

Chapter 2

The Use of Cre-loxP Technology and Inducible Systems to Generate Mouse Models of Cancer

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2.1 Introduction

Drs. Mario R. Capecchi, Martin J. Evans, and Oliver Smithies received the 2007 Nobel Prize in Physiology or Medicine for their pioneering work in introducing specific gene modifications in mice by the use of embryonic stem (ES) cells (Deng 2007). This technology, commonly referred to as gene targeting or knockout, is based on homologous recombination between DNA sequences residing in the chromosome and newly introduced DNA to mutate genes of interest in the mouse genome (Capecchi 1989). Gene targeting has proven to be a powerful means for precise manipulation of the mammalian genome, which has generated thousands of mutant mouse strains. Studies of these mutant mice have yielded enormously useful information in virtually all fields of biological and biomedical sciences. Indeed, gene targeting can theoretically be used to generate mutant mice for all genes in the near future. However, many genes are indispensable for embryonic and/or early postnatal development. In such cases, germline mutations of these genes often result in embryonic, neonatal, or preadult lethality, preventing further studies of their functions in later stages of development and tumorigenesis (Weinstein et al. 2000; Deng 2002b; Coumoul and Deng 2003; Friedberg and Meira 2006).

In the past decade, the Cre-loxP technology, combined with inducible systems, has been used to overcome embryonic and early postnatal lethality (Le and Sauer 2000; Nagy 2000). Many tumor suppressor genes and oncogenes have been mutated or activated in a spatial and temporal manner, making it possible for studying their function in a way that would otherwise not be possible. This chapter discusses

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details for designing and generating mice carrying conditional loss or gain of function mutations, and strategies for tissue-specific Cre-loxP-mediated recombination. Advances of several major inducible systems and their applications to cancer research are also discussed.

2.2 Cre-LoxP System

The Cre-loxP site-specific recombination system of Coliphage P1 is particularly simple and well characterized (Argos et al. 1986; Sternberg et al. 1986; Sauer and Henderson 1988). *Cre* (cyclization recombination) gene encodes a 38-kDa site-specific DNA recombinase, called Cre, which recognizes 34-bp sites, loxP (locus of X-over of P1), and catalyzes both intra and intermolecular recombination between two loxP sites (Fig. 2.1). The loxP site consists of an 8-bp nonpalindromic core region flanked by two 13-bp inverted repeats (Fig. 2.1a). Cre-loxP-mediated recombination between two directly repeated loxP sites excises all DNA sequences located within the two sites as a covalently closed circle (Fig. 2.1b). Because Cre-loxP-mediated recombination occurs at high efficiency and it does not require any other host factors, except for its substrate, i.e., DNA, it has been widely used in a variety of experimental model systems. In most cases, loxP sites are placed in the same chromosome in direct repeat position so that the intervening

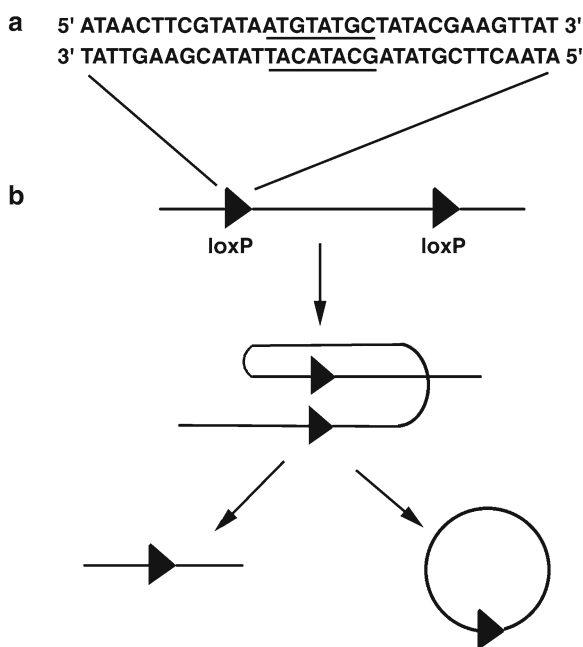


Fig. 2.1 Schematic representation of Cre-loxP-mediated recombination. (a) The loxP site consists of an 8-bp nonpalindromic core region (*underlined*) flanked by two 13-bp inverted repeats. (b). Cre-loxP-mediated recombination between two directly repeated loxP sites generates a linear product containing one loxP and a covalently closed circle containing excised DNA sequence located between two loxP sites

DNA sequence can be deleted. The loxP sites can also be placed in different chromosomes to promote recombination between different chromosomes, and placed in an inverted position in the same chromosome to create a switch to inactivate and activate genes of interest.

2.3 Cre-LoxP-Mediated Gene Inactivation

2.3.1 *Generation of a Conditional Mutant Allele in Mice*

The first step in the Cre-loxP-mediated gene inactivation is to generate a targeting vector for the gene of interest. The vector can be constructed by using multiple established procedures that were described in detail elsewhere (Zhang et al. 2002; Deng and Xu 2004; Iiizumi et al. 2006). Using the *Smad4* gene as an example, a replacement type targeting vector, commonly used for co-transfer of a selectable marker and a nonselectable marker (Deng et al. 1993) is discussed (Fig. 2.2). Such a vector contains a neomycin (*neo*) gene for positive selection and a thymidine kinase (*tk*) gene for negative selection (Mansour et al. 1988) (Fig. 2.2a). The *neo* gene is flanked with two loxP sites and is inserted into intron 8, and the third loxP site is placed in intron 7 of the *Smad4* gene. Thus, exon 8 of *Smad4* gene is flanked by loxP sites (floxed) and can be deleted upon Cre-loxP-mediated recombination (Fig. 2.2b). After introducing such a 3-loxP gene-targeting construct into ES cells, the cells containing predicted homologous recombination are identified by Southern blots and/or PCR (Fig. 2.2c), and injected into blastocysts for germline transmission by standard techniques.

2.3.2 *Deletion of the Neo Gene from a Conditional Mutant Allele*

It has been shown that the presence of the *neo* gene in an intron frequently affects endogenous gene expression and results in the reduction or complete inactivation of the floxed genes (Hirotsume et al. 1998; Chen et al. 1999; Iwata et al. 2000; Rucker et al. 2000); (Xu et al. 2001b). Thus, it is important to be able to remove the *neo* gene from targeted loci whenever it is necessary. The *neo* gene, if it is flanked by loxP, can be removed using several methods either in ES cells or mutant mice. The removal of the *neo* gene in ES cells by transient Cre expression has been used successfully in generating conditional knockouts (Gu et al. 1994). Although it is a quick way to delete the *neo* gene, it requires additional modification of ES cells and it may compromise totipotency and increase the difficulty of obtaining germline transmission. On the other hand, the presence of *neo* in an intron of a gene does not always generate obvious effects and sometime it can even create serial hypomorphic alleles that are useful for studying the function of genes of interest (Hirotsume et al. 1998; Chen et al. 1999; Iwata et al. 2000; Rucker et al. 2000); (Xu et al. 2001b).

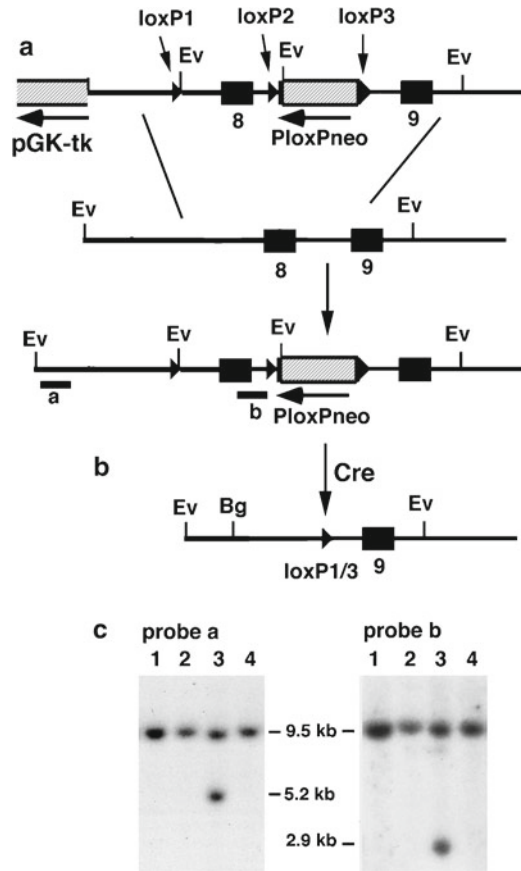


Fig. 2.2 Introduction of loxP sites into the *Smad4* locus. (a) A targeting vector that contains a loxP in the intron 7 and a *ploxPneo* in intron 8. Through a double cross event, the vector introduces all three loxP into the *Smad4* locus. (b) Cre-loxP-mediated complete recombination can delete all DNA sequence between loxP 1 and 3. (c) Targeted events were identified by Southern blot analysis of *Ev* (*EcoRV*)-digested genomic DNAs with a 5' flanking probe (probe a). The wild-type clones only show a fragment of 9.5 kb and the targeted clones showed an additional fragment of 5.5 kb due to the introduction of an *EcoRV* site. The *EcoRV*-digested genomic DNA was also blotted using an internal probe (probe b) to verify the presence of the *ploxPneo* gene. In this case, the targeted ES clones showed a 3-kb fragment in addition to the wild-type fragment of 9.5 kb

In such cases, it is beneficial to keep the *neo* gene in ES cells, and remove it later in mutant mice after its physiological impact is assessed.

Currently, four approaches have been developed in case the *neo* gene needs to be removed from the conditional knockout allele in mice. Xu et al. described two approaches to delete *ploxPneo* from mice. The first approach is to cross the mice containing the 3-loxP mutant allele with the EIIa-Cre transgenic mice (Lakso et al. 1996), and the second one is to microinject the Cre expression construct into the pronucleus of fertilized eggs (Xu et al. 2001b). The third method removes the floxed

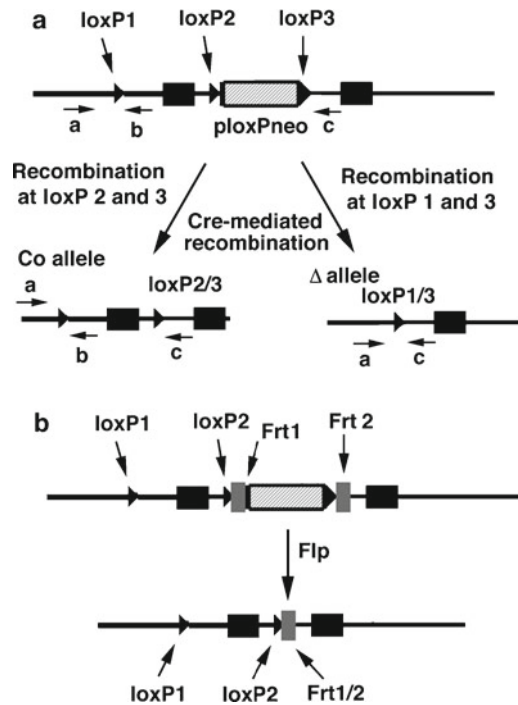


Fig. 2.3 Strategies for the removal of the *neo* gene from the conditional knockout allele. (a) Removal of *ploxPneo* gene from a 3-loxP knockout allele. Cre-mediated recombination between loxP 2 and 3 deletes the *ploxPneo* while the recombination between loxP 1 and 3 deletes all DNA sequences between loxP 1 and 3. Different recombination events can be detected by PCR analysis using primers a, b, and c. If this is performed in mice, it could generate conditional mutant mice and null mice carrying the delta allele at the same time. (b) Removal of a Frt-flxed *neo* gene through the expression of Flp recombinase. Because the loxP 2 is placed outside the Frt1, the Flp/Frt-mediated recombination only deletes the Frt-flxed *neo*, generating a loxP flxed allele, which can be used for conditional knockout

neo gene by infecting 16-cell stage morulae with the recombinant Cre adenovirus (Kaartinen and Nagy 2001). All these approaches are based on the fact that Cre-mediated recombination is normally incomplete, and the allele without the *neo* cassette can be identified by PCR analysis using different sets of primers in the offspring (Fig. 2.3a). To avoid screening for the incomplete recombination product generated by Cre/loxP, Meyers et al. (1998) reported a method using a combined Cre/loxP and Flp/Frt system to excise the *neo* gene in the germline of the adult mouse (Meyers et al. 1998) (Fig. 2.3b). The Flp/Frt site-specific recombination system was initially found in yeast and it works efficiently in *Drosophila* and in mammalian cells (Golic and Lindquist 1989; O’Gorman et al. 1991). In this approach, the *neo* gene is flanked by a combined loxP/Frt site on one side and an Frt on the other side. An advantage is that deletion of *neo* by Flp recombinase does not affect the loxP-flanked fragment.

Table 2.1 Comparison of advantages and disadvantages of several approaches for deletion of the *neo* gene

Methods to remove floxed <i>neo</i> gene	Advantages	Disadvantages
Transit expression of Cre in ES cells	Quick, technically easy	Additional modifications may compromise pluripotency of ES cells
Cross with EIIa-Cre transgenic mice	Avoids ES manipulation and removes the <i>neo</i> gene in mice with a high reliability	Requires two rounds of animal mating, i.e., first with the EIIa-Cre mice and the second with wild-type mice to separate alleles carrying different Cre/loxP-mediated recombination. The screening for incomplete Cre/loxP-mediated recombination can be time consuming
Oocyte injection	Direct injection of a Cre expression plasmid into oocyte. The amount of input Cre can be adjusted to increase efficiency of removing the <i>neo</i>	Requires two rounds of mating with wild type mice to obtain oocytes and separate alleles carrying different Cre/loxP-mediated recombination. In addition, it requires experience in microinjection, embryo manipulation, and implantation
Infecting morulae with recombinant Cre adenovirus	High efficiency of Cre adenovirus to infect morulae, which may delete floxed neo with high efficiency	Two rounds of mating with wild-type mice to obtain morulae and separate alleles carrying different Cre/loxP-mediated recombination. In addition, it requires experience in adenovirus production, embryo manipulation, and implantation
Combination of Cre/loxP–Flp/Frt	Deletion of the <i>neo</i> is independent of Cre/loxP system. It is a straightforward screen for the complete recombination product	Screening large number of offspring is expected due to a low efficiency of the Flp/Frt system in mouse

However, it was found that the efficiency of the Flp/Frt system in mouse is much lower than the Cre/loxP system (Meyers et al. 1998), which requires screening of a relatively large number of animals to obtain the correct allele. A summary of advantages and disadvantages of these approaches is listed in Table 2.1.

2.3.3 Tissue-Specific Conditional Knockout Mice

Once mice carrying conditional knockout alleles of genes are created, the mutant mice can be crossed with mice carrying Cre that is controlled by desired promoters to achieve targeted gene knockout in a spatial–temporal fashion. Numerous transgenic mice carrying tissue-specific and/or inducible Cre expression have been generated

Table 2.2 Transgenic mouse lines carrying inducible Cre

Promoters	Organs/tissues	References
NMDA-type glutamate receptor subunit gene	Cerebellar granule cell-specific and inducible expression inducible by antiprogestins	Tsujita et al. (1999)
SM22	Temporally controlled somatic mutagenesis in smooth muscle – tamoxifen inducible	Kuhbandner et al. (2000)
E _μ /P _{SV40}	B cell – tamoxifen inducible	Schwenk et al. (1998)
Transthyretin	Fetal and adult liver – tamoxifen inducible	Tannour-Louet et al. (2002)
Alpha1-antitrypsin	Hepatocyte – tamoxifen inducible	Imai et al. (2000)
Keratin 14	Epidermis – RU486 inducible	Berton et al. (2000)
WAP	Mammary gland – Tet-inducible	Utomo et al. (1999)
CMV	Ubiquitous – tamoxifen inducible	Hayashi and McMahon (2002)
Wnt	Embryonic neural tube – tamoxifen inducible	Danielian et al. (1998)
An interferon-responsive promoter	Liver and nearly complete in lymphocytes – interferon inducible	Kuhn et al. (1995)
Hsp70	Ubiquitous – heat shock inducible	Dietrich et al. (2000)
CamKIIalpha	Olfactory lobe, cortex, striatum, hippocampus and Purkinje cells – doxycycline inducible	Lindeberg et al. (2002)

(Nagy and Mar 2001, <http://www.mshri.on.ca/nagy/Cre-pub.html>, and Table 2.2). Many of these mice have been used to knock out tumor suppressor genes, including adenomatous polyposis coli (APC) (Clarke 2005), breast cancer-associated gene 1 (BRCA1) (Xu et al. 1999a), breast cancer-associated gene 2 (BRCA2) (Jonkers et al. 2001), Neurofibromatosis type one (NF1) (Gitler et al. 2004), p53 (Jonkers et al. 2001), phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (Li et al. 2002), retinoblastoma (RB) (Ruiz et al. 2006), SMAD4 (Li et al. 2003), and transforming growth factor beta (TGF-beta)-type II receptor (Ijichi et al. 2006). These studies provide valuable information regarding functions of these genes in tumor initiation and progression. The progresses achieved using SMAD4 and BRCA1 conditional knockout mice are briefly reviewed below.

2.3.3.1 Cre-loxP-Mediated Knockout of SMAD4 in Multiple Tissues

SMAD4 serves as a common mediator of the TGF-beta superfamily that comprises over 40 growth and differentiation factors, including members in the subfamily of TGF-beta, activin, inhibin, and bone morphogenetic protein, which play numerous important functions in diverse developmental processes by regulating proliferation, differentiation, and apoptosis (Heldin et al. 1997; Massague 1998; Derynck et al. 2001;

Pollard 2001; Wakefield et al. 2001). In humans, *SMAD4* is a well-known tumor suppressor gene, and its mutations are frequently detected in pancreatic cancer, stomach cancer, liver cancer, and colon cancer (Hahn et al. 1996a, b; Nagatake et al. 1996; Schutte et al. 1996; Maesawa et al. 1997; Friedl et al. 1999). Germline mutations of *SMAD4* also contribute to familial juvenile polyposis, an autosomal dominant disorder characterized by predisposition to hamartomatous polyps and gastrointestinal cancer (Howe et al. 1998).

In mice, loss of *SMAD4* results in lethality at embryonic (E) days 6–7 due to impaired extraembryonic membrane formation and decreased epiblast proliferation (Sirard et al. 1998; Yang et al. 1998). Because *SMAD4* serves as a common mediator for the TGF- β superfamily, *SMAD4* conditional mutant mice generated by using the Cre-loxP approach (Yang et al. 2002; Bardeesy et al. 2006) should serve as a valuable tool for studying TGF- β /*SMAD4* signaling during postnatal development and tumorigenesis.

Currently, conditional knockout of *SMAD4* has been performed in many organs/tissues, and tumorigenesis was observed in the mammary gland (Li et al. 2003), skin (Qiao et al. 2006), forestomach (Teng et al. 2006), liver (Yang et al. 2005; Xu et al. 2006), and pancreas (Bardeesy et al. 2006; Izeradjene et al. 2007; Kojima et al. 2007). Despite the finding that *SMAD4* is mutated in about 60% of pancreatic ductal adenocarcinoma (PDAC) (Hahn et al. 1996a, b), *SMAD4* deletion alone in the pancreas does not induce tumor formation (Bardeesy et al. 2006; Izeradjene et al. 2007; Kojima et al. 2007). Loss of *SMAD4* also does not interfere with pancreas development and physiologic functions. However, when combined with an activated K-ras (G12D) allele, *SMAD4* deficiency enabled rapid development of a distinct class of tumors resembling intraductal papillary mucinous neoplasia (MCN), a precursor to PDAC in humans. Progression of MCNs in both mice and humans is accompanied by loss of heterozygosity of p53 or p16 (Izeradjene et al. 2007). These data suggest that the invasive PDACs in humans and mice share similar overall mutational spectra, and the loss of *Smad4* is a later event in pancreatic tumorigenesis.

Similarly, knockout of *SMAD4* in the liver alone by albumin promoter-driven Cre (*Smad4^{Co/Co};Alb-Cre*) does not cause developmental defects and tumor formation (Wang et al. 2005). Instead, it leads to the surprising finding that liver-specific knockout of *SMAD4* causes iron overload in multiple organs, most pronounced in liver, kidney, and pancreas. The phenotypes of mutant mice resemble those found in hereditary hemochromatosis, a common genetic disorder among Caucasians (Pietrangelo 2006; Beutler 2007). Further studies indicate that the absence of *SMAD4* results in marked decreased expression of hepcidin in the liver. Hepcidin is produced predominantly by the liver, although a number of other organs, such as lung and heart, also express it at much lower levels (Leong and Lonnerdal 2004). Prohepcidin is then cleaved to form the mature form, a 25 aa peptide, which is secreted into the circulation, and transported to duodenum and intestine, where it negatively regulates iron absorption in crypt cells and/or villous enterocytes. The absence of *SMAD4* reduced production of hepatic hepcidin, leading to an increased expression of genes involved in intestinal iron absorption, including *Dcytb*, *DMT1*, and *ferroportin* (Wang et al. 2005). These data uncover a novel role of TGF- β /

SMAD4 in regulating hepcidin expression and thus intestinal iron transport and iron homeostasis.

The lack of cancer formation in the liver suggests that SMAD4 deficiency alone is not enough to cause malignant transformation. However, it was found that the liver of *Smad4^{Co/Co};Alb-Cre* mice exhibited increased expression of the PTEN tumor suppressor, which is mutated in a wide range of human cancers (Sansal and Sellers 2004). These data suggest that the increased expression of PTEN could inhibit the effect of SMAD4 deficiency on tumor induction. To test this, Xu et al. introduced a conditional mutation of PTEN (Groszer et al. 2001) into *Smad4^{Co/Co};Alb-Cre* mice to knockout PTEN and SMAD4 simultaneously (Xu et al. 2006). In the PTEN and SMAD4 double mutant (*Smad4^{Co/Co};Pten^{Co/Co};Alb-Cre*) mice, hyperplastic foci emerged exclusively from bile ducts at 2 months of age (Fig. 2.4a–d). The hyperplastic foci progressed through multiple stages, including hyperplasia, dysplasia, carcinoma *in situ*, and eventually well-established cholangiocarcinoma (CC) in all animals at 4–7 months of age (Fig. 2.4e, f).

Because the endogenous albumin promoter is only expressed in hepatocytes but not in bile ducts (Yakar et al. 1999), it was surprising that the tumors derived exclusively from bile ducts. To investigate this, the *Alb-Cre* mice were mated with transgenic mice bearing a Rosa-26 reporter mouse [β -galactosidase expression upon Cre–LoxP-mediated recombination (Soriano 1999)]. β -Galactosidase positive cells were initially detected in both bile ducts and hepatocytes in the liver in a stochastic fashion in E15.5 embryos (Fig. 2.4g, h), and spread to a majority of hepatocytes and bile duct epithelial cells at P30 (Fig. 2.4i). These data suggest that the bile duct is more sensitive to tumorigenesis induced by deficiency of both PTEN and SMAD4 than hepatocytes in mice.

Further analysis indicated that CC formation follows a multistep progression of histopathological changes that are associated with significant alterations, including high levels of phosphorylated AKT, FOXO1, GSK-3 β , mTOR, and ERK, and increased levels of cyclin D1, β -catenin, and c-Myc. CC accounts for about 15% of total liver cancer cases in the world with significant variations from country to country, and is associated with poor prognosis; most patients die soon after diagnosis (Taylor-Robinson et al. 2001; Okuda et al. 2002; Olnes and Erlich 2004; Sirica 2005). Studies on human CC also revealed similar alterations, including p53, p16, p27, p57, SMAD4, and increased levels of β -catenin, cyclin D1, ERK, Ras, AKT, and c-Myc (Sugimachi et al. 2001a, b; Ito et al. 2002; Kang et al. 2002; Wu et al. 2004; Sirica 2005). These findings elucidate a common mechanism between human and mouse CC formation and thus provide an animal model for the discovery of drugs for the treatment of CC.

2.3.3.2 Cre-loxP-Mediated Knockout of BRCA1 in Breast Cancer Research

Breast cancer is the leading cause of cancer incidence affecting approximately one in nine women in Western countries (Alberg and Helzlsouer 1997; Paterson 1998; Alberg et al. 1999; Kerr and Ashworth 2001; Nathanson and Weber 2001). Familial breast cancer is responsible for about 5–10% of total breast cancer cases caused by

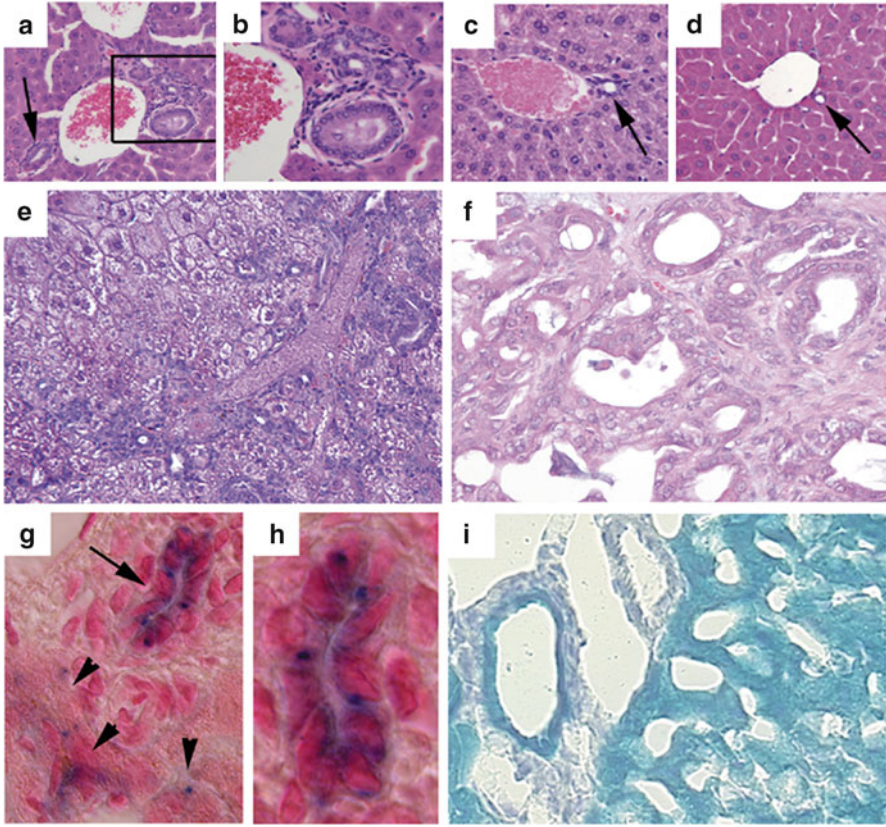


Fig. 2.4 Targeted disruption of SMAD4 and PTEN results in cholangiocarcinoma in the liver. (a–d) Histologic analysis of livers isolated from 2 months old *Smad4^{Co/Co};Pten^{Co/Co};Alb-Cre* (a, b), *Smad4^{Co/Co};Alb-Cre* (c) and wild-type (d) mice. Arrows point to bile ducts. (b) is the boxed area in (a). (e) An H&E liver section showing significantly increased bile duct branching in the liver of a 3-month-old *Smad4^{Co/Co};Pten^{Co/Co};Alb-Cre* mouse. (f) A well-developed CC found in *Smad4^{Co/Co};Pten^{Co/Co};Alb-Cre* liver. (a–c) Albumin-Cre activity assayed by using Rosa-26 reporter mice at P15 (g, h), and P30 (i)

mutations of BRCA1 and BRCA2, and other unidentified tumor suppressor genes (Alberg and Helzlsouer 1997; Paterson 1998; Kerr and Ashworth 2001; Nathanson and Weber 2001). Germline mutations of BRCA1 have been found to contribute to about 45% of the familial breast cancer cases and about 90% of the familial breast and ovarian cancer (Alberg and Helzlsouer 1997; Paterson 1998). BRCA1 was mapped in 1990 and was subsequently cloned in 1994 (Hall et al. 1990; Miki et al. 1994). Germline mutations in *BRCA1* have been detected in approximately half of familial breast cancer cases and most cases of combined familial breast/ovarian cancers (Alberg and Helzlsouer 1997; Paterson 1998). BRCA1 mutation carriers have a 50–80% risk of developing breast cancer by the age of 70 (Easton et al. 1995; Struwing et al. 1997; Ford et al. 1998).

In mice, loss of function mutation of BRCA1 generated by gene targeting is not compatible with embryonic development. Most mutant mice carrying various mutations died during gestation displaying growth retardation and apoptosis (Gowen et al. 1996; Hakem et al. 1996; Liu et al. 1996; Ludwig et al. 1997; Shen et al. 1998; Xu et al. 2001c). Studies on these mice demonstrated that BRCA1-deficiency resulted in defective DNA damage repair, abnormal centrosome duplication, impaired homologous recombination, defective cell cycle checkpoint, growth retardation, increased apoptosis, and genetic instability (Deng 2002a, 2006; Deng and Wang 2003). To overcome the early lethality and create animal models for BRCA1-associated hereditary breast cancer, several mutant mice carrying conditional knock-out BRCA1 have been generated (Xu et al. 1999a; Mak et al. 2000; Liu et al. 2007). A most commonly used model of BRCA1 conditional mutant mice carries floxed exon 11 of the BRCA1 gene (Xu et al. 1999a), and the mutant mice are crossed with transgenic mice carrying either MMTV-Cre or WAP-Cre (Wagner et al. 1997) to specifically delete the BRCA1 in mammary epithelial cells. Analysis of these BRCA1 conditional mutant mice (*Brca1^{Co/Co};MMTV-Cre* and *Brca1^{Co/Co};MMTV-Cre*) revealed abnormal ductal and alveolar development of mutant mammary glands. There was also significantly increased apoptosis of epithelial cells, suggesting that cell death triggered by the loss of BRCA1 may be a primary cause for the abnormalities in branch morphogenesis. Despite these abnormalities, about 25% of BRCA1 conditional mutant mice developed mammary tumors when they were on average 18 months of age (Xu et al. 1999a). Further studies revealed that BRCA1 plays an important role in DNA damage repair and multiple cell cycle checkpoints (Xu et al. 1999b, 2001a, 2003; Weaver et al. 2002; Wang et al. 2004). The absence of BRCA1 results in genetic instability, which activates the tumor suppressor p53, leading to apoptosis. Consistent with this, disruption of p53 in BRCA1 mutant mice attenuates apoptosis and accelerates tumor formation (Brodie et al. 2001; Xu et al. 2001c). Recent studies revealed that increased insulin/IGF signaling (Shukla et al. 2006), activation of estrogen/ER- α signaling (Li et al. 2007; Jones et al. 2008), and increased expression of angiogenic factors, including angiopoietin-1 (Furuta et al. 2006) also facilitate breast cancer formation in BRCA1-deficient mice.

2.4 Cre-loxP-Mediated Gene Activation

Another important application of the Cre-loxP system in cancer research is to achieve gene activation. Many human cancers are caused by activation of numerous oncogenes; for example, activating mutations of the *RAS* oncogene are found in approximately one-third of all human cancers (Bos 1989; Khosravi-Far and Der 1994). Much of our knowledge on oncogenic signaling and its influence on tumor formation came from mouse models carrying activated oncogenes. Using K-ras as an example, the general strategy used for the generation of mutant mice by the Cre-LoxP technology is discussed below.

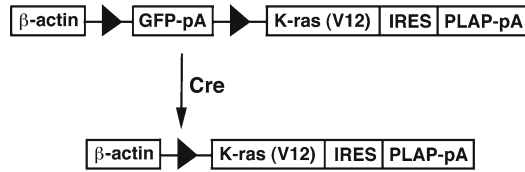


Fig. 2.5 Schematic representation of the conditional K-ras^{G12V} construct. The conditional K-ras^{G12V} transgene is driven by a broadly active beta-actin promoter, followed by a floxed-GFP, and then a K-ras^{G12V} cDNA combined with a PLAP expression construct through IRES (internal ribosomal entry site). Without Cre recombinase, GFP mRNA is expressed and the K-ras^{G12V} oncogene remains silent. After Cre-mediated deletion of the floxed-GFP, the K-ras^{G12V} oncogene is placed directly under control of the beta-actin promoter. The K-ras^{G12V} oncogene is transcribed together with the PLAP cDNA. The expression of alkaline phosphatase can serve as a marker for Cre-loxP-mediated recombination (modified from Meuwissen et al. 2001)

2.4.1 Activation of Oncogenes Using the Cre-loxP Technology

The Ras gene family contains three genes, K-ras, N-ras, and H-ras. Activation of *KRAS*, which occurs more frequently than that of the other two members, is found in many different types of human tumors, including adenocarcinomas of the pancreas (90%), colon (50%), and lung (30%) (Rodenhuis et al. 1988; Mills et al. 1995; Huncharek et al. 1999). Meuwissen et al. (2001) made a mouse model carrying an activated K-Ras (K-ras^{G12V}) mutation that specifically targets lung epithelial cells (Meuwissen et al. 2001). As shown in Fig. 2.5, the conditional K-ras^{G12V} transgene contained a broadly active beta-actin promoter, followed by a GFP (green fluorescence protein) expression cassette flanked by two loxP sites (floxed-GFP), and then a K-ras^{G12V} cDNA combined with a PLAP (human placenta-like alkaline phosphatase) expression construct. The floxed-GFP not only works as an indicator for the presence of the transgene but more importantly, it also serves to prevent expression of K-ras^{G12V}. Thus, the activated K-ras can only be expressed upon the removal of the block through Cre-LoxP-mediated recombination (Fig. 2.5). In this study, the researchers directly injected adenoviruses carrying Cre recombinase (Ad-Cre) intra-tracheally to K-ras^{G12V} transgenic mice to activate the K-ras in lung epithelial cells. This gave rise to rapid onset of pulmonary adenocarcinomas with 100% incidence 9–13 weeks postinjection. The tumor lesions also shared many features with human non-small cell lung cancer. These data demonstrate that sporadic expression of the activated K-Ras oncogene is sufficient to elicit lung tumorigenesis, which mimics human lung cancer.

Ad-Cre was also directly injected into the pancreatic ducts and acini through the common bile duct of K-ras transgenic mice to induce pancreatic cancer (Ueda et al. 2006). Alternatively, the K-ras oncogene can also be activated by breeding K-ras^{G12V} transgenic mice with mice carrying temporal-spatial-regulated Cre expression in organs/tissues of interest, such as the intestine (Luo et al. 2007). Similar approaches have also been used to express some other oncogenes in order to study their functions for tumor formation (Jager et al. 2004).

2.4.2 Activation of Tumor Suppressor Genes Using the Cre-LoxP Technology

Cancer development is often associated with the inactivation of tumor suppressor genes. For example, loss of function mutation of the tumor suppressor p53 is found in approximately 50% of all human cancers (Morgan and Kastan 1997). However, it is unclear whether sustained inactivation of p53 is required for tumor maintenance. To investigate this, a reactivatable p53 knockout allele (p53-LSL) was generated using the Cre-loxP strategy (Ventura et al. 2007). In this case, transcription of p53 is shut off by a floxed blocker that is inserted in intron 1 of the gene. The p53-LSL mice were crossed with mice carrying a Cre recombinase-estrogen-receptor-T2 (Cre-ERT2) allele targeted to the ubiquitously expressed ROSA26 locus. The temporally controlled p53 reactivation *in vivo* can be achieved by tamoxifen administration, which allows the Cre recombinase to translocate from the cytoplasm to the nucleus (Indra et al. 1999), thus permitting the recombination of genomic loxP sites. The data showed that deletion of the blocker restored endogenous p53 expression and resulted in regression of autochthonous lymphomas and sarcomas in mice without affecting normal tissues (Ventura et al. 2007). The p53 restoration primarily induced apoptosis in lymphomas, while in sarcomas it primarily suppressed cell growth with features of cellular senescence. Cre-loxP-mediated bax gene activation was also used to reduce growth rate and increase sensitivity to chemotherapeutic agents in human gastric cancer cells and cervical carcinoma (Komatsu et al. 2000; Huh et al. 2001). This study serves as an example that a therapeutic effect can be achieved by the activation of tumor suppressor genes.

2.5 Conclusion and Future Directions

The Cre-loxP technology, combined with inducible systems, has been widely used to generate animal models for spatial and temporal regulated gene activation and inactivation. Studies of these mutant mice not only advance our knowledge of functions of numerous tumor suppressor genes and oncogenes, but also provide enormously useful information in virtually all areas of cancer biology oncology. It is anticipated that more animal models carrying spatial-temporal inducible systems will be generated in the near future. Using these animals, studies should be directed toward the detection of specific tumor signature profiles, and oncogenic signaling pathways that may be associated with certain tumor suppressors and oncogenes during tumorigenesis and tumor progression. Studies should also be designed to reveal extensive interactions between different genes and their relationship with genetic background modifiers and nongenetic factors (i.e., hormones). Animals can also serve as models for early tumor diagnosis, chemoprevention, and gene therapy studies, including the targeted delivery of drugs and tissue-specific activation of tumor suppressor genes to inhibit cancer growth and metastasis. Furthermore, the Cre-loxP

inducible system combined with RNA interference (RNAi) technology has been used in mice to knockdown endogenous genes with high efficiency (Chang et al. 2004; Ventura et al. 2004; Coumoul and Deng 2006; Coumoul et al. 2005; Shukla et al. 2007a). Of note, a recent study performed in a mouse model for human FGFR2-related craniosynostosis indicates that mutant alleles bearing point mutations can be specifically targeted using RNAi technology with high efficiency without affecting wild-type mRNA levels (Shukla et al. 2007b). Because many human cancers are caused by point mutations of oncogenes, this data points to the future direction of using the Cre-loxP mediated RNAi inducible system for the therapeutic treatment of cancers that are caused by dominant mutations while allowing normal expression of wild-type alleles.

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References

- Alberg AJ, Helzlsouer KJ (1997) Epidemiology, prevention, and early detection of breast cancer. *Curr Opin Oncol* 9:505–511
- Alberg AJ, Lam AP, Helzlsouer KJ (1999) Epidemiology, prevention, and early detection of breast cancer. *Curr Opin Oncol* 11:435–441
- Argos P, Landy A, Abremski K, Egan JB, Haggard-Ljungquist E, Hoess RH, Kahn ML, Kalionis B, Narayana SV, Pierson LS 3rd et al (1986) The integrase family of site-specific recombinases: regional similarities and global diversity. *EMBO J* 5:433–440
- Bardeesy N, Cheng KH, Berger JH, Chu GC, Pahler J, Olson P, Hezel AF, Horner J, Lauwers GY, Hanahan D, DePinho RA (2006) Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. *Genes Dev* 20:3130–3146
- Berton TR, Wang XJ, Zhou Z, Kellendonk C, Schutz G, Tsai S, Roop DR (2000) Characterization of an inducible, epidermal-specific knockout system: differential expression of lacZ in different Cre reporter mouse strains. *Genesis* 26:160–161
- Beutler E (2007) Iron storage disease: facts, fiction and progress. *Blood Cells Mol Dis* 39:140–147
- Bos JL (1989) ras oncogenes in human cancer: a review. *Cancer Res* 49:4682–4689
- Brodie SG, Xu X, Qiao W, Li WM, Cao L, Deng CX (2001) Multiple genetic changes are associated with mammary tumorigenesis in Brca1 conditional knockout mice. *Oncogene* 20:7514–7523
- Capecchi MR (1989) Altering the genome by homologous recombination [Review]. *Science* 244:1288–1292
- Chang HS, Lin CH, Chen YC, Yu WC (2004) Using siRNA technique to generate transgenic animals with spatiotemporal and conditional gene knockdown. *Am J Pathol* 165:1535–1541
- Chen L, Adar R, Yang X, Monsonego EO, Li C, Hauschka PV, Yayon A, Deng CX (1999) Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. *J Clin Invest* 104:1517–1525
- Clarke AR (2005) Studying the consequences of immediate loss of gene function in the intestine: APC. *Biochem Soc Trans* 33:665–666
- Coumoul X, Deng CX (2003) Roles of FGF receptors in mammalian development and congenital diseases. *Birth Defects Res C Embryo Today* 69:286–304

- Coumoul X, Deng CX (2006) RNAi in mice: a promising approach to decipher gene functions in vivo. *Biochimie* 88(6):637–643
- Coumoul X, Shukla V, Li C, Wang RH, Deng CX (2005) Conditional knockdown of *Fgfr2* in mice using Cre-LoxP induced RNA interference. *Nucleic Acids Res* 33:e102
- Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP (1998) Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr Biol* 8:1323–1326
- Deng CX (2002a) Roles of BRCA1 in centrosome duplication. *Oncogene* 21:6222–6227
- Deng CX (2002b) Tumor formation in *Brca1* conditional mutant mice. *Environ Mol Mutagen* 39:171–177
- Deng CX (2006) BRCA1: cell cycle checkpoint, genetic instability, DNA damage response, and cancer evolution. *Nucleic Acids Res* 34:1416–1426
- Deng C (2007) In celebration of Dr Mario R. Capecchi's Nobel Prize. *Int J Biol Sci* 3:417–419
- Deng CX, Wang RH (2003) Roles of BRCA1 in DNA damage repair: a link between development and cancer. *Hum Mol Genet* 12:R113–R123
- Deng CX, Xu X (2004) Generation and analysis of *Brca1* conditional knockout mice. *Methods Mol Biol* 280:185–200
- Deng C, Thomas KR, Capecchi MR (1993) Location of crossovers during gene targeting with insertion and replacement vectors. *Mol Cell Biol* 13:2134–2140
- Derynck R, Akhurst RJ, Balmain A (2001) TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet* 29:117–129
- Dietrich P, Dragatsis I, Xuan S, Zeitlin S, Efstratiadis A (2000) Conditional mutagenesis in mice with heat shock promoter-driven cre transgenes. *Mamm Genome* 11:196–205
- Easton DF, Ford D, Bishop DT (1995) Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. *Am J Hum Genet* 56:265–271
- Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struwing J, Arason A, Schmeck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 62:676–689
- Friedberg EC, Meira LB (2006) Database of mouse strains carrying targeted mutations in genes affecting biological responses to DNA damage Version 7. *DNA Repair (Amst)* 5:189–209
- Friedl W, Kruse R, Uhlhaas S, Stolte M, Schartmann B, Keller KM, Jungck M, Stern M, Loff S, Back W, Propping P, Jenne DE (1999) Frequent 4-bp deletion in exon 9 of the SMAD4/MADH4 gene in familial juvenile polyposis patients. *Genes Chromosomes Cancer* 25:403–406
- Furuta S, Wang JM, Wei S, Jeng YM, Jiang X, Gu B, Chen PL, Lee EY, Lee WH (2006) Removal of BRCA1/CtIP/ZBRK1 repressor complex on ANG1 promoter leads to accelerated mammary tumor growth contributed by prominent vasculature. *Cancer Cell* 10:13–24
- Gitler AD, Kong Y, Choi JK, Zhu Y, Pear WS, Epstein JA (2004) Tie2-Cre-induced inactivation of a conditional mutant *Nf1* allele in mouse results in a myeloproliferative disorder that models juvenile myelomonocytic leukemia. *Pediatr Res* 55:581–584
- Golic KG, Lindquist S (1989) The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell* 59:499–509
- Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH (1996) *Brca1* deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Genet* 12:191–194
- Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, Zack JA, Kornblum HI, Liu X, Wu H (2001) Negative regulation of neural stem/progenitor cell proliferation by the *Pten* tumor suppressor gene in vivo. *Science* 294:2186–2189
- Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K (1994) Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting [see comments]. *Science* 265:103–106

- Hahn SA, Hoque AT, Moskaluk CA, da Costa LT, Schutte M, Rozenblum E, Seymour AB, Weinstein CL, Yeo CJ, Hruban RH, Kern SE (1996a) Homozygous deletion map at 18q21.1 in pancreatic cancer. *Cancer Res* 56:490–494
- Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE (1996b) DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1 [see comments]. *Science* 271:350–353
- Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmaier A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW (1996) The tumor suppressor gene *Brcal* is required for embryonic cellular proliferation in the mouse. *Cell* 85:1009–1023
- Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC (1990) Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 250:1684–1689
- Hayashi S, McMahon AP (2002) Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. *Dev Biol* 244:305–318
- Heldin CH, Miyazono K, ten Dijke P (1997) TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 390:465–471
- Hirosune S, Fleck MW, Gambello MJ, Bix GJ, Chen A, Clark GD, Ledbetter DH, McBain CJ, Wynshaw-Boris A (1998) Graded reduction of *Pafah1b1* (*Lis1*) activity results in neuronal migration defects and early embryonic lethality. *Nat Genet* 19:333–339
- Howe JR, Roth S, Ringold JC, Summers RW, Jarvinen HJ, Sistonen P, Tomlinson IP, Houlston RS, Bevan S, Mitros FA, Stone EM, Aaltonen LA (1998) Mutations in the *SMAD4/DPC4* gene in juvenile polyposis [see comments]. *Science* 280:1086–1088
- Huh WK, Gomez-Navarro J, Arafat WO, Xiang J, Mahasreshti PJ, Alvarez RD, Barnes MN, Curiel DT (2001) Bax-induced apoptosis as a novel gene therapy approach for carcinoma of the cervix. *Gynecol Oncol* 83:370–377
- Huncharek M, Muscat J, Geschwind JF (1999) K-ras oncogene mutation as a prognostic marker in non-small cell lung cancer: a combined analysis of 881 cases. *Carcinogenesis* 20:1507–1510
- Iizumi S, Nomura Y, So S, Uegaki K, Aoki K, Shibahara K, Adachi N, Koyama H (2006) Simple one-week method to construct gene-targeting vectors: application to production of human knockout cell lines. *Biotechniques* 41:311–316
- Ijichi H, Chytil A, Gorska AE, Aakre ME, Fujitani Y, Fujitani S, Wright CV, Moses HL (2006) Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active *Kras* expression. *Genes Dev* 20:3147–3160
- Imai T, Chambon P, Metzger D (2000) Inducible site-specific somatic mutagenesis in mouse hepatocytes. *Genesis* 26:147–148
- Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P, Metzger D (1999) Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. *Nucleic Acids Res* 27:4324–4327
- Ito Y, Takeda T, Sasaki Y, Sakon M, Yamada T, Ishiguro S, Imaoka S, Tsujimoto M, Monden M, Matsuura N (2002) Expression of p57/Kip2 protein in extrahepatic bile duct carcinoma and intrahepatic cholangiocellular carcinoma. *Liver* 22:145–149
- Iwata T, Chen L, Li C, Ovchinnikov DA, Behringer RR, Francomano CA, Deng CX (2000) A neonatal lethal mutation in *FGFR3* uncouples proliferation and differentiation of growth plate chondrocytes in embryos. *Hum Mol Genet* 9:1603–1613
- Izerradjene K, Combs C, Best M, Gopinathan A, Wagner A, Grady WM, Deng CX, Hruban RH, Adsay NV, Tuveson DA, Hingorani SR (2007) *Kras*(G12D) and *Smad4/Dpc4* haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. *Cancer Cell* 11:229–243
- Jager R, Maurer J, Jacob A, Schorle H (2004) Cell type-specific conditional regulation of the c-myc proto-oncogene by combining Cre/loxP recombination and tamoxifen-mediated activation. *Genesis* 38:145–150
- Jones LP, Tilli MT, Assefnia S, Torre K, Halama ED, Parrish A, Rosen EM, Furth PA (2008) Activation of estrogen signaling pathways collaborates with loss of *Brcal* to promote development

- of ERalpha-negative and ERalpha-positive mammary preneoplasia and cancer. *Oncogene* 27:794–802
- Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* 29:418–425
- Kaartinen V, Nagy A (2001) Removal of the floxed neo gene from a conditional knockout allele by the adenoviral Cre recombinase in vivo. *Genesis* 31:126–129
- Kang YK, Kim WH, Jang JJ (2002) Expression of G1-S modulators (p53, p16, p27, cyclin D1, Rb) and Smad4/Dpc4 in intrahepatic cholangiocarcinoma. *Hum Pathol* 33:877–883
- Kerr P, Ashworth A (2001) New complexities for BRCA1 and BRCA2. *Curr Biol* 11:R668–R676
- Khosravi-Far R, Der CJ (1994) The Ras signal transduction pathway. *Cancer Metastasis Rev* 13:67–89
- Kojima K, Vickers SM, Adsay NV, Jhala NC, Kim HG, Schoeb TR, Grizzle WE, Klug CA (2007) Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. *Cancer Res* 67:8121–8130
- Komatsu K, Suzuki S, Shimosegawa T, Miyazaki JI, Toyota T (2000) Cre-loxP-mediated bax gene activation reduces growth rate and increases sensitivity to chemotherapeutic agents in human gastric cancer cells. *Cancer Gene Ther* 7:885–892
- Kuhbandner S, Brummer S, Metzger D, Chambon P, Hofmann F, Feil R (2000) Temporally controlled somatic mutagenesis in smooth muscle. *Genesis* 28:15–22
- Kuhn R, Schwenk F, Aguet M, Rajewsky K (1995) Inducible gene targeting in mice. *Science* 269:1427–1429
- Lakso M, Pichel JG, Gorman JR, Sauer B, Okamoto Y, Lee E, Alt FW, Westphal H (1996) Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. *Proc Natl Acad Sci USA* 93:5860–5865
- Le Y, Sauer B (2000) Conditional gene knockout using cre recombinase [In Process Citation]. *Methods Mol Biol* 136:477–485
- Leong WI, Lonnerdal B (2004) Hecpudin, the recently identified peptide that appears to regulate iron absorption. *J Nutr* 134:1–4
- Li G, Robinson GW, Lesche R, Martinez-Diaz H, Jiang Z, Rozengurt N, Wagner KU, Wu DC, Lane TF, Liu X, Hennighausen L, Wu H (2002) Conditional loss of PTEN leads to precocious development and neoplasia in the mammary gland. *Development* 129:4159–4170
- Li W, Qiao W, Chen L, Xu X, Yang X, Li D, Li C, Brodie SG, Meguid MM, Hennighausen L, Deng CX (2003) Squamous cell carcinoma and mammary abscess formation through squamous metaplasia in Smad4/Dpc4 conditional knockout mice. *Development* 130:6143–6153
- Li W, Xiao C, Vonderhaar BK, Deng CX (2007) A role of estrogen/ERalpha signaling in BRCA1-associated tissue-specific tumor formation. *Oncogene* 26:7204–7212
- Lindeberg J, Mattsson R, Ebendal T (2002) Timing the doxycycline yields different patterns of genomic recombination in brain neurons with a new inducible Cre transgene. *J Neurosci Res* 68:248–253
- Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH (1996) Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev* 10:1835–1843
- Liu X, Holstege H, van der Gulden H, Treur-Mulder M, Zevenhoven J, Velds A, Kerkhoven RM, van Vliet MH, Wessels LF, Peterse JL, Berns A, Jonkers J (2007) 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 USA* 104:12111–12116
- Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A (1997) Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev* 11:1226–1241
- Luo F, Brooks DG, Ye H, Hamoudi R, Pouligiannis G, Patek CE, Winton DJ, Arends MJ (2007) Conditional expression of mutated K-ras accelerates intestinal tumorigenesis in Msh2-deficient mice. *Oncogene* 26:4415–4427
- Maesawa C, Tamura G, Nishizuka S, Iwaya T, Ogasawara S, Ishida K, Sakata K, Sato N, Ikeda K, Kimura Y, Saito K, Satodate R (1997) MAD-related genes on 18q21.1, Smad2 and Smad4, are altered infrequently in esophageal squamous cell carcinoma. *Jpn J Cancer Res* 88:340–343

- Mak TW, Hakem A, McPherson JP, Shehabeldin A, Zabolocki E, Migon E, Duncan GS, Bouchard D, Wakeham A, Cheung A, Karaskova J, Sarosi I, Squire J, Marth J, Hakem R (2000) Bcl-2 required for T cell lineage development but not TCR loci rearrangement. *Nat Immunol* 1:77–82
- Mansour SL, Thomas KR, Capecchi MR (1988) Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature* 336:348–352
- Massague J (1998) TGF- β signal transduction. *Annu Rev Biochem* 67:753–791
- Meuwissen R, Linn SC, van der Valk M, Mooi WJ, Berns A (2001) Mouse model for lung tumorigenesis through Cre/lox controlled sporadic activation of the K-Ras oncogene. *Oncogene* 20:6551–6558
- Meyers EN, Lewandoski M, Martin GR (1998) An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. *Nat Genet* 18:136–141
- Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266:66–71
- Mills NE, Fishman CL, Scholes J, Anderson SE, Rom WN, Jacobson DR (1995) Detection of K-ras oncogene mutations in bronchoalveolar lavage fluid for lung cancer diagnosis. *J Natl Cancer Inst* 87:1056–1060
- Morgan SE, Kastan MB (1997) p53 and ATM: cell cycle, cell death, and cancer. *Adv Cancer Res* 71:1–25
- Nagatake M, Takagi Y, Osada H, Uchida K, Mitsudomi T, Saji S, Shimokata K, Takahashi T, Takahashi T (1996) Somatic in vivo alterations of the DPC4 gene at 18q21 in human lung cancers. *Cancer Res* 56:2718–2720
- Nagy A (2000) Cre recombinase: the universal reagent for genome tailoring. *Genesis* 26:99–109
- Nagy A, Mar L (2001) Creation and use of a Cre recombinase transgenic database. *Methods Mol Biol* 158:95–106
- Nathanson KL, Weber BL (2001) “Other” breast cancer susceptibility genes: searching for more holy grail. *Hum Mol Genet* 10:715–720
- O’Gorman S, Fox DT, Wahl GM (1991) Recombinase-mediated gene activation and site-specific integration in mammalian cells. *Science* 251:1351–1355
- Okuda K, Nakanuma Y, Miyazaki M (2002) Cholangiocarcinoma: recent progress. Part 2: molecular pathology and treatment. *J Gastroenterol Hepatol* 17:1056–1063
- Olmes MJ, Erlich R (2004) A review and update on cholangiocarcinoma. *Oncology* 66:167–179
- Paterson JW (1998) BRCA1: a review of structure and putative functions. *Dis Markers* 13:261–274
- Pietrangelo A (2006) Hereditary hemochromatosis. *Annu Rev Nutr* 26:251–270
- Pollard JW (2001) Tumour-stromal interactions. Transforming growth factor- β isoforms and hepatocyte growth factor/scatter factor in mammary gland ductal morphogenesis. *Breast Cancer Res* 3:230–237
- Qiao W, Li AG, Owens P, Xu X, Wang XJ, Deng CX (2006) Hair follicle defects and squamous cell carcinoma formation in Smad4 conditional knockout mouse skin. *Oncogene* 25:207–217
- Rodenhuis S, Slebos RJ, Boot AJ, Evers SG, Mooi WJ, Wagenaar SS, van Bodegom PC, Bos JL (1988) Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. *Cancer Res* 48:5738–5741
- Rucker EB 3rd, Dierisseau P, Wagner KU, Garrett L, Wynshaw-Boris A, Flaws JA, Hennighausen L (2000) Bcl-x and Bax regulate mouse primordial germ cell survival and apoptosis during embryogenesis [In Process Citation]. *Mol Endocrinol* 14:1038–1052
- Ruiz S, Santos M, Paramio JM (2006) Is the loss of pRb essential for the mouse skin carcinogenesis? *Cell Cycle* 5:625–629
- Sansal I, Sellers WR (2004) The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol* 22:2954–2963
- Sauer B, Henderson N (1988) Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proc Natl Acad Sci USA* 85:5166–5170
- Schutte M, Hruban RH, Hedrick L, Cho KR, Nadasdy GM, Weinstein CL, Bova GS, Isaacs WB, Cairns P, Nawroz H, Sidransky D, Casero RA Jr, Meltzer PS, Hahn SA, Kern SE (1996) DPC4 gene in various tumor types. *Cancer Res* 56:2527–2530

- Schwenk F, Kuhn R, Angrand PO, Rajewsky K, Stewart AF (1998) Temporally and spatially regulated somatic mutagenesis in mice. *Nucleic Acids Res* 26:1427–1432
- Shen SX, Weaver Z, Xu X, Li C, Weinstein M, Chen L, Guan XY, Ried T, Deng CX (1998) A targeted disruption of the murine *Brcal* gene causes gamma-irradiation hypersensitivity and genetic instability. *Oncogene* 17:3115–3124
- Shukla V, Coumoul X, Cao L, Wang R, Xiao C, Xu X, Ando S, Yakar S, LeRoith D, Deng D (2006) Absence of the full-length *BRCA1* leads to increased expression of IGF signaling axis members. *Cancer Res* 66:7151–7157
- Shukla V, Coumoul X, Deng CX (2007a) RNAi-based conditional gene knockdown in mice using a U6 promoter driven vector. *Int J Biol Sci* 3:91–99
- Shukla V, Coumoul X, Wang RH, Kim HS, Deng CX (2007b) RNA interference and inhibition of MEK-ERK signaling prevent abnormal skeletal phenotypes in a mouse model of craniosynostosis. *Nat Genet* 39:1145–1150
- Sirard C, de la Pompa JL, Elia A, Itie A, Mirtsos C, Cheung A, Hahn S, Wakeham A, Schwartz L, Kern SE, Rossant J, Mak TW (1998) The tumor suppressor gene *Smad4/Dpc4* is required for gastrulation and later for anterior development of the mouse embryo. *Genes Dev* 12:107–119
- Sirica AE (2005) Cholangiocarcinoma: molecular targeting strategies for chemoprevention and therapy. *Hepatology* 41:5–15
- Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21:70–71
- Sternberg N, Sauer B, Hoess R, Abremski K (1986) Bacteriophage P1 cre gene and its regulatory region. Evidence for multiple promoters and for regulation by DNA methylation. *J Mol Biol* 187:197–212
- Struwing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA (1997) The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews [see comments]. *N Engl J Med* 336:1401–1408
- Sugimachi K, Aishima S, Taguchi K, Tanaka S, Shimada M, Kajiyama K, Tsuneyoshi M (2001a) The role of overexpression and gene amplification of cyclin D1 in intrahepatic cholangiocarcinoma. *J Hepatol* 35:74–79
- Sugimachi K, Taguchi K, Aishima S, Tanaka S, Shimada M, Kajiyama K, Tsuneyoshi M (2001b) Altered expression of beta-catenin without genetic mutation in intrahepatic cholangiocarcinoma. *Mod Pathol* 14:900–905
- Tannour-Louet M, Porteu A, Vaultont S, Kahn A, Vasseur-Cognet M (2002) A tamoxifen-inducible chimeric Cre recombinase specifically effective in the fetal and adult mouse liver. *Hepatology* 35:1072–1081
- Taylor-Robinson SD, Toledano MB, Arora S, Keegan TJ, Hargreaves S, Beck A, Khan SA, Elliott P, Thomas HC (2001) Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968–1998. *Gut* 48:816–820
- Teng Y, Sun AN, Pan XC, Yang G, Yang LL, Wang MR, Yang X (2006) Synergistic function of *Smad4* and *PTEN* in suppressing forestomach squamous cell carcinoma in the mouse. *Cancer Res* 66:6972–6981
- Tsujita M, Mori H, Watanabe M, Suzuki M, Miyazaki J, Mishina M (1999) Cerebellar granule cell-specific and inducible expression of Cre recombinase in the mouse. *J Neurosci* 19:10318–10323
- Ueda S, Fukamachi K, Matsuoka Y, Takasuka N, Takeshita F, Naito A, Iigo M, Alexander DB, Moore MA, Saito I, Ochiya T, Tsuda H (2006) Ductal origin of pancreatic adenocarcinomas induced by conditional activation of a human *Ha-ras* oncogene in rat pancreas. *Carcinogenesis* 27:2497–2510
- Utomo AR, Nikitin AY, Lee WH (1999) Temporal, spatial, and cell type-specific control of Cre-mediated DNA recombination in transgenic mice. *Nat Biotechnol* 17:1091–1096
- Ventura A, Meissner A, Dillon CP, McManus M, Sharp PA, Van Parijs L, Jaenisch R, Jacks T (2004) Cre-lox-regulated conditional RNA interference from transgenes. *Proc Natl Acad Sci USA* 101:10380–10385
- Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T (2007) Restoration of p53 function leads to tumour regression in vivo. *Nature* 445:661–665

- Wagner KU, Wall RJ, St-Onge L, Gruss P, Wynshaw-Boris A, Garrett L, Li M, Furth PA, Hennighausen L (1997) Cre-mediated gene deletion in the mammary gland. *Nucleic Acids Res* 25:4323–4330
- Wakefield LM, Piek E, Bottinger EP (2001) TGF-beta signaling in mammary gland development and tumorigenesis. *J Mammary Gland Biol Neoplasia* 6:67–82
- Wang RH, Yu H, Deng CX (2004) A requirement for breast-cancer-associated gene 1 (BRCA1) in the spindle checkpoint. *Proc Natl Acad Sci USA* 101:17108–17113
- Wang RH, Li C, Xu X, Zheng Y, Xiao C, Zerfas P, Cooperman S, Eckhaus M, Rouault T, Mishra L, Deng CX (2005) A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metab* 2:399–409
- Weaver Z, Montagna C, Xu X, Howard T, Gadina M, Brodie SG, Deng CX, Ried T (2002) Mammary tumors in mice conditionally mutant for *Brcal* exhibit gross genomic instability and centrosome amplification yet display a recurring distribution of genomic imbalances that is similar to human breast cancer. *Oncogene* 21:5097–5107
- Weinstein M, Yang X, Deng C (2000) Functions of mammalian smad genes as revealed by targeted gene disruption in mice [In Process Citation]. *Cytokine Growth Factor Rev* 11:49–58
- Wu T, Leng J, Han C, Demetris AJ (2004) The cyclooxygenase-2 inhibitor celecoxib blocks phosphorylation of Akt and induces apoptosis in human cholangiocarcinoma cells. *Mol Cancer Ther* 3:299–307
- Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX (1999a) Conditional mutation of *Brcal* in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation [see comments]. *Nat Genet* 22:37–43
- Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX (1999b) Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol Cell* 3:389–395
- Xu B, Kim S, Kastan MB (2001a) Involvement of *Brcal* in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol Cell Biol* 21:3445–3450
- Xu X, Li C, Garrett-Beal L, Larson D, Wynshaw-Boris A, Deng CX (2001b) Direct removal in the mouse of a floxed neo gene from a three-loxP conditional knockout allele by two novel approaches. *Genesis* 30:1–6
- Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, Harris CC, Deng CX (2001c) Genetic interactions between tumor suppressors *Brcal* and p53 in apoptosis, cell cycle and tumorigenesis. *Nat Genet* 28:266–271
- Xu X, Aprelikova O, Moens P, Deng CX, Furth PA (2003) Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. *Development* 130:2001–2012
- Xu X, Kobayashi S, Qiao W, Li C, Xiao C, Radaeva S, Stiles B, Wang R, Ohara N, Yoshino T, LeRoith D, Torbenson MS, Gores GJ, Wu H, Gao B, Deng C (2006) Induction of intrahepatic cholangiocellular carcinoma by liver specific disruption of *Smad4* and *Pten* in mice. *J Clin Invest* 116(7):1843–1852
- Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B, LeRoith D (1999) Normal growth and development in the absence of hepatic insulin-like growth factor I. *Proc Natl Acad Sci USA* 96:7324–7329
- Yang X, Li C, Xu X, Deng C (1998) The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. *Proc Natl Acad Sci USA* 95:3667–3672
- Yang X, Li C, Herrera PL, Deng CX (2002) Generation of *Smad4/Dpc4* conditional knockout mice. *Genesis* 32:80–81
- Yang L, Mao C, Teng Y, Li W, Zhang J, Cheng X, Li X, Han X, Xia Z, Deng H, Yang X (2005) Targeted disruption of *Smad4* in mouse epidermis results in failure of hair follicle cycling and formation of skin tumors. *Cancer Res* 65:8671–8678
- Zhang P, Li MZ, Elledge SJ (2002) Towards genetic genome projects: genomic library screening and gene-targeting vector construction in a single step. *Nat Genet* 30:31–39

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