventing the laborious process of generating transgenic animals. When *Eμ-myc* transgenic, $Trp53^{+/-}$ HSCs are injected into syngeneic mice, they form tumors rapidly, with every lymphoma showing loss of heterozygosity (LOH) for *Trp53*. However, when apoptosis is blocked via overexpression of a dominant-negative form of Caspase 9 or the antiapoptotic Bcl-2 protein, *Trp53* LOH is not observed.[14] The lack of LOH suggests that no selective pressure exists to eliminate p53 in the absence of its ability to induce cell death, suggesting that in this genetically defined model of a hematological cancer, p53's primary means

of suppressing tumor formation is through the induction of apoptosis.

In work from Van Dyke and co-workers,[15,16] p53 function has been analyzed in the context of retinoblastoma protein (Rb) loss via the tissue specific expression of the portion of T-antigen involved in binding to the Rb family of proteins, termed T_{121}. In particular, this model was developed to examine p53's role in epithelia, with expression of T_{121} being driven by a choroid plexus epithelium-specific promoter. In this context, p53 also prevents tumorigenesis through its ability to induce apoptosis. In *Trp53*$^{+/-}$ animals, significant apoptosis is seen in early neoplastic lesions; however, small foci of aggressive and invasive growth show both LOH of *Trp53*, as well as a dramatic decrease in cell death.[15] Further corroborating the role of apoptosis in the prevention of these tumors, T_{121} transgenic mice on a *bax* null background display a reduced apoptotic index, and also show an accelerated onset of tumor formation.[16] Thus, in this model of epithelial cancer, p53's primary role is to eliminate pre-cancerous cells through apoptosis, prior to their development into a fully malignant state.

Although apoptosis has been shown to be a critical component of p53's tumor suppressor function in certain mouse models, the mechanism through which p53 signals a cell to die has remained unclear. Specifically, a long-standing debate in the field has been the contribution of p53's capacity to upregulate target gene expression to its ability to induce apoptosis. Whereas numerous studies have suggested that transcriptional activation is critical to p53's ability to initiate programmed cell death, recent work has suggested that transactivation-independent activities may also contribute significantly to p53 apoptotic functions.[17] Our laboratory has attempted to further dissect the role of transcriptional activation in p53's ability to induce apoptosis using knock-in mice. We described the generation of a conditional knock-in mouse in which a p53 mutant previously shown *in vitro* to be severely compromised for transactivation, termed *Trp53*^{QS} replaced the endogenous *Trp53* locus.[18] Analysis of this mouse, and cells derived from it, yielded several striking findings. While compromised for the transactivation of a number of p53 target genes, p53^{QS} shows significant transcriptional activity on certain p53 targets, such as the *Bax* gene. In addition, this mutant also displays stress-specific apoptotic activity in oncogene-expressing MEFs isolated from the conditional knock-in mice. In response to DNA damage, this mutant is completely inert, behaving indistinguishably from cells completely lacking p53 protein expression, but intriguingly, p53^{QS} shows a dramatic capacity to induce cell death in response to hypoxia. This stress-dependent apoptotic activity will provide great utility in elucidating the role specific stresses play in eliciting a p53 response *in vivo*.

While the tumor suppressor capacity of this mutant has yet to be examined, the physiological significance of this mutant's activity was also demonstrated *in vivo*. Since the p53^{QS} mutant cannot bind to Mdm2[19] and retains a subset of p53 biological functions, it was possible that expression of this mutant during embryogenesis would be lethal, similar to the lethality seen in *Mdm2* knockout mice expressing wild-type p53.[20] In fact, p53^{QS} expression induces embryonic lethality, prior to day 10.5 (unpublished data), a finding that sheds new light on the role of p53 regulation during development. As p53^{QS} lacks the capacity to respond to DNA damage, but is able to induce apoptosis upon exposure to hypoxia, it is possible that the ability of p53^{QS} to initiate cell death in response to low oxygen tensions may be the causative factor behind the lethality seen in these embryos. Extrapolating these results to those seen previously with *Mdm2* knockout mice, it may be that during early embryogenesis, in which the cells of a developing organism exist in a environment with low oxygen concentrations, the primary role of Mdm2 is to prevent p53 from being activated by this hypoxic stress and apoptotically eliminating cells of the primitive embryo. Thus, the p53^{QS} mouse has proven to be a useful tool with which to dissect p53 function both *in vitro* and *in vivo*.

Growth arrest and the maintenance of genomic stability

Apart from killing cells, p53 is also able to initiate a cell cycle arrest in response to cellular stress, as well as preserve the stability of a cell's genome. However, the contribution of these p53 effector functions to tumor suppression *in vivo* has remained largely unclear. Recent experiments by Lozano and co-workers[21] have suggested these functions may be a significant component of p53-dependent tumor suppression in certain contexts. In this model, the gene encoding a p53 mutant protein, R172P, previously shown *in vitro* to maintain the capacity to induce growth arrest but not apoptosis,[22] was introduced into the endogenous *Trp53* locus. Analysis of tissues from these mice, including fibroblasts, thymocytes, and embryonic neurons, in fact shows that this mutant has lost all apoptotic activity in response to DNA damage, but still retains significant cell cycle arrest function. Quite intriguingly, aged homozygous *Trp53*^{R172P} mutants develop cancers with a significantly increased latency, and the malignancies that eventually develop do not contain the genetic heterogeneity, in terms of aberrant chromosome number, characteristic of lymphomas from *Trp53* null animals, suggesting a role for the suppression of genomic instability in p53 tumor suppressor function. This function of p53 has also been described previously in a mouse model of breast cancer in which *Wnt-1* is expressed from the MMTV-promoter. In this model, described by Donehower *et al.*,[23] tumors lacking p53 expression showed significantly higher levels of aneuploidy than wild-type tumors, as well as numerous regional amplifications and deletions. Moreover, no correlation is observed between *Trp53* status and apoptotic indices in the tumors.[24] Together, these findings support the idea that the downstream function of p53 responsible for tumor suppression does not exclusively center on its ability to induce cell death.

Additional research supporting a role for nonapoptotic functions of p53 in suppressing spontaneous tumorigenesis was reported by Bulavin and co-workers.[1] In this study, a transgenic mouse strain was generated in which multiple copies of human *TP53* were inserted into the genome of *Trp53* null mice. The authors found that full-length human p53 in the context of the mouse is defective in multiple aspects of normal p53 function, including the ability to induce apoptosis, activate

G1 arrest, and transactivate p53-target genes, but retains other facets of p53 function, such as the ability to transrepress genes and to maintain a normal centrosome number. These mice are susceptible both to radiation- and oncogene-induced cancers, but are significantly protected from spontaneous tumorigenesis, in that the development of thymic lymphomas, the most common tumor type seen in *Trp53* null mice, was dramatically delayed. These results, along with those described above, strongly suggest that nonapoptotic functions of p53, such as the regulation of the centrosome duplication cycle and the ability to maintain genomic stability, significantly contribute to tumor suppression in specific contexts.

Returning to the *Eμ-myc* lymphoma model described previously, Lowe and co-workers[25] showed that, surprisingly, *Trp53*$^{+/-}$ lymphomas overexpressing either Bcl-2 or a dominant-negative Caspase 9, as described above, still undergo a p53-dependent cytostatic response to chemotherapy. Administration of cyclophosphamide, an alkylating agent utilized clinically in the treatment of a number of different cancers, including hematopoetic malignancies, results in cancers that do not regress, but also, do not progress. A strong selective pressure to undergo *Trp53* LOH after drug administration is also evident, and this LOH is invariably associated with disease progression. Careful molecular analysis showed that in the absence of the ability to stimulate cell death, p53 acts by inducing a permanent exit from the cell cycle known as cellular senescence. This direct demonstration of p53's ability to induce cellular senescence *in vivo* strongly suggests that, at least in this setting, p53's tumor suppressor activity may not rely entirely on its ability to induce apoptosis. Importantly, these findings suggest further that the p53 downstream function involved in tumor suppression may be highly dependent on cellular context, a topic that we will revisit later.

Upstream Signaling to p53

As might be expected for a protein sitting at the nexus of a number of different signaling pathways, the p53 protein is the target of a number of post-translational modifications, including phosphorylation, acetylation, neddylation, ubiquitylation, sumoylation, and methylation (reviewed in Bode and Dong[26]). The majority of these modifications occur at a variety of amino-acid residues in the amino- and carboxyl-termini of the protein. While a number of *in vitro* studies have been performed to analyze the relative contribution of these numerous modifications to p53 function, the results have often been conflicting. Thus, the analysis of these post-translational events in a setting of physiologically relevant expression, and in diverse tissue types, becomes even more crucial to definitively understanding their role in modulating p53 activity. In what has become a recurring theme in this review, the generation of knock-in mice, in which residues critical for specific post-translational modifications of p53 are mutated, will be crucial to understanding this process.

Phosphorylation of S18 in mouse p53 (corresponding to S15 in the human protein) has been proposed to have a number of different effects on p53 function, including enhancing its stability by interfering with Mdm2 binding, as well as stimulating effector responses such as the induction of a growth arrest or apoptosis in response to DNA damage.[27,28] A number of different kinases have been implicated in the phosphorylation of this residue, most prominent among these being the ATM kinase.[29–31] To determine the role of this modification *in vivo* and in p53's role in tumor suppression, knock-in mice changing codon 18 from serine to alanine were generated by Jones and co-workers[32] and Xu and co-workers.[33] Both studies reveal that mutation of this amino acid does not have any significant impact on the stability of either basal or DNA damage-induced p53 protein levels, contradicting what had been suggested from *in vitro* data. However, this mutation partially compromises p53's ability to induce cell death in thymocytes treated with ionizing radiation, with levels of apoptosis intermediate between cells expressing wild-type p53 and those lacking p53 protein expression. Xu and co-workers also describe reduced transactivation, but normal DNA binding, in both thymocytes and MEFs from these mice, as well as diminished c-terminal acetylation of a variety of lysine residues (the role of which will be discussed later), suggesting the phosphorylation of p53 at S18 may be important for the recruitment of transcriptional cofactors such as p300, a histone acetyl transferase previously shown to acetylate p53. Intriguingly, in both studies, the S18A mutation does not predispose the animals to spontaneous tumorigenesis, suggesting this modification is not essential for p53's ability to suppress the formation of thymic lymphoma and other tumors seen in mice lacking p53 expression.

Similar to S18, phosphorylation of S23 in mice (corresponding to S20 in humans) has been proposed to be critical for inducing the dissociation of Mdm2 from p53, resulting in p53 stabilization. Xu and co-workers[34] and Jacks and co-workers[35] have recently described the generation of knock-in mice in which S23 of p53 has been mutated to alanine. In the study by Jacks and co-workers, the S23A mice display a partial compromise in p53-dependent apoptosis in thymocytes treated with ionizing radiation, whereas Xu and co-workers observed no difference between wild-type p53 and the S23A mutant in this same assay. In the former study, a partial reduction in p53 stability in thymocytes post-irradiation was observed, suggesting a possible increased affinity of Mdm2 for p53 after stress, and potentially providing an explanation for the modest apoptotic defect.[35] However, decreased protein stability was not observed in mouse embryo fibroblasts in either study, suggesting that the relative contribution of this modification to stability may in fact be cell-type dependent. Again, and similar to the S18A mice described above, these mice are much more resistant to tumorigenesis than mice completely lacking p53, but surprisingly, and now in contrast to the S18A mice, the S23A mice eventually die from tumors with a mean latency of approximately 1.5 years.[35] These tumors are predominantly of B-cell origin, and include follicular lymphomas, plasmocytomas, and diffuse large B-cell lymphomas. The reason for this particular tumor spectrum is unclear, but could relate to specific roles of p53, and in particular this modification, in monitoring the various stages of B-cell maturation. However, like the S18A mutant, this modification seems to be completely dispensable for p53's role in protecting mice from developing thymic lymphoma. Thus, future studies of the specific downstream activities of

this mutant in both B- and T-lymphocytes may reveal the functions of p53 critical for suppressing cancers arising from these cell types. Also, the generation and analysis of mice containing both S18A and S23A mutations will be very informative for understanding any functional overlap between modification of these residues.

As mentioned above, the p53 protein responds to a number of different cellular stresses. Certain residues, such as S18, are commonly phosphorylated in response to a variety of stresses (reviewed in Giaccia and Kastan[12]), while others seem to be restricted to a specific stress. A prominent example of this is the phosphorylation of S389 (S392 in humans) in response to UV radiation, a modification which does not occur in cells exposed to double-strand-break inducing agents.[36,37] De Vries and co-workers[38] generated a knock-in mouse in which S389 of p53 was mutated to alanine, and analysis of this mouse has yielded several intriguing results. Whereas alteration of this residue has no effect on protein stability either in a basal state or in response to UV-C irradiation, MEFs derived from these mice are modestly compromised in their apoptotic response to UV when compared to wild-type cells. In addition, the capacity of the S389A mutant to activate the transcription of several canonical p53 target genes after UV-C irradiation is also reduced. However, thymocytes derived from these mice display a normal response to gamma-irradiation, confirming that phosphorylation at this residue is specifically required for the response to UV-C radiation. Further validating these findings, S389A mice are not prone to spontaneous tumorigenesis, but show a somewhat enhanced predisposition to skin tumor formation in response to UV-B treatment. Further study of this mouse will help determine the molecular rationale for the requirement of modification of this particular residue solely in the response to UV radiation, but not double-strand break inducing agents.

Acetylation of p53 has been implicated in several aspects of p53 function, including stimulating p53 stabilization, DNA binding, and transactivation (reviewed in Bode and Dong[26]). In particular, several lysine residues at the carboxyl-terminus of p53 have been reported to be the target of acetylation after DNA damage, as well as the target of ubiquitylation of p53 in the absence of a cellular stress. Recent work described by Xu and co-workers,[39] as well as Wahl and co-workers,[40] has attempted to clarify the functional role of the modification of these residues through the generation of knock-in mice (termed K6R and K7R, respectively) in which these C-terminal lysines are mutated to arginines, which cannot undergo acetylation or ubiquitylation but still retain the positive charge characteristic of lysine residues. Whereas Wahl and co-workers[40] chose to mutate all seven lysines in the C-terminus of murine p53, Xu and co-workers[39] only mutated the six lysines conserved between mouse and human p53. Analysis of the K6R mutant in embryonic stem cells, thymocytes, and MEFs show these mutations do not dramatically affect the overall stability of p53.[39] However, they do affect the ability of p53 to activate transcription of several p53 target genes in ES cells and thymocytes, but not in fibroblasts, upon treatment with DNA damaging agents. Intriguingly, the two genes that show dramatically reduced levels of expression in K6R thymocytes are the proapoptotic targets *Killer/DR5* and *Puma*, but nonetheless, the K6R thymocytes are not appreciably resistant to apoptosis when compared with wild-type thymocytes. These data suggest C-terminal lysine acetylation plays an important role in p53's ability to upregulate certain target genes but not others. The study by Wahl and co-workers[40] showed similar results with regard to the lack of an effect of these mutations on p53's stability or transactivation potential in MEFs, and examination of p53-dependent induction of G1 arrest and apoptosis in this cell type also showed the K7R mutant to behave like wild-type p53. However, and in contrast to the data from[39], they observed that the K7R mutant was stabilized more rapidly than wild-type p53 in thymocytes in response to gamma-irradiation. Likewise, they also found this mutant is hyperactive in upregulating p53 target genes upon treatment of thymocytes with gamma-irradiation, when compared with wild-type p53. Along these lines, MEFs expressing this mutant appear more resistant to spontaneous immortalization than MEFs expressing wild-type p53 after serial passaging for extended periods of time. The authors hypothesize that modification of these residues serves to 'fine-tune' the stress response of p53, and further, that even small changes in p53 activity that only produce phenotypically subtle effects, may be in fact biologically important in the context of p53's function as a tumor suppressor. Once again, the results derived from analysis of the above-described mice provide support for the hypothesis that post-translational modifications of p53 may be functionally relevant only in certain cell types or in response to specific environmental cues, thus providing a rationale for why studies in culture utilizing different cell types and diverse stresses would produce conflicting results concerning the requirement of these modifications for p53 function.[41]

While it is known that p53 is modified after stress, the temporal requirement for p53 presence after different stresses has been largely unexplored. Recent work by Evan and co-workers[42] describes the generation of a knock-in mouse in which a p53- (ER) (p53-ER) fusion replaced the wild-type allele. Fusion of the ligand-binding domain of this modified version of the ER to a protein renders the protein activatable by synthetic, but not natural, forms of estrogen. Using this system, the p53 protein is sequestered in the cytoplasm in a complex with heat-shock proteins until the ER domain fused to p53 is bound by the synthetic estrogen analog 4-hydroxytamoxifen (4-OHT), upon which p53 dissociates from the heat shock proteins and translocates to the nucleus. Thus, this model allows for the rapid transition of tissues in an animal from a *Trp53* null state to a wild-type state, and *vice versa*. ER fusions *versus* other forms of conditional protein expression, such as the Cre-LoxP system, have an added bonus in that the changes in protein activity are reversible. This study analyzed the temporal requirement for p53 activation in response to stresses such as DNA damage.[42] The data suggest that the reaction of p53 to an acute stress such as DNA damage is highly dependent on p53 being present to receive an initial 'signal' from an upstream stress sensor, such as the ATM protein kinase; if functional p53 protein is absent directly after the stress, but is then activated via 4-OHT treatment at a later time, a p53 response will not be initiated. In contrast, the response of p53 to a more persistent stress, such as the activation of an oncogene, yields a different

result. In this context, addition of 4-OHT, regardless of timing relative to oncogene expression leads to the activation of the p53 pathway. For example, in this work, the authors introduced oncogenic *H-RAS* into MEFs and showed that even if p53 is activated nearly two weeks later, the p53 protein can still elicit a potent growth arrest indistinguishable from that seen in animals constitutively expressing wild-type p53. Further studies utilizing this mouse strain, and cells derived from it, in the context of different mouse models of cancer will be extremely useful for understanding the requirement for p53 activity at various stages of tumor development.

A New Role for p53: Organismal Aging

By inducing either apoptosis or growth arrest, the p53 protein is able to prevent the expansion of cancerous cells. However, recent studies have suggested that while constitutively high levels of active p53 may indeed provide enhanced protection from malignancy, this may have the negative consequence of inducing a premature aging phenotype. Furthermore, it may be that the same p53 effector functions that prevent malignant growth, namely the ability to induce either apoptosis or cellular senescence, are also the causative factors behind premature aging. This connection between p53 and aging was originally described by Donehower and co-workers.[43] In this study, a mistargeting event at the *Trp53* locus in embryonic stem cells produced a p53 truncation mutant, termed 'p53^{m}', which, in a heterozygous background, confers significantly enhanced tumor suppressor capacity. However, these mice also display decreased longevity and many phenotypes associated with aging, such as osteoporosis, lordokyphosis and reduced body mass. The interpretation of these data is that the fragment of p53 produced from the *Trp53m* allele is able to interact with the wild-type version of the protein and enhance its activity, thus producing both enhanced tumor suppressor capacity as well as premature aging. In a similar study, Scrable and co-workers[44] generated transgenic mice expressing a truncated form of p53, p44, that has previously been reported to be an endogenous p53 isoform. These mice also manifest a premature aging phenotype similar to that seen by Donehower and co-workers.[43] Furthermore, this study suggests that this truncated version of p53, in a manner that also requires the presence of the full-length p53 protein, may in fact have a role in normally regulating lifespan due to its effects on the insulin/insulin-like growth factor signaling pathway, a network that has been frequently associated with regulating the longevity of an organism.

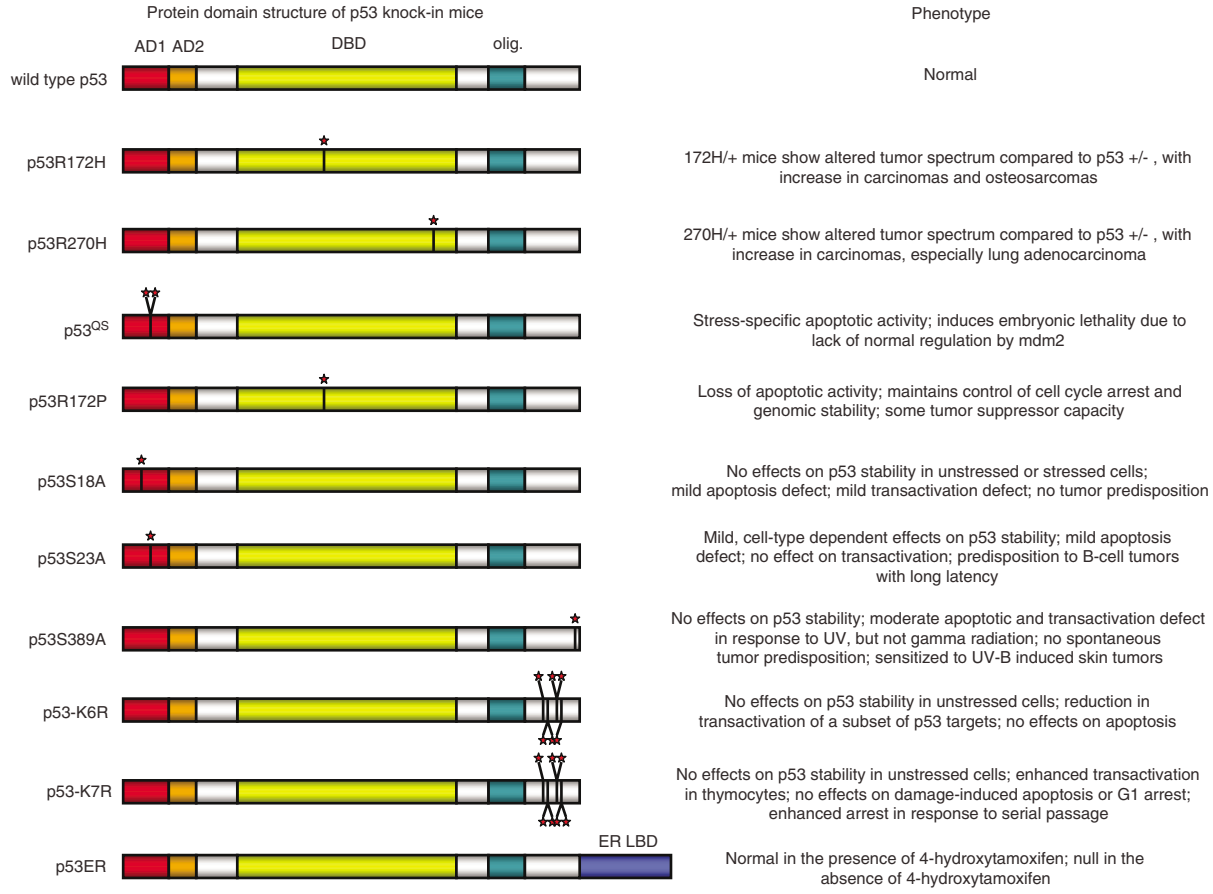

Figure 1 Summary of existing *Trp53* knock-in mice. The p53 mutants produced from the knock-in mouse strain constructs described in this review are represented, along with any associated phenotypes. Red stars denote a mutated amino-acid residue in relation to the wild-type amino-acid sequence depicted at the top of the chart. AD1, transactivation domain 1; AD2, transactivation domain 2; DBD, sequence specific DNA binding domain; Olig., tetramerization domain; ER LBD, estrogen receptor ligand-binding domain

In further experiments meant to assess the role of p53 in aging, Serrano and co-workers[45] used BAC constructs to generate transgenic mice carrying a third copy of the *Trp53* gene. The use of BACs allowed the inserted *Trp53* locus to be insulated from position effects resulting from its integration site in the genome, as well as maintaining normal temporal and spatial expression due to the presence of its endogenous regulatory elements. While these mice possess an enhanced DNA damage response as well as an increased resistance to chemical-carcinogen induced tumorigenesis, they do not show any signs of premature aging. Thus, it may be that expression of an activated form of p53 results in improved tumor suppressor capacity, but has the repercussion of also causing premature aging, whereas increasing the dosage of p53, but maintaining its normal regulation, leads to enhanced tumor suppression but not rapid aging. Understanding this potential paradox will have significant therapeutic implications, since several groups have already discovered small molecules that can activate the p53 response *in vivo*. Further work will be necessary to determine whether this form of therapy may solely have anti-tumor effects, or whether it may also have the undesirable side-effects of activating p53 in non-tumor derived cells and inducing premature aging (Figure 1).

Conclusion

While many questions remain unanswered regarding p53's function as a tumor suppressor, the studies described above have provided significant insight into the mechanism of p53 action. Further exploration into how different p53 effector functions prevent tumorigenesis, as well as which signals are critical for the activation of p53, are central to understanding how a normal cell can give rise to numerous progeny that eventually develop a host of characteristics commonly associated with cancer. The continued generation of mouse models of cancer that are more representative of their human counterparts, as well as dissecting p53 downstream pathways *in vivo* in multiple cellular contexts, will be crucial to developing a more complete understanding of how p53 suppresses cancer in humans.

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