Pancreatic Adenocarcinoma: How good are the Genetically Engineered Mouse Models: A Review

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ABSTRACT: Pancreatic Adenocarcinoma, the most common form of pancreatic cancer, is also one of the deadliest. Though a very complex and multilayer molecular malfunctioning initiate the disease yet, there are some basic genetical mutations on some specific genes that have been identified as the most likely cause of initiation of this type of cancer. On the other hand, mouse models are days old model systems that have been used for better understanding of the molecular mechanism underlying a number of complex diseases such as cancer. In this review the author tried to focus on the significance of different available genetically engineered mouse model which are helpful to understand the molecular pathogenesis of this disease.

Keywords: Pancreatic Adenocarcinoma; pancreatic cancer; genetical mutations; transgenic mouse models.

INTRODUCTION: Deaths from pancreatic cancer rank fourth among cancer-related deaths in both men and women, in the United States. The tumor has a poor prognosis, with an associated 5-year survival rate of patients with pancreatic adenocarcinoma of around 6 percent only. A growing body of evidence supports the view that invasive pancreatic adenocarcinomas arise from histologically well-defined noninvasive lesions within the pancreatic ducts. The early detection of these precursors could reduce the incidence and mortality from pancreatic adenocarcinoma. These precursor lesions include microscopic pancreatic intraepithelial neoplasias (PanINs), and macroscopic intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCN). Though, the exact cause of pancreatic adenocarcinoma remains unknown but in recent years, there have been significant advancement in the understanding of the molecular biology of this common type of pancreatic cancer. Advancement in cancer research suggests that pancreatic cancer results from the successive accumulation of gene mutations.

The pancreatic adenocarcinoma often originates in the ductal epithelium and from premalignant lesions and ultimately converted into invasive carcinoma. It has been found that invasive carcinoma is significantly associated with the successive accumulation of mutations. Some of the mutations are extremely important such as mutation causing activation of the KRAS oncogene, inactivation of the tumor-suppressor gene “Inhibitors of Cyclin-dependent Kinase 4” (INK4A) and inactivation of the tumor-suppressor gene TP53 and/or deletion of SMAD family member 4 gene (SMAD4). Almost all human subjects with fully established pancreatic cancer carry one or more of these four genetic defects. Generally, this chain of mutations starts with KRAS gene and then occurs in INK4A, TP53 and SMAD4 successively.

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One of the best ways to study the role of these mutations in initiation and progression of pancreatic cancer is to study with in vivo genetically engineered mouse models. Scientific studies proved that induced constitutive activation of KRAS following inactivation of TP53 or CDKN2A/INK4A results in the development of pancreatic cancer in mouse, which is identical to the human malady\(^6\). Hence, genetically engineered mouse model is a powerful tool for cancer researcher and scientists to dissect the molecular mechanism of initiation and progression of pancreatic cancer. This review aims to present how the genetically engineered mouse models have been used in studying such a complex process of pancreatic adenocarcinoma formation.

**TRANSGENIC MOUSE MODELS:** Mouse model can be used to identify the role of different genes (expressing proteins or miRNAs) in initiation and progression of complex diseases like cancer and functional aspects of mutations can be evaluated by this system with much perfection. Introduction of foreign DNA into the mouse genomic DNA in both conditional and inducible form, is considered now a major technical advancement in the fields of biology and more specifically genetics. This technology, when applied to produce genetically modified mouse called transgenic mouse technology, has reformed biological applications and provided new approaches to mimic many human diseases in a whole animal context. Several hundreds of transgenic mouse models with expression of foreign genes specifically targeted to desired organelles or tissues have been generated and characterized. Moreover, the ability to spatiotemporally activate or inactivate gene expression in vivo using the “Cre-loxP” technology has recently emerged as a powerful approach to understand various biological processes. In the past two decade, the Cre-loxP technology along with inducible systems has been used to investigate the detail molecular aspects of a number of diseases including cancer. Many tumor suppressor genes and oncogenes have been deactivated or activated respectively in a spatial and/or temporal manner, making it possible for studying their function in tumorigenesis in such a vivid way that would otherwise not be achieved. Genetically engineered mouse models have significantly contributed to our understanding of cancer biology. They are useful in validating gene functions and identifying novel genes associated with cancer and there cellular mechanisms underlying tumor initiation and progression. The murine tumor model systems are though available for most common human cancers but generation of proper mouse model/s of pancreatic cancer has remained an area of significant obstacle and challenges.

An animal model must recapitulate the human disease for understanding its etiology. Hence scientists have tried to develop better models, especially genetically engineered models. Mouse model that develop pancreatic adenocarcinoma spontaneously has emerged as a new tool to investigate the molecular mechanism of formation and progression of this disease and identify potential therapeutic targets. Recently there are several different genetically modified mouse models available that offer the option to evaluate the molecular pathogenesis and therapeutic targets of pancreatic adenocarcinoma in human.

The generation of these models essentially involves targeting a variety of genes to different set of cells in pancreas to produce neoplastic or tumorigenic changes. To develop a proper mouse model of pancreatic adenocarcinoma three aspects should be kept in mind. First, which gene or genes have to be targeted, secondly to select specific promoter/s which will be expressed in mouse pancreas only and third point is to target pancreas specific cells. The models are based on the targeted genes such as KRAS, p53 SMAD4 and named accordingly. These models are being discussed in the following section. Few most common promoters that are been targeted were, Elastase (or elastase-1) promoter, promoter of PDX1 gene (one that is necessary for the development of pancreas), cytokeratin 19 (CK19) etc. among them, PDX1 promoter is the most commonly used because PDX1, though necessary for pancreas development, yet it is widely expressed in pancreatic cancers\(^9\).

Attempts were made to target acinar cells of pancreas to express the foreign gene because these cells can undergo metaplastic conversion to ductal cells. Studies have also found that cells of islets or pancreatic stem cells could be other alternatives in this context. Pancreatic adenocarcinoma may arise from any of such differentiated cells or from tissue stem cells\(^10\). Here we describe the most common genetically engineered mouse models of pancreatic adenocarcinoma that could be useful for assessing the role of genes associated with this terrible malady. Initially, myc and transforming growth factor alpha or TGFalpha genes were manipulated in mouse pancreatic acinar cells targeted with elastase promoter. This condition was able to develop metaplastic lesions in mouse. But the tumor development was much enhanced when p53 gene was also manipulated simultaneously. Later, the choice of gene that was most frequently been targeted selected was KRAS, and hence in vivo models with active KRAS mutant variety was developed. Along with this gene other genes were also
being targeted. The scope and limitations of these models are being described in this section.

**Kras Transgenic Model:** Epidemiological and experimental research have shown that active-mutation of the KRAS oncogene is the most common mutation associated with different cancer including pancreatic cancer\(^{11}\); hence genetically engineered mouse models are developed based on the KRAS oncogene mostly. Mouse expressing mutant KRAS develop lesions similar to early forms of the pancreatic cancers. Pancreas-specific activation of the mutant KRAS allele (KRAS\(^{G12D}\)) in mice forms the malignancy of the tissue. Activation of KRAS alone in the pancreatic epithelium develop pancreatic neoplasias which indicating the need for additional support to promote tumor progression to the malignant PDAC stage. The KRAS\(^{G12D}\) model is consist of the active mutation containing construct, can be induced using inducible alleles of Cre-recombinase, such as estrogen receptor-Cre fusion genes (CreER) or PDX1-Cre fusion gene. The common genetically engineered models include PDX-1-Cre/Lox-Stop-Lox (LSL)-Kras mice. Though the neoplastic growth of cells were observed but invasive tumor was never been developed in this model. Thus it can be said that only KRAS mutation is not sufficient to produce a tumor equivalent to human pancreatic adenocarcinoma, hence, different other genes have been targeted to generate combined models that progress to invasive PDAC. The common genetically engineered KRAS mutated mouse models are further modified with deletions or mutations of Ink4, p53 or Smad4\(^{12,13}\). Tuveson et al. took a different approach to express activated KRAS in mouse. In their study activated KRAS was inserted within Mist1 locus by homologous recombination. Mist1 transcription factor is associated with development of exocrine part of pancreas. It was found that this transgenic mouse have a median survival span of about 10.5 months, which is significantly low in comparison with normal (24.2 months)\(^{14}\).

**Kras, Tp53 Transgenic Model:** To investigate the genetic requirement of p53 and KRAS both, scientists targeted these two genes to alter together in mouse pancreas. This mouse model was generated based on the previously described LSL-Kras\(^{G12D/+}\), PDX-1-Cre mouse. A conditionally expressed point mutant allele of Tp53\(^{R172H}\) causing loss of function of p53 was being used along with. Activation of both the Kras\(^{G12D}\) and the Tp53\(^{R172H}\) alleles occur in pancreatic tissue progenitor cells of the developing mouse pancreas through interbreeding with PDX-1-Cre transgenic animals. The presence of each rearranged allele can be detected in the pancreas but not in other tissues in general. Thus, tissues not expressing Cre-recombinase i.e. non-pancreatic tissue remain heterozygous for these loci and hence can function normally. Four to six weeks old mice PDX-1-Cre, LSL-Kras\(^{G12D/+}\), LSL-Trp53\(^{R172H/+}\) show either normal or early pre-neoplastic lesions (PanIN) similar to Kras\(^{G12D/+}\) expressing mice (described earlier). But at the later stage significant tumor load was observed in animals by ten weeks of age at the earliest and the full spectrum of pre-invasive lesions become prominent. The median lifespan of PDX-1-Cre, LSL-Kras\(^{G12D/+}\), LSL-Trp53\(^{R172H/+}\) mice was significantly shortened. Consequently, metastatic spared of tumor cells at peri-pancreatic lymph nodes were also been detected. Histological analyses reveal a predominant moderately well-differentiated to well-differentiated morphology organized as is observed in the human disease\(^{15-16}\). Hence this model was mostly successful to mimic the actual situation in human.

**Kras, Ink4a Transgenic Model:** In pancreatic adenocarcinoma loss of function of the cyclin-dependent kinase inhibitor, INK4A (encoding both p16\(^^{INK4A}\) and p19\(^{ARF}\)), is a very common incident. INK4A/ARF is a tumor suppressor which can also activate the p53 mediated pathway and hence protect the cells form DNA damage induced cancer development. Based on this knowledge transgenic mice have been developed to study the role of INK4A/ARF in pancreatic adenocarcinoma formation. Studies showed that mice with sole deletion of INK4A/ARF gene cannot develop spontaneous neoplastic lesions. But they have demonstrated that the combination of expression of consecutively active mutant KRAS\(^{G12D}\) and INK4A/ARF deficiency resulted neoplasms which bears striking resemblance to human pancreatic adenocarcinoma. The survival rate of these mice were reduced to about 11 weeks and within this short period of time high metastasis of the tumor cells were found. In this model, the LSL-KRASS\(^{G12D}\) allele is first expressed at the endogenous level. The conditional elimination of both p16\(^{INK4A}\) and p19\(^{ARF}\) proteins in double engineered mouse expressed the Kras\(^{G12D}\) allele was expressed specifically in the pancreas after using the PDX-1-Cre transgene. Within seven to eleven week of age, PDX-1-Cre, KRAS\(^{G12D}\) INK4A/ARF\(^{flox}\) mice show weight loss, ascites, jaundice and aggressive pancreatic tumors. These pancreatic tumors are highly invasive and metastatic\(^{17,18}\). Furthermore, invasion of the lymphatic and vascular system has been detected. These observations suggest the metastatic potential of these neoplasms. Thus, Aguirre et al. found that KrasG12D expression in combination with INK4A/ARF deficiency resulted in an earlier appearance of PanIN lesions.
and these neoplasms progressed rapidly to highly invasive and metastatic cancers, resulting in death in all cases by 11 week.\textsuperscript{19}

**Kras, Smad4 Transgenic Model:** The SMAD4 tumor suppressor gene encodes a transcription factor that is a central effector of transforming growth factor-β (TGF-β). Studies suggest that inactivating mutations in this gene are common in subsets of pancreatic cancer. SMAD4/DPC4 alone has no direct impact on the development of pancreatic cancer. But, SMAD4 deficiency allows rapid progression of active mutant KRAS\textsuperscript{G12D} mediated neoplasm formation in pancreas when combined with the activated KRAS\textsuperscript{G12D} allele. It has been observed that combination of KRAS\textsuperscript{G12D} and SMAD4 deficiency resulted in the rapid development of tumors resembling IPMN which is known as a precursor to pancreatic adenocarcinoma in humans. To develop conditional knockout allele of Smad4 (Smad4\textsuperscript{lox/lox}) in mice, construct harboring loxP sites flanking exons 8 and 9 in the mouse germ line was being generated. Smad4\textsuperscript{lox/lox} homozygous mice were then crossed to PDX1-Cre transgenic mice as mentioned before. LSL-KRAS\textsuperscript{G12D} SMAD4\textsuperscript{lox/lox} mice showed low-grade PanINs from 4 week of age and reached terminal morbidity between 8 and 24 weeks.\textsuperscript{20,21} Hence it can be stated that, combination of KRAS\textsuperscript{G12D} expression and SMAD4 deletion showed a rapid beginning of IPMN and PanIN lesions and exhibit pancreatic malignant progression at a moderate speed in mouse model.

**Kras, Tgfbr2 Transgenic Model:** Transforming growth factor-beta (TGF-beta) signaling plays an important role in developing pancreatic adenocarcinoma. As indicated by the fact that Smad4, which encodes a central signal mediator of TGF-beta pathway, has been found to be deleted or mutated in 55% of cases. In relation to this finding some study has revealed that type II TGF-beta receptor (Tgfbr2) gene is also altered in a relatively smaller subset of human pancreatic adenocarcinoma patients. To investigate further, scientists developed pancreas-specific Tgfbr2 knockout mice (in the combination with active Kras (KRAS\textsuperscript{G12D}) using the same Cre-loxP system as described before. Pancreas-specific Tgfbr2 knockout mice did not show any effect. Interestingly, when the Tgfbr2 knockout was combined with KRAS\textsuperscript{G12D}, well-differentiated pancreatic adenocarcinoma was developed in mouse. Clinical and histopathological manifestations of the tumor progression in those mice recapitulated human PDAC.\textsuperscript{22-24} Hence it can be inferred that blockade of TGF-beta signaling might be useful in controlling PDAC progression and this model is also proved to be another option for in-depth analysis of molecular pathogenesis of pancreatic cancer in us.

**BrcA2, Kras, Tp53 Transgenic Model:** Although the role of BRCA2 gene in development of pancreatic cancer is still somewhat inconclusive but it was found that BRCA2 mutation along with KRA5 and/or p53 is able to develop cancerous growth in mouse pancreas. To study the role of this gene in pancreatic cancer functionally null BRCA2 mouse was developed by deleting its exon 11 from both alleles. Homozygous BRCA2 inactive mice cannot develop cancer by itself. Mouse with inactive BRCA2 at both alleles was found to develop pancreatic cancer only when the p53 is also mutated.\textsuperscript{25} It suggests that inactivation of BRCA2 alone is not enough to promote pancreatic cancer, instead to develop such disease it requires disruption of p53 signaling as well. Interestingly it was also been observed that deletion of this tumor suppressor gene can inhibit the impact of activated KRAS and eventually inhibit formation of pancreatic tumor.

Thus, mouse models use multiple approaches to target the expression of mutant genes and as a result they develop a broad range of pathologic changes, some of them mimic the mode of initiation and propagation of pancreatic carcinoma in human. Studies found that mouse expressing active-mutant KRAS in the pancreas develop a range of pre-neoplastic changes in the form of pancreatic intraepithelial neoplasia. But, additional genetic changes such as inactivation of p16/CDKN2A, TP53 or DPC4/SMAD4 lead to develop tumors that mimic the full spectrum of pancreatic tumor development including invasive and metastatic stage of human pancreatic adenocarcinoma. Since, the genetic basis of pancreatic ductal adenocarcinoma was revealed, with activation of KRAS and inactivation of the p16/INK4a, p53 and SMAD4 etc tumor suppressors, several mouse models of invasive pancreatic cancer have been developed for investigating the opportunity of therapy to this deadly disease. All these models stand to be a good platform for investigating the genetic background of this deadly disease in human.

**CONCLUSION:** Pancreatic adenocarcinoma is one of the most lethal forms of cancer in human with the median survival period of 6 months only. The molecular pathogenesis of this misery is quiet complex and yet to be dissected in depth. Until recently, the cause of initiation and progression of this disease was mostly unknown to us. Scientific investigations in last few decades have shown that multiple genes are involved in development of the disease. Though it involves mutation at gene level other predominate molecular
mechanisms have also been discovered. Epigenetical modifications, micro-RNA mediated regulation of gene expression, and over-expression of some proteins in pancreas is some of the most important mechanism among them. Of all these mechanisms the most extensively studied initial steps were modifications at gene level. To study role of different genes in developing pancreatic adenocarcinoma, genetically engineered mouse models are best to use because they signifies role of specific gene/s conclusively. Based on these models we now know that genes like KRAS, INK4A, TP53 or SMAD4 are the important player in initiation and promotion of pancreatic cancer. Thus, mouse model are very useful in understanding the pathogenesis of pancreatic cancer. The mouse models with so many advantages have still some limitations. It is mostly useful to study the role of genes but epigenetical or only protein level exploration is yet not possible with this scientific tool. Nevertheless, the importance of these genetically engineered mouse models in better understanding the etiology of pancreatic cancer is beyond any doubt.

REFERENCES:


