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REVIEW

Transgenic and Knockout Mice Models to Reveal the Functions of Tumor Suppressor Genes

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Abstract: Cancer is caused by multiple genetic alterations leading to uncontrolled cell proliferation through multiple pathways. Malignant cells arise from a variety of genetic factors, such as mutations in tumor suppressor genes (TSGs) that are involved in regulating the cell cycle, apoptosis, or cell differentiation, or maintenance of genomic integrity. Tumor suppressor mouse models are the most frequently used animal models in cancer research. The anti-tumorigenic functions of TSGs, and their role in development and differentiation, and inhibition of oncogenes are discussed. In this review, we summarize some of the important transgenic and knockout mouse models for TSGs, including *Rb*, *p53*, *Ink4a/Arf*, *Brca1/2*, and their related genes.

Keywords: p53, Rb, Ink4a/Arf, BRCA, tumor suppressor genes, transgenic mice, knockout mice, mouse models, ageing

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Introduction

Cancer is induced by genetic and environmental factors. Cancer can be categorized as familial (inherited) or sporadic, the latter comprising the vast majority of cases. Germline mutations predispose an individual to familial cancers. When the function of remaining wild-type allele is lost in a somatic cell, additional mutation(s) on the wild-type locus can accumulate that lead to tumor formation. In sporadic cancers, all tumorigenic mutations are somatic and are present only in the patient's neoplastic cells. In humans, mutations leading to gain of functions of proto-oncogenes or loss of functions of tumor-suppressor genes (TSGs) predispose to cancer. TSGs can be divided into two groups: "gatekeepers" and "caretakers".[1–3]

Gatekeepers regulate the growth of tumors by inhibiting their proliferation or promoting their apoptosis. Caretakers control cellular processes that repair genetic alterations and maintain genomic integrity. Mutations of caretakers can result in an increase in the overall mutation rate in a given cell, an apparent prerequisite for tumorigenesis. The accumulation of mutations in a dysregulated cell favors the subsequent clonal selection of variant progeny with aggressive growth properties leading to malignancy. Familial cancers often result from the initial germline mutation of one allele of a TSG followed later by somatic mutation or loss of the second allele, a process known as loss of heterozygosity (LOH). Knudson's two-hit cancer model[4,5] is based on this repeated observation. Although LOH was originally a prerequisite for the identification of canonical TSGs, the remaining wild-type allele can also be transcriptionally silenced by hypermethylation of its CpG regions. Functional loss then leads to tumorigenesis.

Animal models have been instrumental in the study of genes involved in human cancer initiation and progression. Spontaneous as well as carcinogen-induced malignancies have been studied in dogs, rats, and mice, and TSGs identified in Drosophila have led to the discovery of mammalian orthologues. The availability of null and tissue-specific mouse mutants for tumor-suppressor genes has greatly facilitated our understanding of the mechanisms leading to cancer.

The mouse remains the animal model of choice for several reasons. First, mice and humans have roughly the same number of genes, and intracellular signaling pathways are highly conserved between the two species. Second, the success of genetic engineering in mice has allowed the study of gene functions in vivo. Mice can be genetically manipulated to overexpress (by transgenesis), or not express (by gene targeting), a specific cancer gene. These animals offer unique opportunities to uncover cellular pathways controlled by specific TSGs and to dissect mechanisms underlying malignancies that may closely resemble those in humans. In addition, the interbreeding of an increasing number of mice with specific mutations in both TSGs and oncogenes allows the assessment of how these mutations cooperate to produce cancer.

Tumor models in mice include (1) mice in which cancer is caused by intrauterine or postnatal exposure to chemical mutagens, and mice in which tumors are produced by viral or bacterial infection, (2) xenograft models that were generated by directly implanting cancer cell lines established from human tumors into mice have been widely used for drug discovery, and (3) genetically engineered mouse (GEM: constitutive or conditional transgenic, knockin, and knockout) cancer models. The major limitations of xenograft models are the requirement for an immunocompromized host and the inability of these models to fully recapitulate the complex relationship between the tumor and its microenvironment (eg, angiogenesis). Most importantly, the ability of xenografts to accurately predict drug efficacy in human cancer patients has been disappointing. In contrast, GEM cancer models are becoming more and more sophisticated in their ability to accurately mimic the histology and biological behavior of human cancers.[6,7] Numerous tissue-specific GEM models have been developed that exhibit many biologic hallmarks of human cancer, including angiogenesis and stromal interactions, as well as similar histopathologic and genetic abnormalities. The major advantages of GEM models are that (i) the initiating genetic event is known, (ii) the mice are immunocompetent, and (iii) the tumors develop spontaneously in their appropriate tissue compartments. Moreover, GEM cancer models are particularly useful for conducting preclinical studies of rare cancers and for assessing synergy between therapeutic agents since they allow assessment of therapeutic efficacy on a uniform genetic background. They can also potentially provide the tools needed

to learn more about the histological and biochemical effects of specific agents prior to human testing.

In this review, we have focused on recent progress in transgenic and knockout mouse models for TSGs involved in apoptosis and the cell cycle, DNA damage repair, cell signaling, and differentiation, and their clinical implications. Special emphasis is placed on conditional models, knock-in models, and transgenic models of TSGs.

p53 models

Mouse knock-in *p53* mutation models are beginning to establish clear p53 tumor suppressor genotype-phenotype relationships. Previous studies identified a hot spot variant p53 mutation (R175H) in sporadic human tumors. R175H functions normally in blocking cell cycle progression, but is deficient in promoting apoptosis, similar to the R175L variant. Both $p53^{R270H/+}$ and $p53^{R172H/+}$ mice are models for human Li-Fraumeni syndrome, and importantly develop epithelial tumors that are not found in $p53^{-/-}$ mice, including carcinomas in the lung, small intestine, colon, breast, skin, liver, and pancreas, and more frequent endothelial tumors. Thus, point mutant *p53* alleles expressed under physiological control have enhanced oncogenic potential beyond the simple loss of *p53* function. *p53* gain-of-function knock-in models develop carcinomas rather than sarcomas within one year, and are thus more faithfully reproduce human carcinomas than classical *p53* knock-out models. These novel *p53* knock-in mice will be useful in drug screens for carcinomas. Mutant mice that constitutively express activated p53 showed enhanced resistance to spontaneous tumors compared with wild-type littermates and displayed an early onset of phenotypes associated with ageing.

p53, located on the human chromosome 17p13.1, is one of the most extensively studied TSGs.[6–10] Li-Fraumeni syndrome is caused by inherited *TP53* mutations and associated with high susceptibility to breast and lung carcinomas, soft tissue sarcomas (STSs) brain tumors, osteosarcoma, and leukemias. The *p53* transcript encodes a 393-aa nuclear phosphoprotein of 53 kDa. The p53 protein exists as a tetramer exhibiting its tumor-suppressive effect by binding to DNA at specific sites. p53 activity is modulated by protein stability, regulated largely through interactions with the E3 ligase Mdm2.

Binding of Mdm2 to p53 leads to p53 degradation and loss of activity, a process that can be inhibited by the gatekeeper protein Arf (Fig. 1). Post-translational modifications mediate p53 activity by phosphorylation, acetylation, sumoylation, or glycosylation. p53 activation is triggered by hyperproliferative mitogenic signals mediated through deregulated Myc, Ras, or E2F-1, which in turn mediates the induction of Arf expression which promotes p53 stabilization and activation (Fig. 1).[9] p53 activity is also induced in response to DNA damage and involves the regulatory kinases ATM, CHK2, and ATR (Fig. 1).

Activated p53 acts as a regulator of cellular processes such as cell cycle, apoptosis, autophagy, and differentiation. Numerous p53 transcriptional targets have been identified, including $p21^{Cip1/Waf1}$, *Mdm2*, *Bax*, *Puma (Bbc)*, *Noxa*, *Pig8*, *Gadd45*, *Pidd*, *p53R2*, *DR5*, *cyclin G*, *Pten*, and *CD9*.[8] Conversely, p53 transcriptionally represses *bcl-2*, *PCNA*, and *Arf*. p53 activation controls the G1 checkpoint of the cell cycle by inducing transcription of the cyclin/Cdk inhibitor $p21^{Cip1/Waf1}$. Cells with damaged DNA are prevented from entering S phase and replicating the defective chromosomes. p53 controls the G2 checkpoint by regulating expression of $p21^{Cip1/Waf1}$ and *Snk/Plk2*.[11,12] The arrest of the cell cycle at these checkpoints allows repair of the damaged DNA. Transcriptional activation of pro-apoptotic genes including *Bax*, *Puma*, *Noxa*, *PIDD*, *DR5*, and *CD95* are dependent on p53.

p53 knockout models

Four strains of mice bearing null mutations of *p53* have been created.[13–16] Although initial studies of $p53^{-/-}$ mice concluded that p53 had no role in development, subsequent work has revealed that a subset of *p53*-deficient embryos dies in utero. This embryonic death is associated with exencephaly marked by defects in neural tube closure and overgrowth of neural tissue in the midbrain region.[17,18] The finding that $Mdm2^{-/-}$ mutants, which die before E6.5, can be fully rescued in a *p53*-null background suggests that p53 activity must be tightly regulated by Mdm2 for successful development.[19,20]

As expected, $p53^{+/-}$ mice are predisposed to tumorigenesis. These animals remain cancer-free for the first 9 months, but half develop osteosarcomas, soft tissue sarcomas, and lymphomas by 18 months of age.

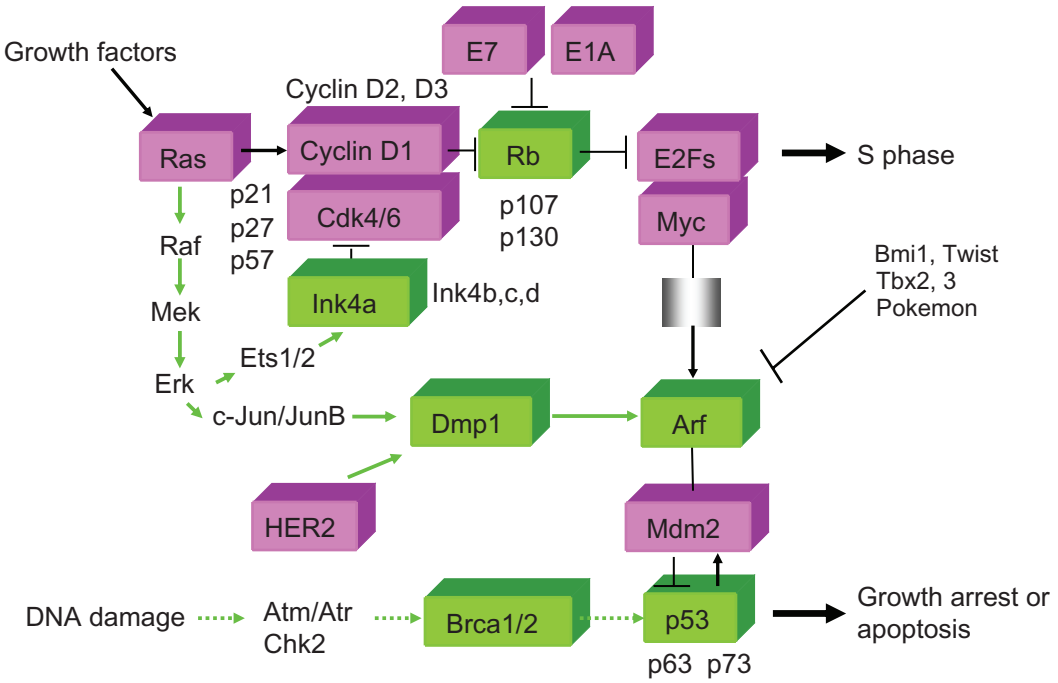

Figure 1. Molecules that are characterized in this review. Mitogenic signals acting through Ras stimulate the formation of cyclin D/Cdk complexes that phosphorylate RB in mid-late G1 phase. Accentuated by cyclin E/Cdk2 (not shown), RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is capable of accelerating S phase entry.[181–183] All D-type cyclins are mitogen-responsive, but only cyclin D1 is Ras-responsive. One of the oncogenic effects of adenovirus E1A and papilloma virus E7 is to interfere with RB function. By inhibiting cyclin D-dependent kinase activity, $p16^{INK4a}$ acts as a potent tumor suppressor. $p16^{Ink4a}$, $p19^{Arf}$, and tumor suppressor *Dmp1* are induced in response to oncogenic Ras-Raf-Mek-Erk signaling. This *Arf* induction (and also $p16^{Ink4a}$ and *Dmp1*) quenches inappropriate mitogenic signaling by diverting incipient cancer cells to undergo p53-dependent and independent growth arrest or cell death. *Arf* is also induced by activated other potentially oncogenic signals stemming from overexpression of oncogenes such as c-Myc, E2F1. *Arf* expression is repressed by a number of nuclear proteins, such as Bmi1, Twist, Tbx2/3, and Pokemon. *Dmp1* (cyclin D binding myb-like protein 1; Dmtf1)[116,117,184–186] is unique in that it directly binds and activates the *Arf* promoter and induces cell cycle arrest in an *Arf*-dependent fashion. The *Dmp1* promoter is also activated by HER2 overexpression.[187] Both *Dmp1*-null and heterozygous mice show hypersensitivity to develop tumors in response to carcinogen DMBA and γ-irradiation,[117] suggesting haploid insufficiency. D-type cyclins inhibit *Dmp1*'s transcriptional activity in a Cdk-independent fashion when E2Fs do not bind to the same promoter; however, D-cyclins cooperate with *Dmp1* to activate the *Arf* promoter. BRCA1/2 proteins are directly or indirectly phosphorylated by ATM/ATR kinases in response to DNA damage, which will interact with p53 to stop cell cycle by activating the $p21^{Cip1/WAF1}$ promoter. DNA damage (as indicated by the horizontal lines) has been shown to access the Mdm2-p53 machinery independently of *Arf*. However, *Arf*-loss enables Mdm2 to work more efficiently in antagonizing p53 function in response to DNA damage.

Most tumors from $p53^{+/-}$ mice show LOH of the wild-type *p53* allele. In $p53^{-/-}$ mice, the onset of tumor development is earlier than in heterozygotes; more than 75% of $p53^{-/-}$ mice develop tumors before 6 months of age. While thymic T cell lymphomas are the major tumor type, $p53^{-/-}$ mice also develop B cell lymphomas, sarcomas, and testicular teratomas.[15] The enhanced susceptibility to cancer of $p53^{-/-}$ mice results in their death before 10 months of age. *p53*-deficient mice are also extremely susceptible to tumorigenesis induced by ionizing radiation (IR) or carcinogens.[21,22]

Patients with Li-Fraumeni syndrome have germline *TP53* mutations, and therefore 50% of them develop malignancies by the age of 30,[23] comparable to the tumor incidence in middle-aged (18 months) $p53^{+/-}$ mice. The spectrum of tumors that arise in Li-Fraumeni patients is generally similar to that in $p53^{+/-}$ mice. However, human patients also develop breast cancers and brain tumors, which are rarely observed in $p53^{+/-}$ mice. These differences were puzzling until $p53^{-/-}$ mice were crossed into the BALB/c background. Spontaneous mammary tumors developed in half of the heterozygous females of this strain,[24] and the tumors showed the loss of the remaining wild-type *p53* allele. Furthermore, when mammary glands from $p53^{-/-}$ BALB/c mice were transplanted into wild-type BALB/c hosts, 75% of the transplanted mice developed mammary tumors. Thus, differences in genetic background can have profound effects on the types of tumors associated with *p53* mutation in mice.

In addition to their use as an animal model for p53-associated cancers, $p53^{-/-}$ mice have also been helpful in determining p53 functions in vivo. p53 is required

for thymocyte apoptosis induced by double-strand DNA breaks (DSBs) in response to stimuli such as ionizing radiation and adriamycin, but dispensable both for the apoptosis that mediates negative selection of immature thymocytes and thymocyte apoptosis induced in response to ultraviolet irradiation, dexamethasone, or anti-CD95.[25,26] With respect to cell cycle control, cells from *p53*$^{-/-}$ mice show enhanced proliferation in culture compared to controls.[27] With respect to maintenance of genomic integrity, *p53*$^{-/-}$ MEFs cultured in vitro show a high degree of aneuploidy, a hallmark for genomic instability.[27] p53 has also been implicated as a component of a spindle checkpoint that ensures the maintenance of diploidy. *p53*$^{-/-}$ fibroblasts exposed to spindle inhibitors performed multiple rounds of DNA synthesis without completing chromosome segregation, resulting in tetraploid and octaploid cells.[28] Increased genomic instability is also seen in mice deficient for *Gadd45α*, a transcriptional target of p53.[29]

Tumor suppressors and oncogenes are presumed to cooperate in the induction of tumorigenesis. A *p53*$^{-/-}$ background dramatically increases tumor frequency and reduces the latency in *c-Myc* transgenic mice.[30] A cooperative effect on transformation between *p53* and *Rb* mutations has also been reported. Mice carrying mutations of both *p53* and *Rb* have reduced viability and exhibit abnormalities not observed with either single mutant, including pinealoblastomas, islet cell tumors, bronchial epithelial hyperplasia, and retinal dysplasia.[31]

The generation of *p53* conditional knockout mice with floxed alleles has shed further light on p53 tumor-suppressor function in vivo.[32] Medulloblastomas are among the most common malignancies in childhood, and they are associated with substantial mortality and morbidity. Marino et al generated a mouse model for medulloblastoma by *Cre–LoxP*-mediated inactivation of *Rb* and *p53* TSGs in the cerebellar external granular layer (EGL) cells. *GFAP–Cre*-mediated recombination was found both in astrocytes and in immature precursor cells of the EGL in the developing cerebellum. *GFAP-Cre*; *Rb*$^{LoxP/LoxP}$; *p53*$^{-/-}$ *or LoxP/LoxP* mice developed highly aggressive embryonal tumors of the cerebellum with typical features of medulloblastoma. These tumors were identified as early as 7 weeks of age on the outer surface of the molecular layer, corresponding to the location of the EGL cells during development.[32]

p53 knock-in mice

Heterozygous mice expressing mutant mouse *p53* with an R172H substitution (corresponding to the R175H mutation "hot-spot" in human tumors) develop osteosarcomas, carcinomas, and lymphomas with high metastatic potential.[34] LOH studies of p53$^{R172H\Delta g}$ tumors indicated loss of the wild-type *p53* allele in only 1 of 11 tumors. These data indicated clear differences between a *p53* missense mutation and a null allele in tumorigenesis in vivo, and suggest that the p53$^{R172H\Delta g}$ mutant represents a gain-of-function allele.[34] Mice with a *p53* allele altered at Leu25 and Trp26, residues essential for transcriptional transactivation and Mdm2 binding, synthesized a p53 protein that was stable but did not accumulate after DNA damage.[35,36] The mutant p53^{L25W26} was abundantly expressed, and the level was not affected by DNA damage, but bound to DNA constitutively; however, mutant p53^{L25W26} showed defects in cell-cycle regulation and apoptosis. Both p53^{L25W26} mutant and *p53*-null mouse MEFs were readily transformed by oncogenes, and the corresponding mice were prone to tumors. Thus, the determining pathway for p53 tumor-suppressor function in mice requires the transactivation domain.

To examine the mechanistic nature of *p53* missense mutations found in individuals with Li-Fraumeni syndrome, Lang et al[37] generated mice harboring a G-to-A substitution at nucleotide 515 of *p53* (*p53*$^{+/515A}$) corresponding to the *p53*^{R175H} hot spot mutation in human cancers. Although *p53*$^{+/515A}$ mice display a similar tumor spectrum and survival curve as *p53*$^{+/-}$ mice, tumors from *p53*$^{+/515A}$ mice metastasized with high frequency. Correspondingly, MEFs from the *p53*$^{515A/515A}$ mutant mice displayed enhanced cell proliferation, DNA synthesis, and transformation. They also reported that disruption of *p63* and *p73* in *p53*$^{-/-}$ cells increased transformation capacity and re-initiated DNA synthesis to levels observed in *p53*$^{515A/515A}$ cells. Additionally, p63 and p73 were functionally inactivated in *p53*^{515A} cells. Their results provided in vivo validation for the gain-of-function properties of certain p53 missense mutations and suggested a mechanistic basis for these phenotypes.

Similar phenotypes were found in *p53* knock-in mice created by Olive et al.[38] To ascertain the physiological effects of *p53* point mutation, the structural mutant *p53*^{R172H} and the contact mutant *p53*^{R270H} (corresponding to codons 175 and 273 in humans) were engineered into

the endogenous *p53* locus in mice. Both $p53^{R270H/+}$ and $p53^{R172H/+}$ mice are models of Li-Fraumeni syndrome; they developed allele-specific tumor spectra distinct from $p53^{+/-}$ mice. In addition, $p53^{R270H/-}$ and $p53^{R172H/-}$ mice developed novel tumors that were not found in $p53^{-/-}$ mice, including carcinomas in the lung, small intestine, colon, breast, skin, liver, and pancreas, and more frequent endothelial tumors.[38] Dominant effects that varied by allele and function were observed in primary cells derived from $p53^{R270H/+}$ and $p53^{R172H/+}$ mice. These results demonstrate that point mutant *p53* alleles expressed under physiological control have enhanced oncogenic potential beyond the simple loss of *p53* function.

Transgenic overexpression models for p53

Lavigueur et al[39] established *p53*-transgenic mice driven by the endogenous promoter using murine *p53* genomic fragments isolated from a Friend cell line CB7 or BALB/c mouse liver DNA. Mutations in Arg to Pro in 193 or Ala to Val in 135 in *p53* gene resulted in multi-organ neoplasias including lymphomas, osteosarcomas, and lung adenocarcinomas. Godley et al[33] created transgenic mice expressing wild-type murine *p53* under the control of the mouse mammary tumor virus terminal repeat (MMTV-LTR). The *p53* transgene was unexpectedly expressed in the kidney, which underwent progressive renal failure due to abnormal kidney development. Similar phenotypes were observed in two transgenic lines that express wild-type *p53* within the ureteric bud, but not in transgenic animals expressing a dominant-negative *p53* mutant allele. Defective differentiation of the ureteric bud, as evidenced by altered marker expression during development, was accompanied by expression of the *p53* transgene. At E17.5–18.5, metanephric mesenchymal cells underwent high rates of apoptosis, and fewer cells than normal were converted to tubular epithelium.[33] Proteinuria was observed as early as 2 weeks of age, with azotemia began at 6 weeks. As a result, kidneys in these animals grew to only half of their expected size and contained about half of the normal number of nephrons, with compensatory hypertrophy of the glomeruli. Most affected glomeruli showed global glomerulosclerosis. In this setting, abnormally high levels of wild-type p53 altered cellular differentiation in embryonic ureteric buds, and caused secondary effects (apoptosis and inefficient conversion to epithelium) in the adjacent undifferentiated mesenchyme.[33]

p53 activation vs. overexpression models and ageing

The role of p53 on ageing has also been studied. Tyner et al[40] generated mice with a deletion mutation in the first six exons of the *p53* gene that express a truncated RNA capable of encoding a carboxyl-terminal *p53* fragment. This mutation confers phenotypes consistent with activated, rather than inactivated, p53. Mutant ($p53^{+/m}$) mice showed enhanced resistance to spontaneous tumors compared with wild-type ($p53^{+/+}$) littermates. As $p53^{+/m}$ mice aged, they displayed an early onset of phenotypes associated with ageing, including reduced longevity, osteoporosis, generalized organ atrophy and a diminished stress tolerance.[40] By contrast, the phenotypes of $p53^{-/m}$ mice were in distinguishable from those of $p53^{-/-}$ mice, suggesting that a normal *p53* allele is required for the mutant p*53* gene to have an effect. The tumor resistance of $p53^{+/m}$ mice was considered to be intrinsic to the individual cells of the animals, since fibroblasts from $p53^{+/m}$ were more resistant to transformation by activated *Ras* plus *Myc* oncogenes (although this does not rule out the possibility of systemic hormonal, immunological or growth factor changes). The association of early ageing and tumor resistance in the $p53^{+/m}$ mice is consistent with the idea that senescence is a mechanism of tumor suppression. They suggested that ageing-related reduction in stem cell proliferation might have a more important role in longevity than previously recognized.[40,41]

Garcia-Cao et al[42] established and characterized mice carrying supernumerary copies of the wild-type *p53* gene as large genomic transgenes. They showed that the *p53* transgenic allele (*p53*-tg), when present in a *p53*-null genetic background, behaves as a functional replica of the endogenous gene. "Super *p53*" mice that carry *p53*-tg alleles in addition to the two endogenous alleles exhibited an enhanced response to DNA damage and were significantly protected from cancer when compared with normal mice. It is conceivable that constitutive or highly frequent activation of p53, such as under chronic exposure to stress, could result in accelerated aging as demonstrated by Tyner et al.[40] In contrast, "super p53" mice have a normal aging process despite having clearly increased p53 functionality.[42]

A critical characteristic of the "super p53" mice is that basal levels of p53 activity are not affected. Attempting to further increase the gene dosage of p53 may eventually reveal a threshold at which deleterious effects will be noticeable, probably as defective tissue regeneration, growth atrophies, and premature aging. This suggests that increases in normally regulated p53, as in the "super p53" mice, could confer cancer protection without affecting ageing, whereas constitutive levels of active p53 provides cancer protection, but promote aging. Thus, cancer resistance could be enhanced by a simple genetic modification of p53 in the absence of undesirable effects.

p73 and p63 models

There is no convincing clinical evidence that p73 or p63 plays a significant role in human tumorigenesis. The combined absence of *p63* and *p73* severely impaired the induction of p53-dependent apoptosis in response to DNA damage in oncogene-expressing cells and in the developing central nervous system in mice, which was explained by the inability of p53 to bind the promoters of apoptosis-associated target genes and to upregulate their transcription. Thus, there are two classes of p53-family target genes. One class includes genes such as *Mdm2* and $p21^{Cip1/Waf1}$, which p53 regulates in the presence or absence of p63 /p73, and the other group includes genes *Perp*, *Bax*, and *Noxa* that require p63/p73 for p53 to be recruited and function properly. Thus, p63 and p73 are important components of the cellular response to DNA damage, and may portend a greater role for these proteins in tumor suppression and chemosensitivity. The validation of p73 and p63 as novel tumor suppressors may offer novel therapeutic approaches to enhance the chemosensitivity of tumor cells harboring mutated or inactivated *p53*.

p73 and p63 are members of the p53 family of proteins and thus attractive candidates for TSGs.[43,44] Both p73 and p63 share significant amino acid identity with p53 in the transactivation domain, the DNA-binding domain, and the oligomerization domain. Like p53, p73 and p63 can recognize canonical p53 DNA-binding sites and, when overproduced, can activate p53-responsive target genes and induce apoptosis.[43] p73 is localized to chromosome 1p36.3, a region of frequent aberrations in human cancers, such as thyroid, colorectal, and breast cancers, pheochromocytomas, and brain tumors.[45] However, there is no convincing clinical evidence that p73 and p63 play a significant role in human tumorigenesis. E2F-1 directly activates transcription of *p73* leading to activation of p53-responsive target genes and apoptosis.[46–49] Moreover, synergistic cooperation between YY1 and E2F-1 through physical interaction was reported in the regulation of the *p73* promoter.[50]

Disruption of p73 function inhibits E2F-1-induced apoptosis in *p53*-deficient cells, suggesting a role for p73 in p53-independent apoptosis. Similarly, T cell receptor activation-induced cell death, which is p53-independent, is inhibited in the absence of p73.[47] Interestingly, a truncated isoform of p73 that is expressed in developing neurons appears to have an anti-apoptotic function.[51] However, other evidence weighs against a tumor-suppressive role for p73. Knockout mice null for *p73* lacked an increased incidence of tumors up to 15 months of age.[52] Several *p73* isoforms and two different *p73* promoters have been described. Mice deficient for specific isoforms of *p73* remain to be reported. Mice deficient for *p63* show defects in limb and epidermal morphogenesis and die within hours of birth,[53,54] but did not show increased incidence of tumor formation. The combined absence of *p63* and *p73* severely impaired the induction of p53-dependent apoptosis in response to DNA damage in E1A-expressing cells and in the developing central nervous system in mice.[55] This was explained by the inability of p53 to bind the promoters of apoptosis-associated target genes and to upregulate their transcription in $p63^{-/-}$; $p73^{-/-}$; E1A cells and the developing central nervous system.

These data support the notion that there might be two classes of p53-family target genes. One class includes genes such as *Mdm2* and $p21^{Cip1/Waf1}$, which p53 regulates in the presence or absence of p63 and p73. The other group includes genes such as *Perp*, *Bax*, and *Noxa* that require p63 or p73 for p53 to be recruited and function properly. These data show that p63 and p73 are important components of the response to DNA damage, and may portend a greater role for these proteins in tumor suppression and chemosensitivity. The same group later conducted a detailed study to determine whether *p63* or *p73* are involved in tumor suppression using aging mice heterozygous for mutations in all p53 family genes.[56] They used $p73^{+/-}$ mice for tumor observation, since

only 25% of *p73*$^{-/-}$ mice survived to adulthood in their colony. Spontaneous tumors developed in *p63*$^{+/-}$; *p73*$^{+/-}$ mice, including mammary and lung adenocarcinomas, thymic and squamous cell carcinoma, and myeloid leukemia. Loss of *p63* and *p73* can also cooperate with loss of *p53* in tumor development. Mice heterozygous for mutations in both *p53* and *p63* or *p53* and *p73* displayed higher tumor burden and metastasis compared to *p53*$^{+/-}$ mice. These findings provide evidence for a broader role for the p53 family in tumor suppression than has been previously reported.[56]

Retinoblastoma models

The retinoblastoma gene family is composed of three members: the product of the retinoblastoma gene (pRb), and two related proteins, pRb2/p130 and pRb3/p107, which have been shown to be structurally and functionally similar to pRb. The three retinoblastoma family members show growth suppressive properties, although the growth arrest mediated by each of the three pocket regions of the proteins is not identical. Although the three pRB members complement each other, they are not functionally redundant. Among these proteins, pRb and p130 are tumor suppressor proteins, and thus are candidate targets for gene therapy. Mutations commonly found in human small cell lung carcinomas (SCLC) are inactivation of *RB* and *TP53*. Recent study established a mouse model for neuroendocrine lung tumors by conditional inactivation of both *Rb* and *p53* in mouse lung epithelial cells. These murine small cell lung carcinoma models may be beneficial for novel drug screening of SCLC.

Retinoblastoma (RB) is an eye tumor that occurs in children. The *RB* gene is located on chromosome 13q14.2, and mutations of *RB* have been found in both inherited and sporadic cases.[57] *RB* mutations can also predispose individuals to osteosarcomas, and prostate and breast cancers. In adults, human papilloma virus (HPV) is thought to initiate cervical carcinoma and squamous cell carcinoma of the head and neck, at least in part by inactivating RB through expression of the E7 oncoprotein (Fig. 1). Viral oncoproteins such as adenovirus E1A and SV40 large tumor antigen can bind to the RB protein and inactivate its function (Fig. 1). In liver cancer, hepatitis C virus induces E6 associated protein (AP) degradation of RB.[58] Moreover, *RB* is inactivated in more than 90% of human small-cell lung carcinomas (SCLC),[59] and mouse genetic studies have confirmed that *Rb* plays crucial roles in preventing the initiation of SCLC.[60]

The *RB* gene encodes a 928-amino acid (aa) nuclear protein of 105 kDa.[61–64] Phosphorylation of the RB protein, which is critical for regulation of its function, is mediated by cyclin/cyclin dependent kinase (CDK) complexes in vivo. Phosphorylation of RB is thus cell cycle-dependent, with the hypophosphorylated, active form being present in G0/G1, and the hyperphosphorylated, inactive form dominant in late G1 of the cell cycle (Fig. 1).[62–64] Inactivation of *RB* by germline mutation of one allele and LOH of the second allele is often found in RB-associated cancers. However, functional inactivation of the RB protein in the absence of *RB* mutation can also lead to tumorigenesis. For instance, amplification of the 11q13 region results in cyclin D1 overexpression, which, in turn, leads to activation of cyclin D-CDK4/6 activity and hyperphosphorylation and inactivation of RB.[65] Functional inactivation of RB can also occur from deficient p16^{INK4a} function caused by gene deletion, promoter methylation, or point mutation.[66]

The function of RB is to repress the transcription of genes required for DNA replication and cell division. At least two different mechanisms may be involved. Binding sites for the transcription factor E2F are present in the promoters of many genes whose expression is essential and limiting for entry into S phase.[67,68] RB binds members of the E2F family (E2F1–4), forming a complex that inhibits transcriptional activation. The RB-E2F complex can also actively repress transcription of genes further downstream.[69] In addition to cell cycle regulation, RB plays a role in apoptosis. Increased apoptosis is observed in gene-targeted *Rb*-deficient (*Rb*$^{-/-}$) embryos, and MEFs from *Rb*$^{-/-}$ mice show activation of E2F-responsive genes and apoptosis.[70,71] Published studies have indicated specific roles of E2F1 in apoptosis among the E2F proteins.[72] E2F1 activates *p73* transcription,[46,47] which, in turn, stimulates the transcription of p53-responsive target genes and induces apoptosis.[49] Published studies found that activation of E2F2 or E2F3a could also lead to cell death, albeit not to the same degree as E2F1 expression.[73,74]

Since both E2F2 and E2F3a can activate pro-apoptotic gene transcription, at least when overexpressed, the unique role of E2F1 to induce apoptosis

in certain conditions may depend on other specific properties, such as E2F1-specific interacting proteins. Intriguingly, E2F3a did not cause apoptosis in the absence of *E2f1*.[75] Thus, the accumulation of crucial levels of E2F1 activity, and not total E2F activity, appears essential for the induction of apoptosis in response to a deregulated RB pathway.

Knockout models for Rb, p107, and p130

Gene-targeted $Rb^{-/-}$ embryos die in utero between E13.5-E15.5.[76–78] Increased apoptosis in the nervous system is seen as early as E11.5 and is particularly evident in the hindbrain, spinal cord, and trigeminal and dorsal root ganglia. Ectopic mitoses were also observed, especially in the hindbrain. In addition to defective neurogenesis, $Rb^{-/-}$ embryos exhibit defective hematopoiesis, manifested as an increased number of immature nucleated erythrocytes. Interestingly, apoptosis in lens fiber cells deficient for *Rb* is dependent on p53, since $Rb^{-/-}$; $p53^{-/-}$ embryos show a complete suppression of apoptosis.[79] Analyses of viable chimeric mice derived from $Rb^{-/-}$ embryonic stem cells revealed that the *Rb*-deficient cells contribute widely to adult tissues.[80,81] The chimeric erythroid and central nervous system compartments appeared normal, but the developing retina was defective and displayed ectopic mitoses.

The effects of *E2f-1* mutation on *Rb* mutant phenotypes in mice have been examined. $Rb^{-/-}$; $E2f1^{-/-}$ embryos die in utero at approximately E17 with anemia and defective skeletal muscle and lung development.[82] Significant tissue-specific suppression of apoptosis, S phase entry, and p53 activation was observed in *Rb*; *E2f1* knockout cells. The fact that mutation of *E2f1* did not fully rescue the *Rb* developmental defects indicates that these abnormalities are not entirely *E2f1*-dependent. Genomic deletion of either *E2f1* or *E2f3* suppresses both increased proliferation and apoptosis in the lens and nervous system of $Rb^{-/-}$ embryos, suggesting that both *E2f1* and *E2f3* are required for induction of apoptosis due to loss of *Rb*.[82,83] Intriguingly, a portion of $Rb^{-/-}$; $E2f3^{+/-}$ embryos exhibited suppression of increased apoptosis, but not ectopic proliferation in the peripheral nervous system.[83] This suggests that *E2f3* has a primary role in induction of apoptosis due to *Rb*-loss, rather than simply contributing to abnormal proliferation and resultant apoptosis of *Rb*-null cells. More recently, de Bruin et al reported that many defects in the $Rb^{-/-}$ embryos are indirectly due to an essential role of *Rb* in placental development; however, the lens defects and peripheral nervous system abnormalities appear to be cell-autonomous, suggesting that E2f may indeed play a direct role in regulation of proliferation and apoptosis in these tissues.[84] Id2 is a dominant-negative antagonist of basic helix-loop-helix transcription factors and proteins of the Rb family. *Id2*;*Rb* double knockout (DKO) embryos survive to term with minimal or no defects in neurogenesis and hematopoiesis, but they die at birth from severe reduction of muscle tissue.[85]

Unlike humans, the *Rb* chimeras develop pituitary gland tumors rather than retinoblastomas.[77] A similar phenotype was observed in $Rb^{+/-}$ mice that, at age 8–10 months, developed tumors in the brain and pituitary gland. These tumors exhibited LOH of the remaining wild-type *Rb* allele, demonstrating that *Rb* is a TSG in mice as well as in humans. Although $Rb^{+/-}$ mice are tumor-prone, they do not accurately recapitulate the tumor spectrum observed in humans with RB, since other members of the Rb family can compensate for *Rb*-loss in murine eyes. Chimeras possessing cells deficient for both *Rb* and its family member *p107* developed retinoblastomas during the early postnatal months.[86] Only $Rb^{-/-}$; $p107^{-/-}$ chimeras, but not $Rb^{+/-}$; $p107^{-/-}$ chimeras, developed retinoblastomas, suggesting that the low number of target cells in the murine retina precludes the acquisition of the required number of mutations to inactivate the remaining *Rb* allele. Mice homozygous for loss of *p107* or *p130* are viable, fertile, and healthy.[62,78] However, $p107^{-/-}$; $p130^{-/-}$ mice experience neonatal lethality,[62] and most $Rb^{+/-}$; $p107^{-/-}$ mice are growth-retarded. Increased mortality of these mice is observed within the first three weeks of birth.[78] Although $Rb^{+/-}$; $p107^{-/-}$ pups that survive to adulthood do not show an increased cancer predisposition compared to $Rb^{+/-}$ mice, they develop multiple dysplastic lesions of the retina.[78] Thus, unlike *Rb*, *p107* and *p130* are not required for embryonic development, and $p107^{+/-}$, $p130^{+/-}$, $p107^{-/-}$, and $p130^{-/-}$ mice do not show increased incidence of tumor development.[62] Embryonic stem cells with a simultaneous deficiency of *Rb*, *p107*, and *p130* (triple knockout, TKO) have normal growth characteristics, but impaired differentiation capacity.[87,88] *Rb*, *p107*; *p130* TKO MEFs have a shorter cell cycle compared to controls and can spontaneously immortalize. TKO mouse embryonic

fibroblasts are also resistant to G1 arrest following DNA damage, contact inhibition or serum starvation.

Conditional mutant mice for *Rb*[32] have been generated using the *Cre-LoxP* system and should prove useful in defining stage- and tissue-specific roles of *Rb*. Mutations commonly found during the pathogenesis of human lung cancer are inactivation of *RB* and *TP53*; and have been identified in up to 90% of human SCLCs. Meuwissen et al[53] established a mouse model for neuroendocrine lung tumors by conditional inactivation of *Rb* and *p53* in mouse lung epithelial cells. Mice carrying conditional alleles for both *Rb* and *p53* frequently developed aggressive lung tumors with striking morphologic and immunophenotypic similarities to human SCLC. Most of these tumors, which they designated MSCLC (murine small cell lung carcinoma), diffusely spread through the lung and gave rise to extrapulmonary metastases. In this model, inactivation of both *Rb* and *p53* was a prerequisite for the pathogenesis of SCLC.

Mutation of *RB* and *TP53* tumor suppressors is associated with the development of human osteosarcomas. To establish a mouse model of osteosarcomas in mice, Berman et al[89] used conditional mouse strains to inactivate *Rb* and/or *p53* specifically in osteoblast precursors. The resulting *Rb*; *p53* DKO animals were viable, but developed early onset osteosarcomas with complete penetrance. These tumors displayed many features of human osteosarcomas, including being highly metastatic. Cell lines from the DKO osteosarcomas and were highly proliferative and retained their tumorigenic potential, multipotency, and expression of Sca-1, a marker that is typically associated with stem cells/uncommitted progenitors. Tumorigenicity of the osteosarcoma cell lines correlated with the presence of the Sca-1 marker. Finally, loss of *Rb* and *p53* in Sca-1-positive mesenchymal stem/progenitor cells was sufficient to yield transformed cells that can initiate osteosarcoma formation in vivo.

Choi et al[90] later inactivated the *Rb* and *p53* genes by Cre-loxP-mediated recombination in mice. More than 90% of mice developed spindle cell/pleomorphic sarcomas after a single subcutaneous injection of adenovirus carrying Cre-recombinase. Similar to human STSs, these sarcomas overexpressed Cxcr4, which contributes to their invasive properties. Sarcomas originated not from bone marrow-derived cells, but from local resident cells. Indeed, dermal mesenchymal stem cells isolated by plastic adherence and low levels of Sca-1 expression (Sca-1^{low}, CD31^{neg}CD45^{neg}) have shown enhanced potential for malignant transformation after the conditional inactivation of both *p53* and *Rb*. Sarcomas formed after transplantation of these cells had features typical for undifferentiated high-grade pleomorphic sarcomas. Together, these results indicated that local Sca-1^{low} dermal mesenchymal stem/progenitor cells are preferential targets for malignant transformation associated with deficiencies in both *p53* and *Rb*.

Transgenic model for *Rb*

Transgenic mice containing 1–7 copies of a human *RB* cDNA transgene under the transcriptional control of the human *RB* promoter have been generated.[91] Most of these transgenic mice were smaller than non-transgenic littermates, which were found as early as embryonic day 15. The degree of dwarfism correlated roughly with the copy number of the transgene and the corresponding level of RB protein. Transferring the transgene to *Rb*-deficient mice, which are nonviable, resulted in the development of normal, healthy mice, indicating that the human *RB* gene can functionally complement the mouse homolog,[91] and suggesting that regulation of Rb expression is required for normal development.

Nikitin et al[92] generated tetracycline-regulated *Rb* transgenic mice to explore the potential mechanism of Rb effects on somatic growth, and compared their phenotypes to those of previously established *Rb* mouse models. By gestational day 12.5, embryos lacking *Rb* and those expressing twice the normal level of Rb were 15% larger and 10%–30% smaller, respectively, compared with their wild-type littermates. The small mouse (dwarf) phenotype of *Rb* transgenic mice was associated with increased plasma levels of insulin-like growth factor-I (IGF-I), but not with growth hormone and glucose concentrations.[92] Notably, down-regulation of the *Rb* transgene expression reduced IGF-I plasma concentrations to normal levels and increased somatic growth pre- and postnatally. Consistent with the in vivo results, cells overexpressing Rb required higher thresholds of IGF-I to stimulate proliferation. Thus, the IGF-I pathway is a critical target for Rb to regulate mouse somatic growth and maintenance during ontogenesis.[92]

Ink4a/Arf models

The frequent mutation or deletion of *INK4a/ARF* in human tumors as well as the occurrence of tumors in the murine knockout models have identified both *p16* and *Arf* as bona fide tumor suppressors. *INK4-ARF* deletions frequently occur in clinically aggressive acute lymphoblastic leukemias (Ph(+) ALLs), but are not in more indolent Ph(+) chronic CML or in CML myeloid blast crisis. Thus, although BCR-ABL mutations typify drug resistance in both CML and Ph(+) ALL, loss of *INK4-ARF* in Ph(+) ALL enhances disease aggressiveness and attenuates the favorable effects of targeted therapy. Super *Ink4a/Arf* mice that have increased copies of the locus manifest higher resistance to cancer compared to normal, non-transgenic mice. Modest increases in the activity of the *Ink4a/Arf* tumor suppressor resulted in a beneficial cancer-resistant phenotype without affecting normal viability or aging. Thus, the expression of the *Ink4a/Arf* tumor suppressor locus is a robust biomarker for tumor indolence and resistance.

The *INK4a/ARF* locus on chromosome 9p21 is frequently disrupted in human cancers.[93] Germline mutations of this locus predispose an individual to familial melanomas, whereas somatic mutations increase the chance of sporadic malignancies of the pancreas and brain. In both mice and humans, the *INK4a* locus includes two independent, but overlapping genes that encode the gatekeeper proteins $p16^{INK4a}$ and $p14^{ARF}$ ($p19^{Arf}$ in mice).[94] Each gene has its own promoter that precedes three coding exons. The first exons for $p16^{INK4a}$ (E1α) and $p14^{ARF}$ (E1β) are specific to each gene. Exons 2 and 3 are shared, although they are read in different frames and produce two different proteins.[65,95,96] Most mutations of the *INK4a* locus were originally thought to inactivate $p16^{INK4a}$. However, the identification of *ARF*, and the finding that *ARF* and $p16^{INK4a}$ share two exons, suggests that some mutations in the *INK4a* locus may affect only *ARF* or only $p16^{INK4a}$, whereas others may affect both proteins. The $p16^{INK4a}$ protein is a cyclin-dependent kinase inhibitor that specifically binds to and inhibits CDK4 and CDK6, proteins that promote the G1/S transition.[97] Inhibition of CDKs leads to maintenance of Rb in its active, hypophosphorylated form (Fig. 1). Thus, $p16^{INK4a}$ performs its tumor-suppressor function through the functional inactivation of Rb. Rb represses $p16^{INK4a}$ expression, and upregulation of $p16^{INK4a}$ expression is observed in *Rb*-deficient cells.[98,99] Expression of $p16^{INK4a}$ can also be repressed by the polycomb family member Bmi-1,[100] and by the helix-loop-helix protein Id1.[90] $p16^{INK4a}$ expression is enhanced by the transcription factors Ets1 and Ets2, two downstream targets of Ras-Raf-MEK signaling.[101]

$p19^{Arf}$ physically interacts with Mdm2 and promotes its rapid degradation (Fig. 1), leading to p53 stabilization and activation.[19,102–107] p53 control Arf expression in return, since cells express high levels of Arf in the absence of functional *p53*.[108] The main activators of Arf expression are oncoproteins such as c-Myc,[109] adenovirus E1A,[110] Ras, E2F-1, and v-abl, consistent with a role for Arf in sensing hyperproliferative signals. Conversely, Bmi-1,[111] Tbx2,[112] Twist,[113] Pokemon,[114] and AML1/ETO[115] repress Arf expression, whereas Dmp1 increases it.[116,117] Induction of *Arf* expression by E2F-1[116,118] provides a functional link between the $p16^{Ink4a}$/cyclin D/Cdk4, 6/Rb and the Arf/Mdm2/p53 tumor suppression pathways. In addition to $p16^{Ink4a}$ and $p19^{Arf}$, the Ink4 family of CKIs includes $p15^{Ink4b}$ and $p18^{Ink4c}$. These proteins share ~40% identity, contain four tandem ankyrin motifs, and specifically inhibit Cdk4 and Cdk6.

Knockout models for *Ink4a/Arf*

A mouse strain ($Ink4a/Arf^{ex2–3}$) in which both *Ink4a* and *Arf* are deficient owing to deletion of their common exons 2 and 3 has been created.[119] Mice heterozygous for the $Ink4a/Arf^{ex2–3}$ mutation show a moderate increase in fibrosarcomas, lymphomas, squamous cell carcinomas, and angiosarcomas. Forty percent of these tumors exhibit LOH at the *Ink4a* locus.[120] Deletion of exon 1β specific to *Arf* is also seen in some of these tumors, suggesting that in these cases it is a deficiency of *Arf* and not $p16^{Ink4a}$ that leads to tumor development in mice. Susceptibility to tumorigenesis induced by the carcinogen DMBA alone or in combination with ultraviolet B irradiation is only slightly increased in $Ink4a/Arf^{ex2–3}$ heterozygous mice compared to controls. Mice homozygous for the $Ink4a/Arf^{ex2–3}$ mutation are viable, suggesting that this locus is not essential for embryonic development or survival. In fact, overexpression of the *Ink4a* locus can be detrimental to mouse development. Increased expression of $p16^{Ink4a}$ and Arf occurs in

Bmi1-deficient mice, which are underdeveloped and have a cerebellum and lymphoid organs of reduced size. Interestingly, these defects are either completely or partially rescued in a homozygous *Ink4a/Arf*$^{ex2-3}$ background.[111]

As expected, *Ink4a/Arf*$^{ex2-3-/-}$ mice show an increased susceptibility to cancer, with most developing sarcomas and lymphomas by age 7 months. Earlier onset of these malignancies (at about 13 weeks) is observed in *Ink4a/Arf*$^{ex2-3-/-}$ mice treated with DMBA or ultraviolet B irradiation. Surprisingly, in contrast to humans, mutations of the *INK4a* locus do not predispose mice to melanoma. However, *Ink4a/Arf*$^{ex2-3-/-}$ mice that overexpress an activated *H-ras*^{G12V} gene in melanocytes develop cutaneous melanomas with high penetrance by age 5.5 months.[121] This finding suggests that a loss of function of the *Ink4a* locus coupled with a gain of function of ras can result in mice predisposed to melanomas. Although studies of *Ink4a/Arf*$^{ex2-3-/-}$ mice indicate that the *Ink4a* locus is important for tumor suppression, the specific roles of p16^{Ink4a} and p19^{Arf} cannot be determined in these animals. Mice deficient for p19^{Arf}, but competent for p16^{Ink4a}, have been generated by deleting the *Arf*-specific exon 1β.[122–124] Mice heterozygous for this mutation develop lymphomas, sarcomas, and hemangiomas after a long latency. The tumors show loss of the remaining *Arf* allele and/or lack of its expression, confirming Arf's role in tumor suppression. Homozygous *Arf*$^{-/-}$ mice are viable and fertile, but most develop sarcomas (~40%) and T cell lymphomas (~30%) at around 6 months of age and often die by 12 months. Interestingly, *Arf*$^{-/-}$ mice differ from *Ink4a/Arf*$^{ex2-3-/-}$ mice in several phenotypes. The latency period for tumor formation is shorter in untreated and DMBA-treated *Ink4a/Arf*$^{ex2-3-/-}$ mice (~32 and ~12 weeks, respectively) than in untreated and DMBA-treated *Arf*$^{-/-}$ mice (~38 and ~24 weeks, respectively). In addition, *Arf*$^{-/-}$ mice develop carcinomas and tumors of the nervous system that do not appear in *Ink4a/Arf*$^{ex2-3-/-}$ mice.[123]

Two different groups created knockout mice specific to *p16*^{Ink4a} by deleting exon 1α or mutating exon 2, respectively.[125,126] Krimpenfort et al[126] generated mice carrying a point mutation specifically affecting p16^{Ink4a}. This allele, designated *Ink4a**, is silent in the p19^{Arf} reading frame, but introduces a stop codon in p16^{Ink4a} at conserved amino-acid position 101, resulting in deletion of the fourth ankyrin repeat motif. The analogous human allele (*CDKN2 A-W110X*) is a naturally occurring mutation found in many human tumor types, and results in an unstable protein that poorly inhibits phosphorylation of RB1, and thus, cell-cycle arrest in transfected cells.[126] These mice, designated *Ink4a*$^{*/*}$, did not show a significant predisposition to spontaneous tumor formation within 17 months. *Ink4a*$^{*/*}$ mouse embryonic fibroblasts proliferated normally, were mortal, and were not transformed by oncogenic H-RAS. However, Sharpless et al reported that *p16*^{Ink4a}-null mouse embryonic fibroblasts exhibited an increased rate of immortalization, although less than observed previously for cells null for *Ink4a/Arf*, *Arf*, or *p53*.[125] Moreover, T cells deficient in *p16*^{Ink4a} exon 1α exhibited enhanced mitogenic responsiveness, consistent with the established role of p16^{Ink4a} in constraining cellular proliferation. Furthermore, *p16*^{Ink4a} deficiency was associated with an increased incidence of spontaneous and carcinogen-induced cancers.[125] Ink4a*/Δ2,3 mice that were deficient for *Ink4a* and heterozygous for *Arf* spontaneously developed a wide spectrum of tumors, including melanoma. Treatment of these mice with the carcinogen DMBA resulted in an increased incidence of melanoma, with frequent metastasis.[126] Thus, the results from these two studies indicated that *Ink4a* is a tumor-suppressor gene that, when lost, could recapitulate the tumor predisposition seen in humans.

Expression of Arf in tissues of adult mice is difficult to detect, possibly because its induction leads to the arrest or elimination of incipient tumor cells. Zindy et al replaced exon 1β of the mouse cellular *Arf* gene with a cDNA encoding GFP, thereby producing *Arf*-null mice in which GFP expression is driven by the endogenous *Arf* promoter.[127] The *Arf* promoter was induced in several biologic settings previously shown to elicit mouse p19^{Arf} expression. GFP was expressed in cultured mouse embryonic fibroblasts, spontaneously arising tumors, Eμ-*Myc*-induced lymphomas, and retrolental masses in the vitreous of the eye. This study provided direct evidence that the *Arf* promoter monitors latent oncogenic signals in vivo.

INK4-ARF genes are epigenetically silenced in hematopoietic stem cells,[128] but become ready to respond to oncogenic stress as blood cells differentiate. Thus, inactivation of *INK4-ARF* provides differentiated cells with an inappropriate self-renewal capacity,

a defining feature of cancer cells. In BCR-ABL-induced (Philadelphia chromosome-positive [Ph(+)]) leukemias, *INK4-ARF* deletions frequently occur in clinically aggressive acute lymphoblastic leukemias (Ph(+) ALLs), but are not seen in more indolent Ph(+) forms of chronic myelogenous leukemia (CML) or in CML myeloid blast crisis. Williams et al[129] infected mouse bone marrow cells with retrovirus encoding either of two oncogenic Bcr-Abl isoforms [p210(Bcr-Abl) and p185(Bcr-Abl)] and induced B cell lympholeukemias by transplanting the cells into lethally irradiated mice. When mouse bone marrow cells expressing Bcr-Abl were placed in short-term cultures selectively designed to support the outgrowth of pre-B cells, only those lacking one or two *Arf* alleles could initiate lympholeukemias when inoculated into immunocompetent recipient mice. Although the ABL kinase inhibitor imatinib mesylate (Gleevec) provides highly effective treatment for BCR-ABL-positive CML, it has proven far less efficacious in the treatment of BCR-ABL-positive acute lymphoblastic leukemias (ALL), many of which sustain deletions of the *INK4A-ARF* locus. Consistently, mice receiving $Arf^{-/-}$ or $Arf^{+/-}$ p210(Bcr-Abl)-positive pre-B cells did not achieve remission when maintained on high doses of oral imatinib therapy and rapidly developed lympholeukemia.[129]

Although cells expressing the Bcr-Abl kinase can proliferate in the absence of IL-7, they remain responsive to this cytokine, which can reduce their sensitivity to imatinib.[130,131] Treatment of $Arf^{-/-}$, p210(Bcr-Abl)-positive pre-B cells with imatinib together with an inhibitor of JAK kinases abrogated this resistance, suggesting that this combination may prove beneficial in the treatment of BCR-ABL-positive acute lymphoblastic leukemia. Thus, although BCR-ABL mutations typify drug resistance in both CML and Ph(+) ALL, loss of *INK4-ARF* in Ph(+) ALL enhances disease aggressiveness and attenuates the favorable effects of targeted therapy. In their following study, Williams et al[132] showed that intravenous infusion of 20 $Arf^{-/-}$, p185(Bcr-Abl)-positive pre-B cells into healthy syngeneic mice induced rapidly fatal, transplantable lymphoblastic leukemias that resist imatinib therapy. However, introduction of BCR-ABL into *Arf*-null severe combined immunodeficient bone marrow progenitors lacking the cytokine receptor common gamma-chain yielded leukemogenic pre-B cells with greater sensitivity to imatinib in vivo. Hence, cytokines in the hematopoietic microenvironment can facilitate leukemic proliferation and confer resistance to targeted therapy.[132]

Transgenic models for *Ink4a/Arf*

Yang et al[133] created MMTV-$p16^{Ink4a}$ transgenic mice and studied the effect of $p16^{Ink4a}$ in ErbB2-induced mammary tumorigenesis. They reported that the $p16^{Ink4a}$ tumor suppressor specifically blocks cyclin-dependent kinase 4 and 6 activity. The $p16^{Ink4a}$ transgene blocked tumorigenesis by ErbB2, demonstrating that deregulation of the cyclin-dependent kinase partner of cyclin D1 is an essential target of ErbB2. ErbB2 overexpression was a determining factor in deregulation of cyclin D1-Cdk4/6 interactions because neither transgenic *cyclin D1* nor loss of $p16^{Ink4a}$ accelerated tumorigenesis in MMTV-*ErbB2*-transgenic mice. ErbB2 was also a deciding factor in deregulation of cyclin D1-Cdk4/6 in human tumors because no loss of *Rb* or $p16^{Ink4a}$ was found in tumors overexpressing ErbB2, although ErbB2-negative invasive breast adenocarcinomas frequently lacked expression of $p16^{Ink4a}$ or pRb.

Mathew et al[134] generated a "super *Ink4a/Arf*" mouse strain carrying a transgenic copy of the entire *Ink4a/Arf* locus. Cells derived from super *Ink4a/Arf* mice had increased resistance to in vitro immortalization and oncogenic transformation. Importantly, super *Ink4a/Arf* mice manifest higher resistance to cancer compared to normal, non-transgenic mice. Super *Ink4a/Arf* mice had normal aging and lifespan. Thus, modest increases in the activity of the *Ink4a/Arf* tumor suppressor resulted in a beneficial cancer-resistant phenotype without affecting normal viability or aging. Matheu et al then crossed "super *Ink4a/Arf*" mice with "super *p53*" mice reasoning that Arf/p53 could have anti-ageing activity by alleviating the load of age-associated damage.[135] The double transgenic mice showed strong cancer resistance and had decreased levels of ageing-associated damage. These observations extend the protective role of Arf/p53 to ageing, revealing a previously unknown anti-ageing mechanism and provided a rationale for the co-evolution of cancer resistance and longevity.

The same group later studied the impact of increased dosage of the *Ink4a/Arf* locus on germ cells.[136] Increased gene dosage of *Ink4/Arf* impaired

the production of male germ cells, and in the case of *Ink4/Arf*-tg/tg mice results in a Sertoli cell-only-like syndrome and a complete absence of sperm. There was a lower incidence of aging-associated cancer proportional to the *Ink4/Arf* gene dosage. Interestingly, increased *Ink4/Arf* gene dosage resulted in lower scores in aging markers and in extended median longevity. The increased survival was also observed in cancer-free mice indicating that cancer protection and delayed aging are separable activities of the *Ink4/Arf* locus. In contrast, mice carrying one or two additional copies of the *p53* gene (*p53*-tg and *p53*-tg/tg) had a normal longevity despite their increased cancer protection.[136] Thus, the *Ink4/Arf* locus has a global anti-aging effect, possibly by favoring quiescence and preventing unnecessary proliferation.

The $p16^{Ink4a}$ tumor suppressor accumulates in many tissues as a function of advancing age. $p16^{Ink4a}$ is an effector of senescence[19,66,124,125] and a potent inhibitor of the proliferative kinase Cdk4, which is essential for pancreatic β-cell proliferation in adult mammals. To study the links between senescence and aging in vivo, Krishnamurthy et al examined *Ink4a/Arf* expression in mouse models of aging.[137] They showed that expression of $p16^{Ink4a}$ and Arf markedly increased in almost all mouse tissues with advancing age, while there was little change in the expression of other Ink4a (p15, p18, and p19) cell cycle inhibitors. Importantly, the increase in expression for Ink4a and Arf occurred in both epithelial and stromal cells. The age-associated increase in expression of $p16^{Ink4a}$ and Arf was attenuated in the kidney, ovary, and heart by caloric restriction, and this decrease correlated with diminished expression of an in vivo marker of senescence, as well as decreased pathology of those organs. Thus, the expression of the *Ink4a/Arf* tumor suppressor locus is a robust biomarker, and possible effector of mammalian aging.[137] To determine the physiological significance of $p16^{Ink4a}$ accumulation on islet function, they assessed the impact of $p16^{Ink4a}$-deficiency and overexpression with increasing age and in the regenerative response after exposure to a specific beta-cell toxin.[138] Transgenic mice that overexpress $p16^{Ink4a}$ to a degree seen with ageing demonstrated decreased islet proliferation. Similarly, islet proliferation was unaffected by $p16^{Ink4a}$-deficiency in young mice, but was relatively increased in $p16^{Ink4a}$-deficient old mice. Survival after toxin-mediated ablation of beta-cells, which requires islet proliferation, declined with advancing age; however, mice lacking $p16^{Ink4a}$ demonstrated enhanced islet proliferation and survival after beta-cell ablation. These genetic data support the view that an age-induced increase of $p16^{Ink4a}$ expression limits the regenerative capacity of beta-cells with ageing.[138]

$p15^{Ink4b}$, $p18^{Ink4c}$, and $p19^{Ink4d}$ models

There is no strong evidence that $p15^{Ink4b}$, $p18^{Ink4c}$, and/or $p19^{Ink4d}$ are involved in tumor suppression in humans. However, methylation of *INK4C* is observed in human gliomas, and the $p18^{INK4C}$ protein is not detectable in 20% of medulloblastoma cases. When combined with *Ptc1* mutation, *Ink4c* shows haplo-insufficiency for tumor suppression. Thus, $p18^{INK4C}$ loss may contribute to medulloblastoma formation in children.

In contrast to $p16^{Ink4a}$, there is no strong evidence that $p15^{Ink4b}$, $p18^{Ink4c}$, and/or $p19^{Ink4d}$ are involved in tumor suppression in humans. Although the $p15^{INK4b}$ locus is often deleted in human tumors, its deletion is concomitant with that of the neighboring *INK4a/ARF* locus. Gene-targeted mice hemizygous for $p18^{Ink4c}$ or $p15^{Ink4b}$ did not show increased incidence of cancer.[139,140] Like other Ink4 family members, $p18^{Ink4c}$ and $p15^{Ink4b}$ are not required for embryonic development. Ablation of these genes, either alone or in combination, did not abrogate cell contact inhibition or senescence of mouse embryo fibroblasts in culture. However, loss of $p15^{Ink4b}$, but not $p18^{Ink4c}$, conferred proliferative advantage to these cells and made them more sensitive to transformation by *H-Ras* oncogenes.[140] $p18^{Ink4c-/-}$ mice developed pituitary adenomas (40%, mostly chromophobe adenomas), testicular tumors (12%), and adrenal tumors (10%) that lead to death before 18 months of age. In contrast, the cancer susceptibility of $p15^{Ink4b-/-}$ mice was only slightly increased over controls; angiosarcomas were observed in fewer than 10% of older $p15^{Ink4b-/-}$ mice.[140] Ablation of both $p15^{Ink4b}$ and $p18^{Ink4c}$ genes resulted in lymphoproliferative disorders and tumor formation. Moreover, mice lacking $p18^{Ink4c}$ exhibited deregulated epithelial cell growth leading to the formation of cysts, mostly in the cortical region of the kidneys and the mammary epithelium. These results indicate that $p15^{Ink4b}$ and $p18^{Ink4c}$ are tumor suppressor proteins that

act in different cellular lineages and/or pathways with limited compensatory roles. Mice with nullizygous or heterozygous mutations of *p27*^{Kip1} were predisposed to tumors in multiple tissues when challenged with γ-irradiation or a chemical carcinogen.[141] Mice lacking both *p18*^{Ink4c} and *p27*^{Kip1}, like chimeric mice for *Rb* deficiency, developed pituitary tumors and died by 3.5 months of age.[139] Hence, p18^{Ink4c} and p27^{Kip1} mediate two separate pathways to collaboratively suppress pituitary tumorigenesis, likely by controlling the function of Rb.

Overlapping and sustained patterns of expression of two cyclin-dependent kinases, p19^{Ink4d} and p27^{Kip1}, in post-mitotic brain cells suggested that these proteins may be important in actively repressing neuronal proliferation. Animals derived from crosses of *Ink4d*-null with *kip1*-null mice exhibited bradykinesia, proprioceptive abnormalities, and seizures, and died as early as 18 days after birth.[142] Metabolic labeling of live animals with neuronal markers showed that subpopulations of central nervous system neurons were proliferating in all parts of the brain, including normally dormant cells of the hippocampus, cortex, hypothalamus, pons, and brain stem.[142] These cells also expressed G2/M marker phosphorylated histone H3, indicating that neurons were dividing after they had migrated to their final positions in the brain. Increased proliferation was balanced by cell death, resulting in no gross changes in the cytoarchitecture of the brains of these mice. Therefore, the Cdk inhibitors p19^{Ink4d} and p27^{Kip1} cooperate to maintain differentiated neurons in a quiescent state that is potentially reversible. Thus, inhibiting function of these Cdk inhibitors in the adult brain could provide an avenue for stimulating the growth of neuronal populations lost in degenerative diseases or through traumatic injury.[142]

Recurrent genetic alterations in human medulloblastoma include mutations in the sonic hedgehog signaling pathway and *TP53* inactivation (approximately 25% and 10% of cases, respectively). However, mouse models of medulloblastoma generally depend upon p53 inactivation for rapid onset and high penetrance. The *p18*^{Ink4c} gene is transiently expressed in mouse cerebellar granule neuronal precursor cells (GNPs) as they exit the cell division cycle and differentiate. Co-inactivation of *Ink4c* and *p53* provided cultured GNPs with an additive proliferative advantage, either in the presence or absence of Shh, and induced medulloblastoma with low penetrance, but with greatly increased the incidence following postnatal irradiation.[143] In contrast, mice lacking one or two functional *Ink4c* alleles and one copy of *Patched* (Ptc1) encoding the Shh receptor rapidly developed medulloblastomas that retained wild-type p53.[143] In tumor cells purified from double heterozygotes, the wild-type *Ptc1* allele, but not *Ink4c*, was inactivated. Therefore, when combined with *Ptc1* mutation, *Ink4c* is haploinsufficient for tumor suppression. Methylation of *INK4C* was observed in four of 23 human medulloblastomas, and the p18^{INK4C} protein was not detectable in 14 of 73 cases. Hence, *p18*^{INK4C} loss may contribute to medulloblastoma formation in children.[142]

BRCA models

A substantial part of hereditary breast cancer cases is caused by *BRCA1* germline mutations. Transgenic *BRCA1* mice showed delayed development of tumors when challenged with DMBA, relative control mice. Mouse models with conventional and conditional mutations in *Brca1/2* have demonstrated critical roles of these proteins in breast cacinogenesis. The most advanced mouse models closely reproduce human *BRCA1/2*-related breast cancers, and may, therefore be useful for addressing clinically relevant questions including novel drug screens.

BRCA1

In humans, inheritance of one mutated allele of *BRCA1* significantly increases the risk of breast or ovarian cancer.[144–146] Mutations of the *BRCA1* gene, located on chromosome 17q21, are a predisposing factor in approximately 50% of families with hereditary breast cancer and in over 80% of families with hereditary breast-ovarian cancer. Most tumors from predisposed individuals demonstrate LOH for the wild-type *BRCA1* allele, and thus it is a classical tumor suppressor. BRCA1 expression is increased in the S phase of the cell cycle, and the BRCA1 protein is phosphorylated in a cell cycle-dependent manner and in response to DNA damage.[146]

Several lines of evidence support a role for BRCA1 in DNA damage repair. BRCA1 is phosphorylated following activation of ATM, CHK2,

and ATR-dependent DNA damage signaling pathways. Furthermore, BRCA1 interacts or forms a complex with multiple proteins involved in DNA damage repair, including RAD51, RAD50-Mre11-p95, MSH2, MSH6, MLH1, ATM, CHK2, BRCA2, and BLM.[147] BRCA1 also interacts with proteins that are involved in transcription, such as the RNA polymerase II holoenzyme complex, RNA helicase A, CtIP (CTBP-interacting protein), and CBP/p300. Unlike humans with an inherited *BRCA1* mutation, however, mice hemizygous for a *Brca1* mutation do not show increased incidence of tumors. Homozygous *Brca1* mutations inevitably lead to post-implantation embryonic lethality.[148–151] However, phenotypic differences exist among different $Brca1^{-/-}$ strains, such as a range of the onset of lethality from E6.5 to E13.[152] Such differences could be due to different types of targeted *Brca1* mutations and/or to the frequent alternative splicing of this gene during embryogenesis.

$Brca1^{-/-}$ embryos exhibit defective cellular proliferation and activation of p53-dependent pathways.[153] Mutation of *p53* or its transcription target $p21^{Cip1}$ partially delays the lethality of the $Brca1^{-/-}$ embryos.[151,153,154] Hypomorphic *Brca1* mutants with a partial loss of *Brca1* function show spina bifida and anencephaly accompanied by increased apoptosis in the neuroepithelium and die at E10-E13.[148] Hypomorphic *Brca1*-mutant ES cells and MEFs are hypersensitive to IR[155,156] and have a defect in transcription-coupled DNA repair. Human *BRCA1* transgenes can rescue the embryonic lethality of *Brca1* mutant mice.[157,158]

Three different conditional *Brca1* alleles have been generated, $Brca1^{F5-6}$, $Brca1^{Co}$, and $Brca1^{F5-13}$.[159–161] Cre-mediated deletion of *Brca1* exons 5–6 or exons 5–13 from the $Brca1^{F5-6}$ or $Brca1^{F5-13}$ allele, respectively, induces a frameshift mutation that abrogates the production of all three splicing products (full-length Brca1, Brca1-D11, and Brca1-IRIS). In contrast, deletion of exon 11 of the $Brca1^{Co}$ allele results in a hypomorphic mutation that still allows for expression of Brca1-D11. Mice with a mammary gland-specific hypomorphic *Brca1* function showed increased mammary cell apoptosis and abnormal ductal development, including incomplete ductal outgrowth, alveolar differentiation and involution, suggesting that *Brca1* function is indispensable for normal mammary gland development.[160] These mice developed mammary gland tumors after 10 to 13 months of age that showed genetic instability characterized by aneuploidy and chromosomal rearrangements, or alteration of *p53* transcription. This model was later improved by introduction of a single *p53*-null allele, yielding *MMTV-Cre*; $Brca1^{Co/Co}$; $p53^{+/-}$ mice, which developed mammary tumors with reduced latency.[162] Cooperation between *Brca1* and *p53* loss in mammary tumorigenesis was also demonstrated in a *K14-Cre*; $Brca1^{F5-13/F5-13}$; $p53^{F2-10/F2-10}$ mouse model with tissue-specific inactivation of both *Brca1* and *p53*.[161] The fact that loss of *p53* function accelerates the formation of mammary tumors in female mice with the mammary gland-specific *Brca1* mutation paralleled the frequent loss of *TP53* in tumors from human BRCA1 patients. Mice with a T cell lineage–specific null mutation of *Brca1* exhibit depletion of T lineage cells, abnormal p53 activation, and decreased cell cycle progression, and apoptosis.[159]

To directly determine the role of *BRCA1* in mammary gland development and tumor suppression, a transgenic mouse model of *BRCA1* overexpression was developed.[163] Using the *MMTV* promoter/enhancer, transgenic mice expressing human *BRCA1* or select mutant controls were generated. Transgenic animals examined during adolescence expressed the human transgene in their mammary glands. The mammary glands of 13-week-old virgin homozygous *MMTV-BRCA1* mice showed moderately increased lobulo-alveolar development. The mammary ductal trees of both hemizygous and homozygous $MMTV\text{-}BRCA1^{t340}$ were similar to those of control non-transgenic littermates. Interestingly, both hemi- and homozygous transgenic mice expressing a splice variant of *BRCA1* lacking the N-terminal RING finger domain ($MMTV\text{-}BRCA1^{sv}$) and exhibited marked mammary lobulo-alveolar development, particularly terminal end bud proliferation. Homozygous *MMTV-BRCA1* mice showed delayed development of tumors when challenged with DMBA, relative to non-transgenic and homozygous $BRCA1^{t340}$ expressing mice. In contrast, homozygous $MMTV\text{-}BRCA1^{sv}$ transgenic animals were sensitized to DMBA treatment exhibited a very rapid onset of mammary tumor development and accelerated mortality. *MMTV-BRCA1* effects on mortality were restricted to DMBA-induced tumors of the mammary gland. These results demonstrate in vivo

roles for *BRCA1* in both mammary gland development and in tumor suppression against mutagen-induced mammary gland neoplasia.[163]

BRCA2

The human *BRCA2* gene is located on chromosome 13q12-q13, and encodes a major transcript of 11 kb that is translated into a highly charged 3,418 amino acid protein.[146,153] In women, mutations of *BRCA2* are responsible for 32% of hereditary breast cancers. *BRCA2* mutations are also related to increased breast cancer in men. Like BRCA1, BRCA2 is thought to act as a caretaker involved in DNA damage repair and the maintenance of genomic integrity. BRCA1 and BRCA2 co-immunoprecipitate and co-localize with RAD51 in subnuclear foci and on the axial elements of developing synaptonemal complexes. Furthermore, BRCA2, like BRCA1 and RAD51, relocates to PCNA-positive replication sites following exposure of S phase cells to HU or ultraviolet irradiation.[142]

Brca2$^{-/-}$ mice die in utero between E7.5 and E9.5.[164–166] The onset of abnormalities is seen as early as E5.5 and the mutant embryos remain underdeveloped until death. Defective cell proliferation occurs in vivo and in vitro. Some hypomorphic *Brca2* mutants survive to adulthood.[167–169] These animals are smaller than their control littermates, show abnormal tissue differentiation, lack germ cells, and are infertile. *Brca2* hypomorphs develop lethal thymic lymphomas by 12–14 weeks of age. Mammary gland tumors were not observed, possibly because these animals died early. It is unclear whether *Brca2* mutations in mice will accurately reproduce human pathologies. *Brca2*-deficient cells have defects in their ability to repair DNA damage induced by genotoxic agents such as γ-, X-ray, and ultraviolet irradiation, and MEFs of *Brca2* hypomorphic mutants exhibit genomic instability.[146,147,169–171]

To achieve mammary gland-specific recombination of a *Brca2*$^{F3–4}$ conditional allele, Ludwig et al[172] created knock-in mice for *Brca2*$^{F3–4}$ and crossed with *WAP-Cre*. *Wap*$^{cre/+}$; *Brca2*$^{ex11/F3–4}$ female mice developed non-metastatic mammary carcinomas or adenosquamous carcinomas after a relatively long latency of 1.4 years.[172] The tumors displayed aneuploidy and chromosomal aberrations, were ErbB2/neu-negative, and ERα and cyclin D1-positive. Cheung et al[173] created conditional *Brca2*$^{F9–10}$ mice and crossed them with *MMTV*- or *WAP-Cre* transgenic lines. Although *Brca2* was not required for epithelial expansion in mammary glands of pregnant mice, *Brca2*-deficient mice developed mammary adenocarcinomas after a long latency (average: 1.6 years). Detailed histopathological analysis of four of these tumors demonstrated that three of them showed abnormal p53 protein expression. Moreover, homozygosity versus heterozygosity for the *Brca2* mutation heavily skewed the tumor spectrum toward mammary adenocarcinoma development in *p53*$^{+/–}$ mice. Thus, although *Brca2* is not essential for mammary epithelial development, *Brca2*-deficiency and hemizygous *p53* deletion collaborate to promote mammary tumorigenesis.

Brca2 is also implicated in T cell lymphoma development. A small acceleration of T cell lymphomagenesis was reported for *Lck-Cre*; *Brca2*$^{F9–10/F9–10}$; *p53*$^{–/–}$ mice, compared to *Lck-Cre*; *p53*$^{–/–}$ control animals.[174] However, Park and Lee[175] found that thymus-specific disruption of *Brca2*$^{F12/F12}$ allele in mice that were not crossed to a *p53*-mutant background also led to development of thymic lymphomas. The tumors were fatal in 25% of these mice from 16 weeks to 66 weeks after birth. The difference between the two studies could be due to different targeting sites between the two types of mice or different genetic backgrounds (129/C57BL/6 vs. FVB). Park and Lee1[175] had targeted exon 11 of *Brca2*, whereas Cheung and colleagues had targeted exon 9 and 10 of *Brca2* allele.[174] The very large exon 11 of *Brca2* contains 6–8 BRC repeats, which are implicated in Rad51-binding and modulating DNA repair. Therefore, it was speculated that targeting exon 11 of *Brca2* would yield better survival and less genetic instability. Nevertheless, the results from Park and Lee indicate that *Brca2* mutation in T cells predisposed them to mutations that led to cancer.

BRCA2 has also been implicated in the etiology of prostate cancer, but the impact of *Brca2* mutations in prostate tumorigenesis is unclear. Francis et al[177] showed that deletion of *Brca2* specifically in prostate epithelia results in focal hyperplasia and low-grade prostate intraepithelial neoplasia (PIN) in animals over 12 months of age. Simultaneous deletion of *Brca2* and *p53* in prostate epithelia gave rise to focal hyperplasia and atypical cells at 6 months, leading to high-grade PIN in animals

from 12 months.[176–177] Epithelial cells in these lesions showed increased DNA damage and had higher levels of proliferation, but also elevated apoptosis. Castration of *Brca2*; *p53* mutant animals led to regression of PIN lesions, but atypical cells continued to proliferate and express nuclear androgen receptor. This study provided evidence that *Brca2* can act as a tumor suppressor in the prostate, which could guide the development of new therapeutic approaches to prostate cancer.[177]

Novel tumor suppressor *ARH1*

ARHI is an imprinted TSG in breast and ovarian carcinomas; expression of *ARHI* in cancer cells inhibits cell growth and is associated with down-regulation of the *Cyclin D1* promoter activity and induction of $p21^{WAF1/CIP1}$. Identification of these novel Ras-related GTPase family members has expanded our understanding of the roles of these proteins in cell physiology. ARH1 serves as functionally distinct regulators of as yet to be characterized signaling cascades.

Using differential display PCR, Yu et al identified a gene *ARH1* (A Ras homologue member 1; *NOEY2*) with high homology to ras and rap that is expressed consistently in normal ovarian and breast epithelial cells, but not in ovarian and breast cancers.[178] Re-expression of *ARH1* by transfection suppresses clonogenic growth of breast and ovarian cancer cells. Growth suppression by *ARH1* was associated with down-regulation of the *Cyclin D1* promoter activity and induction of $p21^{WAF1/CIP1}$. LOH of *ARH1* was detected in 41% of ovarian and breast cancers. In most cancer samples with LOH, the non-imprinted functional *ARH1* allele was deleted, indicating that *ARH1* is a classical tumor suppressor.

Xu et al[179] developed transgenic mice that overexpress *ARH1* under the control of the *CMV* promoter. Offspring with the transgene weighed significantly less than non-transgenic littermates. In addition, strong expression of the *ARH1* transgene was associated with greatly impaired mammary gland development and lactation, failure of ovarian folliculogenesis resulting in decreased fertility, loss of neurons in the cerebellar cortex, and impaired development of the thymus. Decrease in body size and defects in the mammary glands correlated with the level of transgene expression. Immunohistochemical analysis indicated that expression of prolactin (but not growth hormone) was lower in the pituitary glands of mice with defective mammary gland development. The defect in pregnancy-associated mammary tissue proliferation was associated with decreased serum prolactin and progesterone levels. Moreover, lower levels of estrogen receptor and progesterone receptor were observed in postpartum mammary glands and in the ovaries of mice that overexpressed *ARH1*. Thus, *ARH1* can inhibit prolactin secretion and act as a negative regulator in murine growth and development.[179]

ARH1 is downregulated in more than 60% of human ovarian cancers. Lu et al[180] showed that re-expression of *ARH1* in multiple ovarian cancer cell lines induced autophagy by blocking PI3 K signaling and inhibiting mammalian target of rapamycin mTOR, upregulating ATG4, and colocalizing with cleaved microtubule-associated protein light chain 3 in autophagosomes. Furthermore, *ARH1* was required for spontaneous and rapamycin-induced autophagy in normal and malignant cells. Although *ARH1* re-expression led to autophagic cell death when SKOV3 ovarian cancer cells were grown in culture, it enabled the cells to remain dormant when they were grown in mice as xenografts. When ARH1 levels were reduced in dormant cells, xenografts grew rapidly. However, inhibition of ARH1-induced autophagy with chloroquine dramatically reduced re-growth of xenografted tumors upon reduction of ARH1 levels, suggesting that autophagy contributed to the survival of dormant cells.

ARH1 is also frequently lost in pancreatic cancers. *ARH1* was re-expressed in pancreatic cancer cells that had lost its expression to demonstrate the molecular mechanisms of cell growth inhibition.[180] Flow cytometric analysis indicated that ARH1 blocked cell cycle progression at the G1 phase in pancreatic cancer cells. Re-expression of ARH1 increased the expression of $p21^{WAF1}$, through the accumulation of p53 protein by the inhibition of PI3K/AKT signaling. In addition, ARH1 enhanced expression of $p27^{KIP1}$ through the inhibition of PI-3K/AKT signaling. The expression of cyclins A and D1 decreased, followed by decreased activities of CDK2/4. These results suggest that the PI-3K/AKT pathway plays a pivotal role in the pathogenesis of pancreatic cancer and ARH1 exerts its growth-inhibitory effects through

modulation of several key G1 regulatory proteins, such as $p21^{WAF1}$, $p27^{KIP1}$, CDK2/4, Cyclins A and D1.

Conclusions

Mouse models for TSGs, particularly models generated by gene targeting, offer the advantage of studying a genetically modified animal bearing one or only a few mutations. The use of these animals has revealed previously unsuspected developmental roles for TSGs such as *Rb*, *Brca1*, and *Brca2*, and has advanced our overall understanding of tumor-suppressor functions. Mouse models for TSGs have greatly facilitated the identification and characterization of cellular pathways controlled by these genes. This knowledge will be invaluable in choosing pathways or molecules to target for therapy of a specific cancer.

Most mouse models that manipulate TSGs exhibit some degree of predisposition to cancer development. A closer relationship between the murine and human situations may be precluded by several factors. Tumorigenesis associated with homozygous, but not heterozygous, mutation of a mouse TSG strongly suggests that LOH and inactivation of a wild-type allele are rare and limiting events. In addition, the mouse lifespan may be too short to allow the inactivation of the wild-type allele followed by the accumulation of other genetic abnormalities necessary for tumor development. For example, the frequent thymic lymphomas in the mouse model created by Cheung et al[174] and Park and Lee[175] might preclude these animals from developing other tumors owing to their early death from lymphomas. Finally, different genetic backgrounds give rise to different cancer spectra. Thus, it is not surprising that phenotypic differences exist between human diseases and their mouse models.

Nevertheless, the engineering of mouse models with combinations of mutated TSGs and mice transgenic for different oncogenes will provide powerful systems for identifying synergistic effects of specific mutations/deletions on tumorigenesis. Where the embryonic lethality of a mutation hampers the study of gene function in vivo, conditional mutants can be generated in which deletion or mutation of a TSG can be induced in a tissue of choice. In the future, mouse models in which mutation of both alleles of a TSG is induced in a few somatic cells will replicate sporadic human cancers. The sequencing of the human genome will no doubt lead to the identification of still more TSGs and oncogenes, and thus even more sophisticated mouse models. The study of such models should ultimately be of great benefit to cancer patients.

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Disclosure

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References

1. Kinzler KW, Vogelstein B. Cancer susceptibility genes. Gatekeepers and caretakers. *Nature*. 1997;386:761–63.
2. Sherr CJ. Principles of tumor suppression. *Cell*. 2004;116:235–46.
3. van Heemst D, den Reijer PM, Westendorp RG. Ageing or cancer: a review on the role of caretakers and gatekeepers. *Eur J Cancer*. 2007;43:2144–52.
4. Knudson AG. Hereditary cancer: two hits revisited. *J Cancer Res Clin Oncol*. 1996;122:135–40.
5. Knudson AG. Chasing the cancer demon. *Annu Rev Genet*. 2000;34:1–19.
6. Frese KK, Tuveson DA. Maximizing mouse cancer models. *Nat Rev Cancer*. 2007;7:645–58. Review.
7. Gopinathan A, Tuveson DA. The use of GEM models for experimental cancer therapeutics. *Dis Model Mech*. 2008;1:83–6.
8. Menendez D, Inga A, Resnick MA. The expanding universe of p53 targets. *Nat Rev Cancer*. 2009;9:724–37.
9. Toledo F, Wahl GM. Regulating the p53 pathway: *in vitro*. hypotheses, *in vivo* veritas. *Nat Rev Cancer*. 2006;6:909–23.
10. Vousden KH, Prives C. Blinded by the light: the growing complexity of p53. *Cell*. 2009;137:413–31.
11. Chan TA, Hwang PM, Hermeking H. Cooperative effects of genes controlling the G(2)/M checkpoint. *Genes Dev*. 2000;14:1584–88.
12. Burns TF, Fei P, Scata KA. Silencing of the novel p53 target gene Snk/Plk2 leads to mitotic catastrophe in paclitaxel (taxol)-exposed cells. *Mol Cell Biol*. 2003;23:5556–71.

13. Donehower LA, Harvey M, Slagle BL, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*. 1992;356:215–21.
14. Tsukada T, Tomooka Y, Takai S, Ueda Y, Nishikawa S, et al. Enhanced proliferative potential in culture of cells from p53-deficient mice. *Oncogene*. 1993;8:3313–22.
15. Jacks T, Remington L, Williams BO, et al. Tumor spectrum analysis in p53-mutant mice. *Curr Biol*. 1994;4:1–7.
16. Purdie CA, Harrison DJ, Peter A, et al. Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. *Oncogene*. 1994;9:603–9.
17. Armstrong JF, Kaufman MH, Harrison DJ. High-frequency developmental abnormalities in p53-deficient mice. *Curr. Biol*. 1995;5:931–36.
18. Sah VP, Attardi LD, Mulligan GJ, Williams BO, Bronson RT, Jacks T. A subset of p53-deficient embryos exhibit exencephaly. *Nat Genet*. 1995;10: 175–80.
19. Jones SN, Roe AE, Donehower LA, Bradley A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature*. 1995;378: 206–8.
20. Montes de Oca Luna R, Wagner DS, Lozano G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature*. 1995;378: 203–6.
21. Harvey M, McArthur MJ, Montgomery CA, Butel JS, Bradley A, Donehower LA. Spontaneous and carcinogen induced tumorigenesis in p53-deficient mice. *Nat Genet*. 1993;5:225–9.
22. Kemp CJ, Wheldon T, Balmain A. p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. *Nat Genet*. 1994;8:66–9.
23. Malkin D. p53 and the Li-Fraumeni syndrome. *Cancer Genet Cytogenet*. 1993;66:83–92.
24. Kuperwasser C, Hurlbut GD, Kittrell FS, et al. 2000. Development of spontaneous mammary tumors in BALB/c p53 heterozygous mice. A model for Li-Fraumeni syndrome. *Am J Pathol*. 2000;157:2151–59.
25. Clarke AR, Purdie CA, Harrison DJ, et al. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature*. 1993;362:849–52.
26. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature*. 1993,362:847–9.
27. Harvey M, Sands AT, Weiss RS, et al. In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene*. 1993;8: 2457–67.
28. Cross SM, Sanchez CA, Morgan CA, et al. A p53-dependent mouse spindle checkpoint. *Science*. 1995;267:1353–56.
29. Hollander MC, Sheikh MS, Bulavin DV, et al. Genomic instability in Gadd45 deficient mice. *Nat Genet*. 1999;23:176–84.
30. Blyth K, Terry A, O'Hara M, et al. Synergy between a human c-myc transgene and p53 null genotype in murine thymic lymphomas: contrasting effects of homozygous and heterozygous p53 loss. *Oncogene*. 1995;10: 1717–23.
31. Harvey M, Vogel H, Lee EY, Bradley A, Donehower LA. Mice deficient in both p53 and Rb develop tumors primarily of endocrine origin. *Cancer Res*. 1995;55:1146–51.
32. Marino S, Vooijs M, van Der Gulden H, Jonkers J, Berns A. Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev*. 2000;14:994–1004.
33. Godley LA, Kopp JB, Eckhaus M, Paglino JJ, Owens J, Varmus HE. Wild-type p53 transgenic mice exhibit altered differentiation of the ureteric bud and possess small kidneys. *Genes Dev*. 1996;10:836–50.
34. Liu G, McDonnell TJ, Montes de Oca Luna R, et al. High metastatic potential in mice inheriting a targeted p53 missense mutation. *Proc Natl Acad Sci U S A*. 2000;97:4174–79.
35. Chao C, Saito S, Kang J, et al. p53 transcriptional activity is essential for p53-dependent apoptosis following DNA damage. *EMBO J*. 2000;19:4967–75.
36. Jimenez GS, Nister M, Stommel JM, et al. A transactivation-deficient mouse model provides insights into Trp53 regulation and function. *Nat Genet*. 2000;26:37–43.
37. Lang GA, Iwakuma T, Suh YA, et al. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell*. 2004;119:861–72.
38. Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell*. 2004;119:847–60.
39. Lavigueur A, Maltby V, Mock D, Rossant J, Pawson T, Bernstein A. High incidence of lung, bone, and lymphoid tumors in transgenic mice overexpressing mutant alleles of the p53 oncogene. *Mol Cell Biol*. 1989;9: 3982–91
40. Tyner SD, Venkatachalam S, Choi J, et al. p53 mutant mice that display early ageing-associated phenotypes. *Nature*. 2002;415:45–53.
41. Ferbeyre G, Lowe SW. The price of tumour suppression? *Nature*. 2002;415: 26–7.
42. García-Cao I, García-Cao M, Martín-Caballero J, et al. "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *EMBO J*. 2002;21:6225–35.
43. Collavin L, Lunardi A, Del Sal G. p53-family proteins and their regulators: hubs and spokes in tumor suppression. *Cell Death Differ*. 2010;17:901–11.
44. Zawacka-Pankau J, Kostecka A, Sznarkowska A, Hedström E, Kawiak A. p73 tumor suppressor protein: a close relative of p53 not only in structure but also in anti-cancer approach? *Cell Cycle*. 2010;9:720–8.
45. Bagchi A, Mills AA. The quest for the 1p36 tumor suppressor. *Cancer Res*. 2008;68:2551–6.
46. Irwin M, Marin MC, Phillips AC, Seelan RS, Smith DI, et al. Role for the p53 homologue p73 in E2F-1-induced apoptosis. *Nature*. 2000;407:645–48.
47. Lissy NA, Davis PK, Irwin M, Kaelin WG, Dowdy SF. A common E2F-1 and p73 pathway mediates cell death induced by TCR activation. *Nature*. 200;407:642–45.
48. Waltermann A, Kartasheva NN, Dobbelstein M. Differential regulation of p63 and p73 expression. *Oncogene*. 2003;22:5686–93.
49. Stiewe T, Putzer BM. Role of the p53-homologue p73 in E2F1-induced apoptosis. *Nat Genet*. 2000;26:464–69.
50. Wu S, Murai S, Kataoka K, Miyagishi M. Yin Yang 1 induces transcriptional activity of p73 through cooperation with E2F1. *Biochem Biophys Res Commun*. 2008;365:75–81.
51. Pozniak CD, Radinovic S, Yang A, McKeon F, Kaplan DR, Miller FD. An anti-apoptotic role for the p53 family member, p73, during developmental neuron death. *Science*. 2000;289:304–6.
52. Yang A, Walker N, Bronson R, et al. p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. *Nature*. 2000;404:99–103.
53. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature*. 1999;398:708–13.
54. Yang A, Schweitzer R, Sun D, et al. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature*. 1999;398:714–18.
55. Flores ER, Tsai KY, Crowley D, et al. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature*. 2002;416: 560–4.
56. Flores ER, Sengupta S, Miller JB, et al. Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. *Cancer Cell*. 2005;7:363–73.
57. Mastrangelo D, De Francesco S, Di Leonardo A, Lentini L, Hadjistilianou T. The retinoblastoma paradigm revisited. *Med Sci Monit*. 2008;14:RA231–40. Review.
58. Munakata T, Liang Y, Kim S, McGivern DR, Huibregtse J, et al. Hepatitis C virus induces E6AP dependent degradation of the retinoblastoma protein. *PLoS Pathog*. 2007;3:1335–47.
59. Yokota J, Akiyama T, Fung YK, et al. Altered expression of the retinoblastoma (RB) gene in small-cell carcinoma of the lung. *Oncogene*. 1998;3: 471–5.
60. Meuwissen R, Linn SC, Linnoila RI, Zevenhoven J, Mooi WJ, Berns A. Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model. *Cancer Cell*. 2003;4:181–9.
61. Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nat Rev Cancer*. 2008;8:671–82. Review.
62. Cobrinik D, Lee MH, Hannon G, et al. Shared role of the pRB-related p130 and p107 proteins in limb development. *Genes Dev*. 1996;10:1633–44.
63. Cobrinik D. Pocket proteins and cell cycle control. *Oncogene*. 2005;24: 2796–809.

64. Giacinti C, Giordano A. RB and cell cycle progression. *Oncogene*. 2006;25: 5220–7. Review.
65. Sherr CJ. The Pezcoller lecture: cancer cell cycles revisited. *Cancer Res*. 2000;60:3689–95.
66. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol*. 2007;8:703–13.
67. Chen HZ, Tsai SY, Leone G. Emerging roles of E2Fs in cancer: an exit from cell cycle control. *Nat Rev Cancer*. 2009;9:785–97. Review.
68. Hallstrom TC, Nevins JR. Balancing the decision of cell proliferation and cell fate. *Cell Cycle*. 2009;8:532–5. Review.
69. Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. *Nat Rev Mol Cell Biol*. 2002;3:11–20. Review.
70. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. *Nature*. 1992;359:295–300.
71. Almasan A, Yin Y, Kelly RE, et al. Deficiency of retinoblastoma protein leads to inappropriate S-phase entry, activation of E2F responsive genes, and apoptosis. *Proc Natl Acad Sci U S A*. 1995;92:5436–40.
72. Iaquinta PJ, Lees JA. Life and death decisions by the E2F transcription factors. *Curr Opin Cell Biol*. 2007;19:649–57. Review.
73. Vigo E, Muller H, Prosperini E, et al. CDC25A phosphatase is a target of E2F and is required for efficient E2F-induced S phase. *Mol Cell Biol*. 1999;19:6379–95.
74. Moroni MC, Hickman ES, Denchi EL, et al. Apaf-1 is a transcriptional target for E2F and p53. *Nat Cell Biol*. 2001;3:552–8.
75. Lazzerini Denchi E, Helin K. E2F1 is crucial for E2F-dependent apoptosis. *EMBO Rep*. 2005;6:661–8.
76. Clarke AR, Maandag ER, van Roon M, et al. Requirement for a functional Rb-1 gene in murine development. *Nature*. 1992;359:328–30.
77. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. *Nature*. 1992;359:295–300.
78. Lee EY, Chang CY, Hu N, et al. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. *Nature*.1992;359: 288–94.
79. Morgenbesser SD, Williams BO, Jacks T, DePinho RA. p53-dependent apoptosis produced by Rb-deficiency in the developing mouse lens. *Nature*. 1994;371:72–4.
80. Maandag EC, van der Valk M, Vlaar M, et al. Developmental rescue of an embryoniclethal mutation in the retinoblastoma gene in chimeric mice. *EMBO J*. 1994;13:4260–68.
81. Williams BO, Schmitt EM, Remington L, et al. Extensive contribution of Rb-deficient cells to adult chimeric mice with limited histopathological consequences. *EMBO J*. 1994;13:4251–59.
82. Tsai KY, Hu Y, Macleod KF, Crowley D, Yamasaki L, Jacks T. Mutation of E2f-1 suppresses apoptosis and inappropriate S phase entry and extends survival of Rb-deficient mouse embryos. *Mol Cell*. 1998;2:293–304.
83. Ziebold U, Reza T, Caron A, Lees JA. 2001. E2F3 contributes both to the inappropriate proliferation and to the apoptosis arising in Rb mutant embryos. *Genes Dev*. 15:386–91.
84. de Bruin A, Wu L, Saavedra HI, et al. Rb function in extraembryonic lineages suppresses apoptosis in the CNS of Rb-deficient mice. *Proc Natl Acad Sci U S A*. 2003;100:6546–51.
85. Lasorella A, Noseda M, Beyna M, Yokota Y, Iavarone A. Id2 is a retinoblastoma protein target and mediates signalling by Myc oncoproteins. *Nature*. 2000;407:592–8.
86. Robanus-Maandag E, Dekker M, van der Valk M, et al. p107 is a suppressor of retinoblastoma development in pRb-deficient mice. *Genes Dev*. 1998;12: 1599–609.
87. Dannenberg JH, van Rossum A, Schuijff L, te Riele H. Ablation of the retinoblastoma gene family deregulates G(1) control causing immortalization and increased cell turnover under growth-restricting conditions. *Genes Dev*. 2000;14:3051–64.
88. Sage J, Mulligan GJ, Attardi LD, et al. Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. *Genes Dev*. 2000;14:3037–50.
89. Berman SD, Calo E, Landman AS, et al. Metastatic osteosarcoma induced by inactivation of Rb and p53 in the osteoblast lineage. *Proc Natl Acad Sci U S A*. 2008;105:11851–6.
90. Choi J, Curtis SJ, Roy DM, Flesken-Nikitin A, Nikitin AY. Local mesenchymal stem/progenitor cells are a preferential target for initiation of adult soft tissue sarcomas associated with p53 and Rb deficiency. *Am J Pathol*. 2010;177:2645–58.
91. Bignon YJ, Chen Y, Chang CY, Riley DJ, Windle JJ, et al. Expression of a retinoblastoma transgene results in dwarf mice. *Genes Dev*. 1993;7: 1654–62.
92. Nikitin AYu, Shan B, Flesken-Nikitin A, Chang KH, Lee WH. The retinoblastoma gene regulates somatic growth during mouse development. *Cancer Res*. 2001;61:3110–8.
93. Ruas M, Peters G. The p16INK4a/CDKN2A tumor suppressor and its relatives. *Biochim Biophys Acta*. 1998;1378:F115–77.
94. Dominguez-Brauer C, Brauer PM, Chen YJ, Pimkina J, Raychaudhuri P. Tumor suppression by ARF: gatekeeper and caretaker. *Cell Cycle*. 2010;9: 86–9.
95. Sherr CJ. The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol*. 2001;2:731–7. Review.
96. Sherr CJ, Bertwistle D, DEN Besten W, et al. p53-Dependent and -independent functions of the Arf tumor suppressor. *Cold Spring Harb Symp Quant Biol*. 2005;70:129–37. Review.
97. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*. 1993;366:704–7.
98. Liao MJ, Van Dyke T. Critical role for Atm in suppressing V(D)J recombination-driven thymic lymphoma. *Genes Dev*. 1999;13:1246–50.
99. Tam SW, Shay JW, Pagano M. Differential expression and cell cycle regulation of the cyclin-dependent kinase 4 inhibitor p16Ink4. *Cancer Res*. 1994;54:5816–20.
100. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi- 1 regulates cell proliferation and senescence through the ink4a locus. *Nature*. 1999;397:164–68.
101. Ohtani N, Zebedee Z, Huot TJ, et al. Opposing effects of Ets and Id proteins on p16INK4a expression during cellular senescence. *Nature*. 2001;409:1067–70.
102. Pomerantz J, Schreiber-Agus N, Liegeois NJ, et al. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell*. 1998;92:713–23.
103. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell*. 1998;92:725–34.
104. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. *Proc Natl Acad Sci U S A*. 1998;95:8292–97.
105. Stott FJ, Bates S, James MC, et al. The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. *EMBO J*. 1998;17:5001–14.
106. Sherr CJ. Divorcing ARF and p53: an unsettled case. *Nat Rev Cancer*. 2006;6:663–73.
107. Dominguez-Brauer C, Brauer PM, Chen YJ, Pimkina J, Raychaudhuri P. Tumor suppression by ARF: gatekeeper and caretaker. *Cell Cycle*. 2010;9: 86–9. Review.
108. Robertson KD, Jones PA. The human ARF cell cycle regulatory gene promoter is a CpG island which can be silenced by DNA methylation and down-regulated by wild-type p53. *Mol Cell Biol*. 1998;18:6457–73.
109. Zindy F, Eischen CM, Randle DH, Kamijo T, Cleveland JL, et al. Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev*. 1998;12:2424–33.
110. de Stanchina E, McCurrach ME, Zindy F, et al. E1A signaling to p53 involves the p19(ARF) tumor suppressor. *Genes Dev*. 1998;12:2434–42.
111. Jacobs JJ, Scheijen B, Voncken JW, Kieboom K, Berns A, van Lohuizen M. Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes Dev*. 1999;13:2678–90.
112. Jacobs JJ, Keblusek P, Robanus-Maandag E, et al. Senescence bypass screen identifies TBX2 which represses Cdkn2a (p19(ARF)) and is amplified in a subset of human breast cancers. *Nat Genet*. 2000;26: 291–99.
113. Maestro R, Dei Tos AP, Hamamori Y, Krasnokutsky S, Sartorelli V, et al. Twist is a potential oncogene that inhibits apoptosis. *Genes Dev*. 1999;13: 2207–17.

114. Maeda T, Hobbs RM, Merghoub T, Guernah I, Zelent A, et al. Role of the proto-oncogene Pokemon in cellular transformation and ARF repression. *Nature*. 2005;433:278–85.
115. Linggi B, Müller-Tidow C, van de Locht L, et al. The t(8;21) fusion protein, AML1 ETO, specifically represses the transcription of the p14(ARF) tumor suppressor in acute myeloid leukemia. *Nat Med*. 2002; 8:743–50.
116. Inoue K, Roussel MF, Sherr CJ. Induction of ARF tumor suppressor gene expression and cell cycle arrest by transcription factor DMP1. *Proc Natl Acad Sci U S A*. 1999;96:3993–98.
117. Inoue K, Mallakin A, Frazier DP. Dmp1 and tumor suppression. *Oncogene*. 2007;26:4329–35.
118. Bates S, Phillips AC, Clark PA, Stott F, Peters G, et al. p14 ARF links the tumour suppressors RB and p53. *Nature*. 1998;395:124–25.
119. Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D, DePinho RA. Role of the INK4a locus in tumor suppression and cell mortality. *Cell*. 1996;85:27–37.
120. Orlow I, Rabbani F, Chin L, et al. Involvement of the Ink4a gene (p16 and p19arf) in murine tumorigenesis. *Int J Oncol*. 1999;15:17–24.
121. Chin L, Pomerantz J, Polsky D, et al. Cooperative effects of INK4a and ras in melanoma susceptibility in vivo. *Genes Dev*. 1997;11:2822–34.
122. Kamijo T, Zindy F, Roussel MF, et al. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell*. 1997;91:649–59.
123. Kamijo T, Bodner S, van de Kamp E, Randle DH, Sherr CJ. Tumor spectrum in ARF-deficient mice. *Cancer Res*. 1999;59:2217–22.
124. Sharpless NE, Ramsey MR, Balasubramanian P, Castrillon DH, DePinho RA. The differential impact of p16(INK4a) or p19(ARF) deficiency on cell growth and tumorigenesis. *Oncogene*. 2004;23:379–85.
125. Sharpless NE, Bardeesy N, Lee KH, et al. Loss of p16Ink4a with retention of p19Arf predisposes mice to tumorigenesis. *Nature*. 2001;413:86–91.
126. Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature*. 2001;413:83–6.
127. Zindy F, Williams RT, Baudino TA, Rehg JE, Skapek SX, et al. Arf tumor suppressor promoter monitors latent oncogenic signals in vivo. *Proc Natl Acad Sci U S A*. 2003;100:15930–5.
128. Randle DH, Zindy F, Sherr CJ, Roussel MF. Differential effects of p19(Arf) and p16(Ink4a) loss on senescence of murine bone marrow-derived preB cells and macrophages. *Proc Natl Acad Sci U S A*. 2001;98: 9654–9.
129. Williams RT, Roussel MF, Sherr CJ. Arf gene loss enhances oncogenicity and limits imatinib response in mouse models of Bcr-Abl-induced acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A*. 2006;103:6688–93.
130. Wong S, Witte ON. The BCR-ABL story: bench to bedside and back. *Annu Rev Immunol*. 2004;22:247–306. Review.
131. Drucker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355:2408–17.
132. Williams RT, den Besten W, Sherr CJ. Cytokine-dependent imatinib resistance in mouse BCR-ABL+, Arf-null lymphoblastic leukemia. *Genes Dev*. 2007;21:2283–7.
133. Yang C, Ionescu-Tiba V, Burns K, et al. The role of the cyclin D1-dependent kinases in ErbB2-mediated breast cancer. *Am J Pathol*. 2004; 164:1031–8.
134. Matheu A, Pantoja C, Efeyan A, et al. Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging. *Genes Dev*. 2004;18: 2736–46.
135. Matheu A, Maraver A, Klatt P, et al. Delayed ageing through damage protection by the Arf/p53 pathway. *Nature*. 2007;448:375–9.
136. Matheu A, Maraver A, Collado M, et al. Anti-aging activity of the Ink4/Arf locus. *Aging Cell*. 2009;8:152–61.
137. Krishnamurthy J, Torrice C, Ramsey MR, et al. Ink4a/Arf expression is a biomarker of aging. *J Clin Invest*. 2004;114:1299–307.
138. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, et al. p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature*. 2006;443:453–7.
139. Franklin DS, Godfrey VL, Lee H, et al. CDK inhibitors p18(INK4c) and p27(Kip1) mediate two separate pathways to collaboratively suppress pituitary tumorigenesis. *Genes Dev*. 1998;12:2899–911.
140. Latres E, Malumbres M, Sotillo R, et al. Limited overlapping roles of P15(INK4b) and P18(INK4c) cell cycle inhibitors in proliferation and tumorigenesis. *EMBO J*. 2000;19:3496–506.
141. Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. The murine gene p27 Kip1 is haplo-insufficient for tumour suppression. *Nature*. 1998;396: 177–80.
142. Zindy F, Cunningham JJ, Sherr CJ, Jogal S, Smeyne RJ, Roussel MF. Postnatal neuronal proliferation in mice lacking Ink4d and Kip1 inhibitors of cyclin-dependent kinases. *Proc Natl Acad Sci U S A*. 1999;96:13462–7.
143. Uziel T, Zindy F, Xie S, et al. The tumor suppressors Ink4c and p53 collaborate independently with Patched to suppress medulloblastoma formation. *Genes Dev*. 2005;19:2656–67.
144. Rahman N, Stratton MR. The genetics of breast cancer susceptibility. *Annu Rev Genet*. 1998;32:95–121.
145. Deng CX, Xu X. Generation and analysis of Brca1 conditional knockout mice. *Methods Mol Biol*. 2004;280:185–200.
146. Scully R, Livingston DM. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature*. 2000;408:429–32.
147. Deng CX, Brodie SG. Roles of BRCA1 and its interacting proteins. *Bio Essays*. 2000;22:728–37.
148. Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH. Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Genet*. 1996;12:191–94.
149. Hakem R, de la Pompa JL, Sirard C, et al. The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell*. 1996;85:1009–23.
150. Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH. Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev*. 1996;10:1835–43.
151. Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A. Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 null-lizygous embryos. *Genes Dev*. 1997;11:1226–41.
152. Evers B, Jonkers J. Mouse models of BRCA1 and BRCA2 deficiency: past lessons, current understanding and future prospects. *Oncogene*. 2006;25: 5885–97.
153. Hakem R, de la Pompa JL, Mak TW. Developmental studies of Brca1 and Brca2 knock-out mice. *J Mammary Gland Biol Neoplasia*. 1998;3: 431–45.
154. Deng CX, Brodie SG. Knockout mouse models and mammary tumorigenesis. *Semin Cancer Biol*. 2001;11:387–94. Review.
155. Gowen LC, Avrutskaya AV, Latour AM, Koller BH, Leadon SA. BRCA1 required for transcription-coupled repair of oxidative DNA damage. *Science*. 1998;281:1009–12.
156. Shen SX, Weaver Z, Xu X, Li C, Weinstein M, et al. A targeted disruption of the murine Brca1 gene causes gamma irradiation hypersensitivity and genetic instability. *Oncogene*. 1998;17:3115–24.
157. Chandler J, Hohenstein P, Swing D, et al. Human BRCA1 gene rescues the embryonic lethality of Brca1 mutant mice. *Genesis*. 2001;29:72–7.
158. Lane TF, Lin C, Brown MA, Solomon E, Leder P. Gene replacement with the human BRCA1 locus: tissue specific expression and rescue of embryonic lethality in mice. *Oncogene*. 2000;19:4085–90.
159. Mak TW, Hakem A, McPherson JP, et al. Brca1 is required for T cell lineage development but not TCR loci rearrangement. *Nat Immunol*. 2000;1:77–82.
160. Xu X, Wagner KU, Larson D, et al. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat Genet*. 1999;22:37–43.
161. Liu X, Holstege H, van der Gulden H, et al. Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. *Proc Natl Acad Sci U S A*. 2007;104: 12111–6.
162. Brodie SG, Xu X, Qiao W. Multiple genetic changes are associated with mammary tumorigenesis in Brca1 conditional knockout mice. *Oncogene*. 2001;20:7514–23.

163. Hoshino A, Yee CJ, Campbell M, et al. Effects of BRCA1 transgene expression on murine mammary gland development and mutagen-induced mammary neoplasia. *Int J Biol Sci*. 2007;3:281–91.
164. Chen J, Silver DP, Walpita D, et al. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol Cell*. 1998;2:317–28.
165. Sharan SK, Morimatsu M, Albrecht U, et al. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature*. 1997;386:804–10.
166. Suzuki A, de la Pompa JL, Hakem R, et al. Brca2 is required for embryonic cellular proliferation in the mouse. *Genes Dev*. 1997;11:1242–52.
167. Connor F, Bertwistle D, Mee PJ, et al. Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. *Nat Genet*. 1997;17:423–30.
168. Friedman LS, Thistlethwaite FC, Patel KJ, et al. Thymic lymphomas in mice with a truncating mutation in Brca2. *Cancer Res*. 1998;58:1338–43.
169. Patel KJ, Yu VP, Lee H, et al. Involvement of Brca2 in DNA repair. *Mol Cell*. 1998;1:347–.
170. Tutt A, Gabriel A, Bertwistle D, et al. Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. *Curr Biol*. 1999;9:1107–10.
171. Yu VP, Koehler M, Steinlein C, et al. Gross chromosomal rearrangements and genetic exchange between nonhomologous chromosomes following BRCA2 inactivation. *Genes Dev*. 2000;14:1400–6.
172. Ludwig T, Fisher P, Murty V, Efstratiadis A. Development of mammary adenocarcinomas by tissue-specific knockout of Brca2 in mice. *Oncogene*. 2001;20:3937–48.
173. Cheung AM, Elia A, Tsao MS, et al. Brca2 deficiency does not impair mammary epithelium development but promotes mammary adenocarcinoma formation in p53(+/–) mutant mice. *Cancer Res*. 2004;64:1959–65.
174. Cheung AM, Hande MP, Jalali F, et al. Loss of Brca2 and p53 synergistically promotes genomic instability and deregulation of T-cell apoptosis. *Cancer Res*. 2002;62:6194–204.
175. Park PG, Lee H. Development of thymic lymphomas in mice disrupted of Brca2 allele in the thymus. *Exp Mol Med*. 2008;40:339–44.
176. Lee H, Trainer AH, Friedman LS et al. Mitotic checkpoint inactivation fosters transformation in cells lacking the breast cancer susceptibility gene, Brca2. *Mol Cell*. 1999;4:1–10.
177. Francis JC, McCarthy A, Thomsen MK, Ashworth A, Swain A. Brca2 and Trp53 deficiency cooperate in the progression of mouse prostate tumourigenesis. *PLoS Genet*. 2010;6:e1000995.
178. Yu Y, Xu F, Peng H, et al. NOEY2 (ARH1), an imprinted putative tumor suppressor gene in ovarian and breast carcinomas. *Proc Natl Acad Sci U S A*. 1999;96:214–9.
179. Xu F, Xia W, Luo RZ, et al. The human ARH1 tumor suppressor gene inhibits lactation and growth in transgenic mice. *Cancer Res*. 2000;60:4913–20.
180. Lu Z, Luo RZ, Lu Y, et al. The tumor suppressor gene ARH1 regulates autophagy and tumor dormancy in human ovarian cancer cells. *J Clin Invest*. 2008;118:3917–29.
181. Sexl V, Diehl JA, Sherr CJ, Ashmun R, Beach D, Roussel MF. A rate limiting function of cdc25 A for S phase entry inversely correlates with tyrosine dephosphorylation of Cdk2. *Oncogene*. 1999;18:573–82.
182. Cole MD, Nikiforov MA. Transcriptional activation by the Myc oncoprotein. *Curr Top Microbiol Immunol*. 2006;33–50. Review.
183. Lebofsky R, Walter JC. New Myc-anisms for DNA replication and tumorigenesis? *Cancer Cell*. 2007;12:102–3.
184. Sugiyama T, Frazier DP, Taneja P, et al. Signal transduction involving the Dmp1 transcription factor and its alteration in human cancer. *Clinical Medicine Oncology*. 2008;2:209–19.
185. Inoue K, Sugiyama T, Taneja P, Morgan RL, Frazier DP. Emerging roles of DMP1 in lung cancer. *Cancer Res*. 2008;68:4487–90.
186. Sugiyama T, Frazier DP, Taneja P, Morgan RL, Willingham MC, Inoue K. The role of Dmp1 and its future in lung cancer diagnostics. *Expert Rev Mol Diagn*. 2008;8:435–48.
187. Taneja P, Maglic D, Kai F, et al. Critical role of Dmp1 in HER2/neu-p53 signaling and breast carcinogenesis. *Cancer Res*. 2010;70:2084–94.