Control of Genomic Instability and Epithelial Tumor Development by the p53-Fbxw7/Cdc4 Pathway

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Abstract

Mouse models of cancer have provided novel insights into the timing of p53 loss during tumorigenesis. We have recently identified Fbxw7/Cdc4 as a downstream target of p53 loss that controls genomic instability and tumor development in epithelial tumors. Although p53-deficient mice primarily develop lymphomas and sarcomas, the additional loss of one copy of the Fbxw7 gene drives tumor development in a range of epithelial tissues. These data highlight the importance of genetic instability at the chromosome level in the development of common cancer types, and further illustrate the value of mouse models in identifying causal genetic events in epithelial tumor formation.

Background

Although the p53 tumor suppressor gene has been studied for more than two decades, many important questions regarding its disparate roles in tumor formation remain to be answered. For example, what is the timing of loss of p53 during tumor initiation or progression? Which secondary genetic events are required for tumors to develop from p53 heterozygous or null cells? Why do p53-deficient animals fail to develop epithelial tumors, in spite of the fact that a large proportion of human epithelial tumors have p53 mutations? In order to address these questions, we have exploited a genetic model of radiation-induced lymphoma in p53-deficient mice. Mice heterozygous for p53 (+/−/−) develop tumors after a considerably longer latency period than their homozygous null counterparts (1, 2), presumably because of a requirement for loss of the remaining wild-type p53 allele. However, the exact timing of the loss of the remaining wild-type p53 gene is unknown, and the possibility that other genetic events occur prior to complete somatic loss of p53 has not been investigated. To assess this possibility, we compared the spectrum of genetic alterations in p53 null (p53−/−) lymphomas originating from p53+/−/− animals with those that developed in p53−/−/− mice. If loss of the wild-type p53 allele in p53+/−/− mice were the first somatic genetic lesion, we would expect the overall pattern of genetic changes to be very similar to that of tumors from p53−/−/− mice. However, if additional events were required for the growth or survival of somatic cells that suffer a second hit in the p53 gene, we would expect to find certain genetic changes exclusively in tumors from the p53+/−/− mice. Identification of such downstream p53 pathway genes might be expected to provide novel insights into the rate-limiting effector functions of p53 in lymphoma development.

Strategy and Key Observations

This study exploited the advantages of interspecific mouse crosses to investigate the patterns of genetic changes in p53-deficient tumors. With this strategy, it was possible, using microsatellite analysis, to determine the parental origin of alleles showing loss of heterozygosity (LOH), specifically in tumors from p53+/−/− or p53−/−/− mice. A surprising finding was that tumors from p53+/−/− mice in fact showed a greater degree of LOH than tumors from p53−/−/− mice. A subset of chromosomal imbalances involving chromosomes 3, 16, and 18 were found exclusively or mainly in tumors from p53−/−/− mice. In contrast, other changes were present in tumors from both p53+/−/− and p53−/−/− mice, e.g., on chromosomes 6, 12, and 19, which were therefore independent of p53 status.

LOH on chromosome 3 was found in almost 100% of the lymphomas from F1 p53+/−/− and in >50% of the F1 backcross p53+/−/− mice, but only at background levels in tumors from p53+/−/− mice. The high frequency of LOH on this chromosome suggested the presence of an important p53-dependent tumor suppressor in lymphoma development. By microsatellite analysis of tumors from two different crosses, we eventually focused on the Fbxw7/Cdc4 gene as the only viable candidate. This gene encodes a ubiquitin ligase essential for the degradation of important targets in tumor development (3–11), and has been found to be mutated in several primary human tumors (3, 12–14). The sequence of the Fbxw7/Cdc4 gene showed p53-dependent up-regulation of Fbxw7/Cdc4 DNA responsive elements in the Fbxw7/Cdc4 promoter and showed p53-dependent up-regulation of Fbxw7/Cdc4 mRNA levels in mouse embryonic fibroblasts after γ-radiation. These data confirmed a previous study carried out with human Fbxw7/Cdc4 that suggested it was a p53-transcriptional target (15).

Further in vitro studies confirmed the relationship between Fbxw7/Cdc4 and p53 in control of cell growth and genomic instability. However, whereas we were able to show that loss of Fbxw7/Cdc4 led to up-regulation of Notch4 and c-Jun, in agreement with previous reports, we did not see an effect in this system on levels of cyclin E, thought to be the main mediator of genetic instability due to loss of hCDC4 (12). We therefore tested the levels of other possible targets implicated in

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genomic instability, among them Aurora A, a well-known target implicated in genomic instability and aneuploidy (16). These experiments showed that Fbxw7/Cdc4 down-regulation indeed leads to increased expression of Aurora A, but further studies are required in order to show if this is a direct or an indirect effect.

The consequences of Fbxw7/Cdc4 loss for tumor development in vivo were also investigated. Complete loss of Fbxw7/Cdc4 function causes early embryonic lethality (17, 18), and Fbxw7/Cdc4−/− heterozygous mice do not develop spontaneous tumors at a significant frequency. However, 30% develop tumors after exposure to γ-radiation, mainly lymphomas, but also some epithelial tumors after a long latency period (50 weeks). Interestingly, loss of one allele of Fbxw7/Cdc4 only accelerated tumor development in p53+/− mice, but not in p53−/− mice, in agreement with our in vitro studies. On the other hand, tumors from Fbxw7/Cdc4 and p53 double heterozygous mice showed loss of the remaining Fbxw7/Cdc4 allele in only 2 out of 10 tumors investigated; in the remaining tumors, mRNA was detected but no mutations were found, suggesting that Fbxw7/Cdc4 is a haploinsufficient tumor suppressor gene. Importantly, whereas p53+/− mice developed mainly lymphomas and fibrosarcomas, Fbxw7/Cdc4 and p53 double heterozygous mice also developed tumors in tissues of epithelial origin such as lung, liver, biliary ducts, colon, pancreas, and the ovary. Loss of Fbxw7/Cdc4 therefore exerts a profound influence on the spectrum of tumors that develop in p53-deficient mice.

**Implications**

**The timing of p53 loss in tumor development.** The data generated using this mammalian genetic screen for p53 pathway genes allow us to address several of the questions outlined earlier in this article regarding the timing of genetic events in tumor formation. We conclude that p53 itself cannot be the primary genetic “target” for deletions in tumors that develop in irradiated p53+/− mice, because in this case, the subsequent pattern of genetic changes in the tumors would be expected to be the same as those from the irradiated p53 null mice. The loss of the wild-type allele must presumably be preceded by loss of the Fbxw7/Cdc4 gene on chromosome 3, as well as additional genes on some other chromosomes (Fig. 1).

This interpretation is supported by our in vitro and in vivo data demonstrating that loss of Fbxw7/Cdc4 does not provide any selective growth advantage in the mouse embryonic

![Figure 1](https://www.aacrjournals.org)
fibroblasts from \textit{p53}\textsuperscript{−/−} mice. The fact that several loci have to be inactivated prior to loss of the single wild-type \textit{p53} allele suggests that there is some disadvantage for early tumor cells that suffer complete loss of \textit{p53} function. Because \textit{p53} is such a potent tumor suppressor gene, there may be strong selection against its acute loss in somatic cells, possibly involving induction of a strong \textit{p53}-independent cell death mechanism (19). Identification of the additional genes that show \textit{p53}-dependent or -independent deletions using this genetic screen in the mouse will help us to understand the sequence of genetic events leading from DNA damage to epithelial tumor development.

\textbf{\textit{p53} loss and epithelial tumor development}. Mutations in the \textit{p53} gene are very common in human carcinomas of the skin, colon, lung, and pancreas. Nevertheless, germ line inactivation of the \textit{p53} gene in mice rarely results in the formation of epithelial tumors in these tissues (1). There are at least two possible explanations for this phenomenon. One is that signaling through a wild-type \textit{p53} pathway is necessary to initiate genomic instability. In agreement with this interpretation, we have shown that germ line loss of one \textit{Fbxw7/Cdc4} allele causes predisposition to development of a spectrum of tumors in epithelial tissues, but only when at least one allele of \textit{p53} is expressed. The particular reasons for the altered tumor spectrum are still unclear. The observation of frequent LOH and mutations in \textit{Fbxw7/Cdc4} specifically in tumors from \textit{p53}\textsuperscript{−/−} mice suggests that this pathway represents a bottleneck through which these cells must pass in order to progress to the tumorigenic state. Mice devoid of \textit{p53} fail to activate this checkpoint, and cells with mutations in \textit{Fbxw7/Cdc4} are not selected. The data suggest that \textit{Fbxw7/Cdc4} could play a particularly important role in the protection of epithelial tissues from the consequences of DNA damage, possibly through regulation of important targets implicated in genomic instability such as cyclin E, cMyc, or Aurora A (3–5, 9, 10, 20). Results obtained using other mouse models support the interpretation that loss of \textit{Fbxw7/Cdc4} causes increased genomic instability through deregulation of these target proteins. \textit{Fbxw7/Cdc4} forms a complex with Skp1, leading to targeted degradation of target proteins through the Skp1/Cul1 ubiquitination-degradation system. Functional inactivation of Skp1 in the T cell lineage leads to a phenotype remarkably similar to that observed in \textit{Fbxw7/Cdc4}-deficient mice: karyotype heterogeneity associated with c-Myc overexpression, together with the formation of multinucleated cells, centrosome and mitotic spindle abnormalities, and impaired chromosome segregation (21). These data suggest that functional inactivation of different components of this protein degradation pathway have similar consequences in control of genomic instability.

However, a second, more trivial explanation for the absence of epithelial tumors in \textit{p53} null mice is that the animals...
simply die from lymphoma or sarcoma development before they have enough time to allow carcinoma formation. Indeed, early analyses of human epidemiology data have suggested that the number of genetic events required for carcinoma formation is greater (from 6 to 10) than is required for development of hematopoietic malignancies or sarcomas (22, 23). In support of this possibility, studies of other mouse models of cancer involving tissue-specific inactivation of p53 in epithelial tissues have shown that carcinomas can arise from p53 null epithelial cells. The latency for epithelial tumor formation in the skin and mammary gland is relatively long, but can be strongly accelerated when combined with other genetic alterations such as conditional loss of Rb (24) or BRCA2 (25) in the same cells.

Other mouse models have also resulted in an increased incidence of tumors in epithelial tissues, but always in the context of partial or complete deficiency of p53. For example, breeding of mice carrying inactive alleles of the telomerase RNA component (Terc−/−) with p53+/− mice gave rise, after several generations of reduced telomere length, to a series of epithelial cancers (26). A slight increase in the frequency of carcinomas, as well as increased metastasis, was seen in mice with a germ line mutation in the p53 gene similar to that found in some patients with Li-Fraumeni syndrome (27). More recently, additional studies on germ line p53 mutant mice have revealed increased epithelial tumor formation (28, 29).

The latter two studies provided evidence for gain of function of certain mutant p53 alleles in vivo, but also raised some additional questions. Olive et al. (28) showed that the p53R172H mice developed epithelial tumors in the absence of a wild-type functional p53 allele, providing clear evidence for a gain of function rather than dominant-negative role for p53 mutations. Similar mouse generated by Lang et al. (29), however, showed increased carcinoma formation (metastasis) only when the wild-type allele was present. Although some of these differences may be due, as proposed by the authors, to “strain background effects,” it is clear that additional studies will be required to elucidate the mechanisms of interplay between the wild-type and mutant p53 alleles, as well as the influence of the other p53 family members p63 and p73.

In the scheme shown in Fig. 2, all of these models converge at the point of control of genetic instability in epithelial cells. Loss of Fbxw7/Cdc4 in the context of p53 heterozygosity leads to aneuploidy and epithelial tumor development. Loss of telomeres in p53+/− mice also leads to genetic instability by a different mechanism, but has similar consequences in the development of epithelial tumors (26). Moreover, previous studies both in vitro (30) and in vivo (31) have implicated defective mitotic spindle checkpoints and increased aneuploidy as a cause of genetic instability and transformation by the p53R172H mutant p53 allele. Epithelial cells, because of their capacity for continuous renewal, combined with their repeated exposure to exogenous carcinogenic insult, may have evolved higher threshold levels of controls for genetic stability. Only when these are exceeded through gain of function mutations in p53 and/or loss of specific controls of genetic instability such as those mediated by Fbxw7/Cdc4, do cells within epithelia acquire the necessary momentum for progression to carcinomas. In summary, epithelial cell transformation may require a minimum threshold level of genetic instability that is not normally attainable by loss of p53 function, and is only reached after an additional increase in genomic instability by a multiplicity of different mechanisms.

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