Generation of primary tumors with Flp recombinase in FRT-flanked p53 mice

Chang-Lung Lee1,*, Everett J. Moding1,*, Xiaofang Huang2, Yifan Li2, Loretta Z. Woodlief2, Rafaela C. Rodrigues2, Yan Ma2 and David G. Kirsch1,2,‡

SUMMARY

The site-specific recombinases Cre and Flp can mutate genes in a spatially and temporally restricted manner in mice. Conditional recombination of the tumor suppressor gene p53 using the Cre-loxP system has led to the development of multiple genetically engineered mouse models of human cancer. However, the use of Cre recombinase to initiate tumors in mouse models limits the utilization of Cre to genetically modify other genes in tumor stromal cells in these models. To overcome this limitation, we inserted FRT (flippase recognition target) sites flanking exons 2-6 of the endogenous p53 gene in mice to generate a p53FRT allele that can be deleted by Flp recombinase. We show that Flpo-mediated deletion of p53 in mouse embryonic fibroblasts impairs the p53-dependent response to genotoxic stress in vitro. In addition, using F5F-KrasG12D+; p53FRT/FRT mice, we demonstrate that an adenovirus expressing Flpo recombinase can initiate primary lung cancers and sarcomas in mice. p53FRT mice will enable dual recombinase technology to study cancer biology because Cre is available to modify genes specifically in stromal cells to investigate their role in tumor development, progression and response to therapy.

INTRODUCTION

The transformation related protein p53 gene, Trp53, is the most frequently mutated gene in human cancer, altered in approximately 50% of human malignancies (Brosh and Rotter, 2009). The p53 nuclear phosphoprotein functions as a transcription factor that responds to cellular stress by initiating multiple signaling pathways. The p53 response varies across cell and tissue types, and involves a spectrum from transient cell cycle arrest to senescence and apoptosis (Stiewe, 2007). Mice deficient for p53 generally develop a spectrum from transient cell cycle arrest to senescence and apoptosis (Stiewe, 2007). Mice deficient for p53 generally develop a spectrum from transient cell cycle arrest to senescence and apoptosis (Stiewe, 2007).

The site-specific recombinases Cre and Flp allow for spatially and temporally regulated mutation of a target gene in the somatic tissues of mice (Branda and Dymecki, 2004). Conditional recombination of p53 using the Cre-loxP system has been utilized to delete or mutate p53 in a tissue-specific manner or to delete p53 at a specific time during development (Donehower et al., 1992; Jacks et al., 1994).

The site-specific recombinases Cre and Flp allow for spatially and temporally regulated mutation of a target gene in the somatic tissues of mice (Branda and Dymecki, 2004). Conditional recombination of p53 using the Cre-loxP system has been utilized to delete or mutate p53 in a tissue-specific manner or to delete p53 at a specific time during development (Donehower et al., 1992; Jacks et al., 1994).

Technological advancements have led to the development of multiple mouse models of primary cancer (Marino et al., 2000; Lin et al., 2004; Jackson et al., 2005; Kirsch et al., 2007; Martinez-Cruz et al., 2008). Genetically engineered mouse models (GEMMs) might offer an advantage over xenograft and chemically induced cancer models by providing an opportunity to study mechanisms of autochthonous cancer development and response to treatment in an anatomically restricted manner in mice that are neither tumor-prone nor immunosuppressed (Sharpless and DePinho, 2006). However, most GEMMs use Cre-loxP technology to initiate cancer, limiting the availability of Cre recombinase to modify genes in tumor stromal cells.

Because Cre and Flp recombine distinct DNA target sites, loxP and FRT, respectively (Branda and Dymecki, 2004), these two highly efficient site-specific recombinase systems (Cre-loxP and Flp-FRT) have been used to create genetically engineered mice with a targeting construct with a removable positive selection cassette (Meyers et al., 1998). More recently, dual recombinase technology was used to sequentially delete p53 and activate the Kras oncogene, revealing the importance of timing of Kras and p53 mutations in tumorigenesis (Young et al., 2011). Despite the growing abundance of loxP-flanked (‘floxed’) alleles and tissue-specific Cre drivers, the Flp-FRT system has been utilized less frequently than Cre-loxP to modify genes in the somatic tissues in mice. Generating additional FRT-flanked (‘frted’) alleles will enable dual recombinase technology so that distinct gene mutations can be directed to different cell types by Cre and Flp recombinases. Here, we generated p53FRT mice in which the endogenous p53 allele is flanked by FRT sites so that it can be deleted by Flp recombinase.

RESULTS

Generation of p53FRT mice

To generate a frted p53 mouse, we constructed a targeting vector in which exons 2 through 6 of p53 genomic DNA are flanked by FRT sites (Fig. 1A). Exons 2-6 encode for the DNA-binding domain that is required for p53-dependent tumor suppression (Brady et al., 2011). The 5′ FRT site was inserted between exons 1 and 2, and a loxP-flanked (floxed) PGK-neo cassette (neo) followed by a 3′ FRT site was inserted between exons 6 and 7. A PGK-diphtheria toxin A (DTA) cassette was placed following exon 11 as a negative selectable marker. The targeting vector was linearized and electroporated into embryonic stem (ES) cells. Following selection...
by G418, two out of 800 colonies had correctly undergone homologous recombination as demonstrated by PCR (Fig. 1B). Successful homologous recombination of the \( p53^FRT \)-neo allele into the endogenous \( p53 \) locus was confirmed by Southern blot. As shown in Fig. 1A and 1C, a ScaI-digested DNA fragment of 6.7 kb, which includes genomic DNA outside the targeting construct, was detected in these two ES cell clones using probes binding either neo or exon 11 of \( p53 \). ES cell line 8-8B was used to derive germline transmitting chimeric mice. Male chimeric mice were then bred to Meox2-Cre females to delete the floxed PGK-neo cassette in germine cells. Germline transmission of the targeted allele after deletion of the neo cassette (\( p53^FRT \) allele as shown in Fig. 1A) was confirmed by PCR (Fig. 1D).

**Characterization of \( p53^FRT \) MEFs**

To study FlpO-mediated recombination of the \( p53^FRT \) allele, mouse embryonic fibroblasts (MEFs) were isolated from \( p53^FRT/– \) females to delete the floxed PGK-neo cassette in \( p53 \) locus, the targeting construct, and the \( p53^FRT \)-null (\( p53^{-/-} \)) MEFs, which probably reflects incomplete infection of the MEFs with adenovirus.

Primary mammalian cells such as MEFs have a limited life span in vitro owing to \( p53 \)-mediated senescence and are transformed by loss of \( p53 \) (Harvey et al., 1993). We investigated whether cell-culture-induced senescence occurs in \( p53^FRT^{+/–} \) MEFs after FlpO-mediated recombination by assessing population doubling of cells in vitro. Similar to \( p53^{–/–} \) MEFs, \( p53^FRT^{+/–} \) MEFs infected with Ad-FlpO did not show \( p53 \)-mediated senescence and proliferated faster than \( p53^FRT^{+/–} \) MEFs infected with Ad-eGFP (Fig. 2C). Additionally, FlpO-recombined \( p53^FRT^{+/–} \) MEFs were found to be

**Fig. 1. Generation of \( p53^FRT^{+/–} \) mice by gene targeting.** (A) Schematic representation of the wild-type \( p53 \) locus, the targeting construct, and the \( p53^FRT \)-neo, \( p53^FRT \) and \( p53^{–/–} \) alleles. The 5′ \( FRT \) site (yellow triangle) was inserted between exons 1 and 2, whereas the 3′ \( FRT \) site (yellow triangle) and a loxP-flanked (red diamonds) PGK-neo cassette (neo) were inserted between exons 6 and 7 of \( p53 \). A PGK-DTA (DTA) cassette was placed following exon 11 as a negative selectable marker. (B) PCR amplicons including the 5′ \( FRT \) site (292 bp) as well as the PGK-neo cassette (2.5 kb) were detected in two ES cell clones (7-9H and 8-8B) electroporated with the targeting vector, whereas only one amplicon of the wild-type \( p53 \) allele (258 bp) was present in ES cells without transfection (WT). (C) Genomic DNA from aforementioned ES cells was digested by ScaI and hybridized with DNA probes that bind to either the PGK-neo cassette or \( p53 \) exon 11. A DNA fragment of ~6.7 kb that includes the PGK-neo cassette was detected in ES cells 7-9H and 8-8B using either probe, but was absent in WT ES cells. By contrast, a ScaI-digested fragment of ~5 kb from the WT \( p53 \) allele was detected in all ES cells using the probe binding to exon 11. (D) Transmission of the \( p53^FRT \) allele was shown by PCR using DNA extracted from tails of \( p53^FRT^{+/–} \), \( p53^FRT^{+/–} \), and WT mice. PCR amplicons including the 5′ \( FRT \) site (292 bp) as well as the 3′ \( FRT \) site and recombined \( loxP \) site (685 bp) were detected in \( p53^FRT^{+/–} \) and \( p53^FRT^{+/–} \) mice, but were absent in WT littermates.
Flp-mediated deletion of p53

generically unstable compared with p53\textsuperscript{WT} MEFs, as demonstrated by their markedly increased DNA content at later passages (Fig. 2D).

**Generation of FlpO-driven tumors**

To study Flp-mediated recombination of the p53\textsuperscript{FRT} allele in vivo, we crossed p53\textsuperscript{FRT} mice with mice carrying a Flp-activated allele of oncogenic Kras to generate FSF-Kras\textsuperscript{G12D/}; p53\textsuperscript{FRT/FRT} (Kp\textsuperscript{FRT}) compound conditional mutant mice. It has been shown that activation of Kras\textsuperscript{G12D} and deletion of p53 in LSL-Kras\textsuperscript{G12D/}; p53\textsuperscript{FL/FL} (Kp\textsuperscript{FRT}) mice via intramuscular (IM) and intranasal (IN) infection with Ad-Cre is sufficient to initiate high-grade soft-tissue sarcomas and lung adenocarcinomas (Jackson et al., 2005; Kirsch et al., 2007). Although we crossed mice with mice carrying a Flp-activated allele in vivo, Kp\textsuperscript{FRT} mice phenocopy lung tumors generated by Exelixis. In this mouse, exons 2-10 are flanked by a 5’ FRT site combined with one wild-type copy of LSL-Kras\textsuperscript{G12D/}; p53\textsuperscript{FRT/FRT} infected with Ad-Cre to generate lung adenocarcinomas and soft-tissue sarcomas. We infected Kp\textsuperscript{FRT} mice with IM and IN Ad-FlpO, and extremity sarcomas and high-grade lung adenocarcinomas developed as early as 8 weeks after infection (Fig. 3A-D). The time frame of tumor development was similar to that of tumors generated in Kp\textsuperscript{FRT} mice by Ad-Cre infection (Jackson et al., 2005; Kirsch et al., 2007). Although FSF-Kras\textsuperscript{G12D/} mice can also develop lung tumors, the tumors are low grade (Young et al., 2011). Kras\textsuperscript{G12D/} lung tumors have an average tumor volume doubling time of approximately 35 days (Oliver et al., 2010). By contrast, lung tumors from Kp\textsuperscript{FRT} mice had a doubling time of approximately 2 weeks (Fig. 3E,F), which was similar to that of lung tumors generated in Kp\textsuperscript{FRT} mice following Ad-Cre infection (Kirsch et al., 2010; Oliver et al., 2010).

**DISCUSSION**

Human cancers develop in a complex environment composed of blood vessels, fibroblasts and immune cells. The tumor microenvironment has been shown to contribute to all of the hallmarks of cancer (Hanahan and Weinberg, 2011). Primary mouse models of cancer driven by site-specific recombinases develop within the native microenvironment in immunocompetent mice. A number of studies have demonstrated that genetically engineered mouse models could more accurately recapitulate the tumor stroma and microenvironment of human cancer than xenograft models in immunocompromised mice (Olive et al., 2009; Graves et al., 2010; Maity and Koumenis, 2010). In addition, the response of these primary mouse cancer models to conventional and novel therapies has been shown to closely model the response of human cancers in clinical trials (Singh et al., 2010). Dual recombinase technology will enable further examination of the role of the tumor microenvironment in primary tumors (Fig. 4).

Interestingly, a mouse with deletion of the first six exons of p53 was previously reported to express a truncated RNA capable of coding for the C-terminus of the p53 protein that can be detected only after in vitro translation (Tyner et al., 2002). When combined with one wild-type copy of p53 (p53\textsuperscript{m/+}), this mutant allele (m) leads to a gain of p53 function and early-aging associated phenotypes. However, p53\textsuperscript{m/m} mice phenocopy p53\textsuperscript{FRT/–} mice. We have looked for a truncated p53 protein in p53\textsuperscript{FRT/–} MEFs infected with Ad-FlpO by western blot by using an antibody against the full-length p53 protein. However, we were unable to detect the truncated protein. This might be due to lack of antibody specificity for the C-terminal epitope or because the level of truncated p53 protein in mouse cells is below the detection limit by western blot, similar to that found in p53\textsuperscript{m/m} mice (Tyner et al., 2002). Regardless of whether the truncated p53 protein is expressed, this recombined allele lacks the ability to suppress tumor development. In addition, a FRT-flanked p53 mouse has been generated by Exelixis. In this mouse, exons 2-10 are flanked by FRT sites. This allele has been used to generate primary mouse lung tumors in combination with the LSL-Kras\textsuperscript{G12D} allele using an adenoovirus expressing both Cre and Flp (Singh et al., 2010). However, to our knowledge, our study is the first to use a p53\textsuperscript{FRT} allele to generate lung adenocarcinomas and soft-tissue sarcomas with Flp-mediated recombination alone.

In summary, we have generated a conditional p53 mouse allele regulated by Flp recombinase. When used in combination with the

---

**Fig. 2. Characterization of p53\textsuperscript{FRT} MEFs.**

(A) PCR primers flanking the 5’ FRT site and the recombined FRT site (Δ2-6) demonstrate recombination of the p53\textsuperscript{FRT} allele in p53\textsuperscript{FL/–} MEFs infected with 100 MOI FlpO-expressing adenovirus (Ad-FlpO), but not p53\textsuperscript{FRT/–} MEFs infected with eGFP-expressing adenovirus (Ad-eGFP). (B) Western blot of passage 5 MEFs treated with 0.5 μg/ml doxorubicin for 18 hours after infection with Ad-FlpO or Ad-eGFP. (C) 3T3 protocol on p53\textsuperscript{m/m} and p53\textsuperscript{FRT/–} MEFs infected with 100 MOI Ad-FlpO and p53\textsuperscript{FRT/–} MEFs infected with 100 MOI Ad-eGFP. (D) Relative DNA content based on propidium iodide staining measured by flow cytometry of passage 4 and passage 10 MEFs infected with 100 MOI Ad-FlpO.
FSF-Kras<sup>G12D</sup> allele, the p53<sup>FRT</sup> mouse can be used to generate primary sarcomas and lung cancers with Flp recombinase. When combined with the growing number of loxP-flanked alleles and tissue-specific Cre drivers, this novel mouse model will enable dual recombinase technology to be employed to investigate the mechanism by which stromal cells contribute to cancer development, progression, and response to therapy.

**METHODS**

**Mouse strains**

All animal procedures for this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Duke University. FSF-Kras<sup>G12D</sup> and p53<sup>FRT/FRT</sup> mice were kindly provided by Tyler Jacks and were described previously (Jacks et al., 1994; Young et al., 2011). Meox2-Cre mice were obtained from Jackson Laboratory (Tallquist and Soriano, 2000).

**Fig. 3. Generation of primary cancers in FSF-Kras<sup>G12D</sup>; p53<sup>FRT/FRT</sup> mice by Flp recombinase.** (A) Intramuscular injection of Ad-FlpO into FSF-Kras<sup>G12D</sup>; p53<sup>FRT/FRT</sup> mice caused soft tissue sarcomas at the site of injection in the lower extremity 2 months post-injection. (B) Sections of the sarcomas were stained with hematoxylin and eosin, and show high-grade spindle cells. (C) Intranasal infection of Ad-FlpO into FSF-Kras<sup>G12D</sup>; p53<sup>FRT/FRT</sup> mice caused lung adenocarcinomas 2 months post-infection. (D) Higher magnification of lung tumors demonstrates pleomorphic nuclei, prominent nucleoli and nuclear molding characteristic of high-grade adenocarcinoma. Scale bars: 100 μm. (E) Relative tumor volume measured by micro-CT of lung cancers from FSF-Kras<sup>G12D</sup>; p53<sup>FRT/FRT</sup> mice. A total of three tumors were contoured from two mice. Micro-CT scans were performed at 8 weeks and 10 weeks after infection with Ad-FlpO. (F) Doubling time in days for lung cancers in FSF-Kras<sup>G12D</sup>; p53<sup>FRT/FRT</sup> mice. Data are presented as mean ± s.e.m.

**Fig. 4. Rationale for dual recombinase technology.** (A) Ad-Cre infection generates tumors by expressing Cre recombinase in tumor-initiating cells. However, in this model, Cre recombinase cannot be utilized to selectively recombine additional floxed alleles in stromal cells. (B) Dual recombinase technology combines Ad-Flp infection with a tissue-specific Cre driver that recombines floxed alleles in stromal cells. For example, Tie2-Cre recombines floxed alleles in endothelial cells and macrophages. Tumors can be initiated by Flp-mediated activation of oncogenes and deletion of flared tumor suppressor genes. This approach enables recombination of floxed alleles in stromal cells expressing Cre recombinase only.

**Construction of the p53<sup>FRT-neo</sup> targeting vector and generation of mice**

Genomic DNA of the mouse p53 gene was provided by Tyler Jacks and was used to make the targeting construct. We used an Ndel site in intron 1 and a BamHI site in intron 6 to insert a single FRT site before exon 2 and exon 7, respectively. A loxP-flanked PGK-neo cassette was inserted into intron 6 before the FRT site as a positive selectable marker and a PGK-DTA cassette was inserted into the targeting vector after exon 11 as a negative marker. The targeting vector was linearized with PacI and electroporated into ES cells using standard conditions. Diagnostic PCR was performed to identify ES clones with successful homologous recombination using primers flanking the PGK-neo cassette (sense primer 5’-TGCTCCTGCGAGAAATGAT-3’ and anti-sense primer 5’-CACCATGAGACAGGGTGTCATG-3’) and primers flanking the 5’ FRT site (sense 5’-CAAGAGAACTGTGCCTAAGAG-3’ and anti-sense 5’-CTTTCTAACAGCAAAGCAGC-3’). Two out of 800 ES cells were positive at both sites. Genomic DNA of these two clones was digested by ScaI and successful homologous recombination of the p53<sup>FRT-neo</sup> allele was determined by Southern blot using probes binding to either neo or exon 11. One clone was used to derive male germline p53<sup>FRT-neo</sup> chimeras, which were bred with Meox2-Cre females to delete the floxed neo in the germ line. Deletion of neo was verified by PCR using primers flanking the recombinated loxp site and 3’ FRT site: sense 5’-TGAGCCAC-CGAGGTCTCTGTAATT-3’ and anti-sense 5’-ACTCGTGGAACAGGACAGCGAGC-3’. Both FRT sites, the recombinated loxp site and all exons of p53, including intron-exon splice sites, were sequenced to confirm that no mutations were present in p53<sup>FRT/FRT</sup> mice. These mice will be donated to Jackson Laboratory.
Briefly, 25 or 10^8 PFU/ml was incubated in 600 µl minimum essential media (Sigma-Aldrich, St Louis, MO) for 15 minutes to form calcium phosphate precipitates. A total of 50 µl precipitated virus per mouse was injected intramuscularly to generate sarcomas, or 30 µl precipitated virus followed by 30 µl media was administered via IN inhalation to initiate lung tumors.

Micro-CT scans

The computerized tomography (CT) data were acquired by X-RAD 225Cx (Precision X-ray, North Branford, CT) using 40 kVp X-rays with 2.5 mA current. Tumor volumes were calculated with Amira image analysis software (TGS, San Diego, CA).

ACKNOWLEDGEMENTS

We thank Ute Hochgeschwender and the Duke Neurotransgenic Laboratory for assistance with ES cell targeting and blastocyst injection. The Duke Neurotransgenic Laboratory is supported, in part, with funding from NIH-NINDS Center Core Grant 1P01NS061789. We thank Tyler Jacks for providing p53FRT/- and FSF-KrasG12D mice as well as mouse p53 genomic DNA.

COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

AUTHOR CONTRIBUTIONS

C.-L.L., E.J.M. and D.G.K. conceived and designed the experiments. C.-L.L. and E.J.M. performed the experiments and analyzed the data. Y.L. performed the micro-CT scans and analyzed the data. C.L.L., E.J.M. and D.G.K. wrote the manuscript.

REFERENCES

Attardi, L. D., de Vries, A. and Jacks, T. (2004). Activation of the p53-dependent G1 checkpoint response in mouse embryo fibroblasts depends on the specific DNA damage inducer. Oncogene 23, 973-980.
Brady, C. A., Jiang, D., Mello, S. S., Johnson, T. M., Jarvis, L. A., Kozak, M. M., Kenzelmann Broz, D., Basak, S., Park, E. J., McLaughlin, M. E. et al. (2011). Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression. Cell 145, 571-583.

Brandt, C. S. and Dymecki, S. M. (2004). Talking about a revolution: the impact of site-specific recombinases on genetic analyses in mice. Dev. Cell 6, 7-28.

Brosh, R. and Rotter, V. (2009). When mutants gain new powers: news from the mouse model of soft tissue sarcoma. Nat. Rev. Cancer 9, 701-713.

Donehower, L. A. and Lozano, G. (2009). 20 years studying p53 functions in genetically engineered mice. Nat. Rev. Cancer 9, 831-841.

Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr, Butel, J. S. and Bradley, A. (1992). Mouse deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356, 215-221.

DuPage, M., Dooley, A. L. and Jacks, T. (2009). Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase. Nat. Protoc. 4, 1064-1072.

Graves, E. E., Vilalta, M., Cecic, I. K., Erler, J. T., Tran, P. T., Felsher, D., Sayles, L., Sweet-Cordero, A., Le, Q. T. and Giaccia, A. J. (2010). Hypoxia in models of lung cancer: implications for targeted therapeutics. Clin. Cancer Res. 16, 4843-4852.

Hanahan, D. and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. Cell 144, 646-674.

Harvey, M., Sands, A. T., Weiss, R. S., Hegi, M. E., Wiseman, R. W., Pantazis, P., Giovanello, B. C., Tainsky, M. A., Bradley, A. and Donehower, L. A. (1993). In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. Oncogene 8, 2457-2467.

Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S., Bronson, R. T. and Weinberg, R. A. (1994). Tumor spectrum analysis in p53-mutant mice. Curr. Biol. 4, 1-7.

Jackson, E. L., Olive, K. P., Tuveson, D. A., Bronson, R., Crowley, D., Brown, M. and Jacks, T. (2005). The differential effects of mutant p53 alleles on advanced murine lung cancer. Cancer Res. 65, 10280-10288.

Kirsch, D. G., Dinulescu, D. M., Miller, J. B., Grimm, J., Santiago, P. M., Young, N. P., Nielsen, G. P., Quade, B. J., Chaber, C. J., Schultz, C. P. et al. (2007). A spatially and temporally restricted mouse model of soft tissue sarcoma. Nat. Med. 13, 992-997.

Kirsch, D. G., Grimm, J., Guimaraes, A. R., Wojtkiewicz, G. R., Perez, B. A., Santiago, P. M., Anthony, N. K., Forbes, T., Dopke, K., Weissleder, R. et al. (2010). Imaging primary lung cancers in mice to study radiation biology. Int. J. Radiat. Oncol. Biol. Phys. 76, 973-977.

Lin, S. C., Lee, K. F., Nikitin, A. Y., Hilsenbeck, S. G., Cardiff, R. D., Li, A., Kang, K. W., Frank, S. A., Lee, W. H. and Lee, E. Y. (2004). Somatic mutation of p53 leads to estrogen receptor alpha-positive and -negative mouse mammary tumors with high frequency of metastasis. Cancer Res. 64, 3525-3532.

Maity, A. and Koumenis, C. (2010). Location, location, location-makes all the difference for hypoxia in lung tumors. Clin. Cancer Res. 16, 4685-4687.

Marino, S., Vooijs, M., van Der Gulden, H., Jonkers, J. and Berns, A. (2000). Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. Genes Dev. 14, 994-1004.

Martinez-Cruz, A. B., Santos, M., Lara, M. F., Segrelles, C., Ruiz, S., Moral, M., Lorz, C., Garcia-Escudero, R. and Paramio, J. M. (2008). Spontaneous squamous cell carcinoma induced by the somatic inactivation of retinoblastoma and Trp53 tumor suppressors. Cancer Res. 68, 683-692.

Meyers, E. N., Lewandoski, M. and Martin, G. R. (1998). An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. Nat. Genet. 18, 136-141.

Olive, K. P., Jacobetz, M. A., Davidson, C. J., Gopinathan, A., McIntyre, D., Hones, D., Madhu, B., Goldgraben, M. A., Caldwell, M. E., Allard, D. et al. (2009). Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 324, 1457-1461.

Oliver, T. G., Mercer, K. L., Sayles, L. C., Burke, J. R., Mendus, D., Lovejoy, K. S., Cheng, M. H., Subramanian, A., Mu, D., Powers, S. et al. (2010). Chronic cisplatin treatment promotes enhanced damage repair and tumor progression in a mouse model of lung cancer. Genes Dev. 24, 837-852.

Raymond, C. S. and Soriano, P. (2007). High-efficiency FLP and PhiC31 site-specific recombination in mammalian cells. PLoS ONE 2, e162.

Sharpless, N. E. and Depinho, R. A. (2006). The mighty mouse: genetically engineered mouse models in cancer drug development. Nat. Rev. Drug Discov. 5, 741-754.

Singh, M., Lima, A., Molina, R., Hamilton, P., Clermont, A. C., Devasthali, V., Thompson, J. D., Cheng, J. H., Bou Reslan, H., Ho, C. C. et al. (2010). Assessing therapeutic responses in Kras mutant cancers using genetically engineered mouse models. Nat. Biotechnol. 28, 585-593.

Stiewe, T. (2007). The p53 family in differentiation and tumorigenesis. Nat. Rev. Cancer 7, 165-168.

Tallonquist, M. D. and Soriano, P. (2000). Epiblast-restricted Cre expression in MORE mice: a tool to distinguish embryonic vs. extra-embryonic gene function. Genesis 26, 113-115.

Tyner, S. D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., Lu, X., Soron, G., Cooper, B., Brayton, C. et al. (2002). p53 mutant mice that display early ageing-associated phenotypes. Nature 415, 45-53.

Young, N. P., Crowley, D. and Jacks, T. (2011). Uncoupling cancer mutations reveals critical timing of p53 loss in sarcomagenesis. Cancer Res. 71, 4040-4047.