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...
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 Coliphase 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 sometimes 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).
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. 2.2](image-url)
The use of Cre–loxP technology and inducible systems to generate mouse 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.
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.

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

<table>
<thead>
<tr>
<th>Methods to remove floxed neo gene</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transit expression of Cre in ES cells</td>
<td>Quick, technically easy</td>
<td>Additional modifications may compromise pluripotency of ES cells</td>
</tr>
<tr>
<td>Cross with EIIa-Cre transgenic mice</td>
<td>Avoids ES manipulation and removes the neo gene in mice with a high reliability</td>
<td>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</td>
</tr>
<tr>
<td>Oocyte injection</td>
<td>Direct injection of a Cre expression plasmid into oocyte. The amount of input Cre can be adjusted to increase efficiency of removing the neo gene</td>
<td>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</td>
</tr>
<tr>
<td>Infecting morulae with recombinant Cre adenovirus</td>
<td>High efficiency of Cre adenovirus to infect morulae, which may delete floxed neo with high efficiency</td>
<td>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</td>
</tr>
<tr>
<td>Combination of Cre/loxP–Flp/Frt</td>
<td>Deletion of the neo is independent of Cre/loxP system. It is a straightforward screen for the complete recombination product</td>
<td>Screening large number of offspring is expected due to a low efficiency of the Flp/Frt system in mouse</td>
</tr>
</tbody>
</table>

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
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) (Gitter 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;
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-beta 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-beta/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^CoeCo; 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-beta/
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-galactocidase 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-3β, 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
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 knockout 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<sup>Cre/Cre</sup>;MMTV-Cre and Brca1<sup>Cre/Cre</sup>;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-alpha 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.
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\textsuperscript{G12V}) mutation that specifically targets lung epithelial cells (Meuwissen et al. 2001). As shown in Fig. 2.5, the conditional K-ras\textsuperscript{G12V} transgene contained a broadly active beta-actin promoter, followed by a floxed-GFP, and then a K-ras\textsuperscript{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\textsuperscript{G12V} oncogene remains silent. After Cre-mediated deletion of the floxed-GFP, the K-ras\textsuperscript{G12V} oncogene is placed directly under control of the beta-actin promoter. The K-ras\textsuperscript{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)

![Fig. 2.5](image)

**Fig. 2.5** Schematic representation of the conditional K-ras\textsuperscript{G12V} construct. The conditional K-ras\textsuperscript{G12V} transgene is driven by a broadly active beta-actin promoter, followed by a floxed-GFP, and then a K-ras\textsuperscript{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\textsuperscript{G12V} oncogene remains silent. After Cre-mediated deletion of the floxed-GFP, the K-ras\textsuperscript{G12V} oncogene is placed directly under control of the beta-actin promoter. The K-ras\textsuperscript{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)
2.4.2 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

Capecchi MR (1989) Altering the genome by homologous recombination [Review]. Science 244:1288–1292


Jones LP, Tili MT, Assefniia S, Torre K, Halama ED, Parrish A, Rosen EM, Furth PA (2008) Activation of estrogen signaling pathways collaborates with loss of Brca1 to promote development
of ERalpha-negative and ERalpha-positive mammary preneoplasia and cancer. Oncogene 27:794–802
tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer.
Nat Genet 29:418–425
the adenoviral Cre recombinase in vivo. Genesis 31:126–129
and Smad4/Dpc4 in intrahepatic cholangiocarcinoma. Hum Pathol 33:877–883
13:67–89
Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. Cancer Res
67:8121–8130
activation reduces growth rate and increases sensitivity to chemotherapeutic agents in human
gastric cancer cells. Cancer Gene Ther 7:885–892
trolled somatic mutagenesis in smooth muscle. Genesis 28:15–22
269:1427–1429
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) Hepcidin, 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, Vononderhaar BK, Deng CX (2007) A role of estrogen/ERalpha signaling in BRCA1-
associated tissue-specific tumor formation. Oncogene 26:7204–7212
leads to failure in the morphogenesis of the egg cylinder in early postimplantation develop-
ment. Genes Dev 10:1835–1843
p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like
breast cancer. Proc Natl Acad Sci USA 104:12111–12116
cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2,
Conditional expression of mutated K-ras accelerates intestinal tumorigenesis in Msh2-deficient
mice. Oncogene 26:4415–4427
Kimura Y, Saito K, Satodate R (1997) MAD-related genes on 18q21.1, Smad2 and Smad4, are altered


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


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