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Study Human Pancreatic Cancer in Mice: How Close Are They?

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Abstract

Pancreatic cancer is the fourth leading cause of cancer deaths and is characterized by dismal prognosis. Xenograft and genetically engineered mouse (GEM) models have recapitulated critical elements of human pancreatic cancer, providing useful tools to probe the underlying cause of cancer etiology. In this review, we provide a brief description of the common genetic lesions that occur during the development of pancreatic cancer. Next, we describe the strengths and weaknesses of these two models and highlight key discoveries each has made. Although the relative merits of GEM and xenograft pancreatic cancer mouse models are subject to debate, both systems have and will continue to yield essential insights in understanding pancreatic cancer etiology. This information is critical for the development of new methods to screen, treat, and prevent pancreatic cancer.

Keywords

pancreatic cancer; animal model; gene function; tumor microenvironment Zhang and Chen contributed equally

1. Introduction

Pancreatic cancer is the fourth leading cause of cancer mortality and has the worst prognosis of all cancers. Among the 43,920 new cases estimated by the American Cancer Society for 2012, 37,390 deaths are expected [1]. Inadequate early diagnosis leads to less than 5% overall survival rate at five years [2]. If detected early, pancreatic cancer patients have a 5-

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Conflict of Interest

The authors declare no conflict of interest.

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year survival rate of 21% when treated by surgery in combination with chemotherapy [3]. One of the greatest challenges for early detection is the absence of diagnostic symptoms in the early stage of pancreatic cancer. Metastatic pancreatic cancer progresses from a morphologically well-defined preinvasive lesion known as pancreatic intraepithelial neoplasia (PanIN) [4]. This process involves mutations in oncogenes and tumor suppressors and deregulated expression of cancer master switch genes that occur throughout PanIN stages (Fig. 1), contributing to the development of advanced and more invasive pancreatic ductal adenocarcinoma (PDAC) [5]. Therefore, understanding the tumorigenesis of PDAC at the molecular level and utilizing the identified markers are critical to develop a better system to screen, prevent, and treat this deadly disease.

In the past decade, various mouse models have been designed to elucidate the effect genetic mutation exerts on the tumorigenesis of PDAC. In this review, we discuss currently used mouse models including the xenograft models and the genetically engineered mouse (GEM) models to explore the underlying mechanism of pancreatic cancer. The differences between the human and mouse pancreatic cancer genetics and pathological features are also discussed.

2. Overview of Common Genetic Alterations in Pancreatic Cancer

Mutations in both oncogenes and tumor suppressors contribute to the progression of pancreatic cancer [6]. KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is the best known oncogene in pancreatic cancer involved in cell proliferation, survival, and differentiation [7, 8]. KRAS mutation typically occurred at codon 12, is found in over 95% of PDAC, and is an early initiating event in pancreatic tumorigenesis [9, 10]. Tumor suppressor genes such as cyclin-dependent kinase inhibitor 2A (CDKN2A/INK4A), tumor protein p53 (TP53), mothers against decapentaplegic homolog 4 (SMAD4/DPC4), Serine/threonine-protein kinase 11 (LKB1/STK11) and the breast cancer 2 early onset gene (BRCA2) all play important roles in pancreatic cancer progression (Fig. 1) [11]. Germline mutation in INK4A is associated with both Familial Atypical Mole-Malignant Melanoma (FAMMMM) syndrome as well as pancreatic cancer [12, 13]. On the other hand, p19^{ARF} stabilizes p53 by preventing its degradation via MDM2-dependent proteolysis [14]. In addition to defects in p53 tumor suppressor function caused by p19^{ARF} loss, missense mutation within the DNA-binding domain of p53 is observed in more than 50% of PDAC cases [15]. In human PDAC, mutations of TP53 and ARF coexist in ~40% of cases [15–18]. Loss of SMAD4 by either deletion or intragenic point mutation is found in ~50% of pancreatic tumors [19–21]. Levy and colleagues [22] showed that SMAD4 deficiency may inhibit TGF- β -induced cell cycle arrest and migration, promoting tumorigenesis. BRCA2 tumor suppressor gene is best known for its association with familial breast and ovarian cancer, and recently BRCA2 mutation was found in approximately 17% pancreatic cancers [9].

Deregulations in numerous developmental signaling pathways also contribute to PDAC pathogenesis. The two most common pathways are Hedgehog and Notch signaling. The Hedgehog family signaling proteins regulate organogenesis, including the development of pancreas during embryogenesis [23]. It has been strongly implicated that the activation of the Hedgehog pathway contributes to both the initiation of pancreatic ductal neoplasia and the promotion of invasive cancer progression. While Sonic Hedgehog (SHH) is not found in the normal adult pancreas, the intensity of its signaling gradually increases as the cancer progresses to a more invasive stage, suggesting its role in tumor progression. SHH was also found to contribute to the formation of desmoplasia, the cellular fibroinflammatory response which usually accompanies invasive ductal adenocarcinomas [11, 24]. Notch is thought to potentiate the tumor-initiating effects of TGF- α , promoting the formation of early PanIN

lesions [25]. Recently, several new molecular targets were identified in pancreatic cancer, the deregulation of those genes and related pathways promotes pancreatic cancer progression. These include CEACAM-1, CEACAM-6, S100P, ataxia-telangiectasia group D complementing gene (ATDC), ZIP4, and PDX1 [26–31]. Our previous studies demonstrated that ZIP4, a zinc importer, is substantially overexpressed in human pancreatic cancer cells and tumor tissues. Moreover, ZIP4 downregulation inhibited pancreatic cancer growth and increased survival rate in a mouse model. These data strongly implicate ZIP4 as a novel molecular target in pancreatic cancer and as a potential cancer master switch gene in pancreatic cancer progression (Fig. 1) [30, 32, 33].

3. Chemically-Induced Animal Models

Before the arrival of transgenic and GEM mouse models, the study of pancreatic cancer heavily relied on the chemically-induced animal models. One of the most notable and widely investigated model is the Syrian gold hamsters intraperitoneally injected with N-nitroso-bis(2-oxopropyl)amine. Although this model initially provided promising results of adenocarcinoma development with ductal differentiation, the formation of hepatocellular carcinoma caused by uncontrolled diffusion of carcinogen was less than ideal [34]. Most recently, direct implantation of 7,12-dimethylbenzanthracene (DMBA) in rats and mice induced PanIN lesions and ductal adenocarcinoma with similar histological features of human pancreatic cancer, however, the lack of spontaneous tumorigenesis as well as the emergence of GEM mouse model diminish the clinical value of chemically-induced animal models [35, 36].

4. Xenograft Mouse Models

4.1. Subcutaneous Mouse Model

To study the roles of these above mentioned oncogenes and tumor suppressor genes in the initiation and progression of pancreatic cancer, many mouse models have been developed. Two well accepted xenograft mouse models have been widely used for pancreatic cancer research: the subcutaneous model and the orthotopic model. For the subcutaneous mouse models, pancreatic tumors are introduced to the nude mice by injection of either cultured pancreatic cancer cell lines or tumor tissues. The subcutaneous mouse model involving injections of cultured cells are frequently termed “indirect xenograft” because these tumor cells were obtained many years prior to their implantation in the animal models. The indirect subcutaneous model is inexpensive and the tumor growth can be easily measured, however, the implanted cultured tumor cells do not resemble the histopathological feature of the actual human pancreatic tumor and lack for tumor microenvironment. The absence of immune response from the immunodeficient mouse also prevents the interaction between host immune response and tumor cells, giving rise to an unrealistic tumor growth. Another limitation associated with these subcutaneous mouse models is that the tumor cells show excellent local growth but they rarely metastasize. Therefore, this model is not ideal in studying tumor initiation, progression, and metastasis. Although the subcutaneous xenograft model has its limitations, its low cost, easy production and well-defined tumor size make it a valuable tool for tumor biology studies and initial drug screening, especially for large-scale evaluation for drug candidates by pharmaceutical companies.

4.2. Orthotopic Xenograft Model

To address the issues raised from the subcutaneous models, orthotopic (correct place in Latin) xenograft model was developed so that the tumor cells can be implanted to the organ from which the cancer originated. The tumor cells in orthotopic xenograft mouse model metastasize frequently. Compared to the subcutaneous model, the orthotopic xenografts are more closely related to the physiological features of the pancreas, and represent a better

model system to recapitulate human pancreatic cancer. Many techniques have been developed to optimize the tumor implantation in the orthotopic xenograft model over the years. The simplest method is to directly inject the tumor cells into the pancreas; however, intra-abdominal hemorrhage, disruption of pancreatic capsule and tumor cell spillage into the peritoneal cavity were frequently observed. Tsuji and colleague [37] proposed the main pancreatic duct injection method and successfully introduced pancreatic cancer cells along with red fluorescent protein (RFP) into the pancreas so the injected cancer cells can be visualized and monitored. Huynh and colleague proposed an orthotopic human pancreatic cancer xenograft model by injecting the cancer cells with ultrasound guidance [38]. This injection technique guided by ultrasound not only prevented injection complications but also allowed faster injection time and significantly shortened the recovery time. Recently Ni et al also developed a fluorescence-guided tumor implantation and resection method for pancreatic cancer in an orthotopic xenograft model [39], other groups mixed the tumor cells with matrigel before the injection. All those methods are aimed to minimize the potential tumor cell spillage during the implantation.

In contrast to cell line injection, solid tumor implantation involves surgically implanting a sliced tumor fragment from subcutaneous xenografts [40]. This technique is more traumatic and often triggers unnecessary inflammatory response [41, 42]. Another solid tumor implantation technique that involves wrapping the pancreas around the xenograft was carried out to minimize the invasiveness caused by suturing [43]. Although this is less invasive, the change in its normal anatomical position raises questions about its validity. Hotz and colleagues implanted the donor tumor fragments from four pancreatic cancer cell lines into the head of pancreas and these nude mice had a 100% tumor take rate, while the tumor cell injection resulted in various success rates [44]. Not only did the mouse developed relevant clinical symptoms, their tumor cells also exhibited histological appearance similar to human disease. Although the orthotopic mouse model seems to hold many advantages over the subcutaneous model, the xenograft recipient mice still lack essential immune cells for tumor development and metastatic spread [45]. This leads to the development of humanized mice by injecting human CD34+ hematopoietic stem cells (HSCs) or bone marrow to the NOD/SCID mice in order to reconstitute the immune system, providing a more natural tumor microenvironment as seen in humans. Two humanized mouse models NOD.Cg-Tg(Ins2-TAg)1Lt *Prkdc^{scid}*/DvsJ and NOD/ShiLt-Tg(RipTAg)1Lt/J have been developed by the Jackson Laboratory, their advantage in assessing the genetic interactions has yet to be confirmed.

5. Genetically Engineered Mouse (GEM) Models

5.1. Transgenic Mouse Models

To overcome the limitations of the xenograft model, more sophisticated GEM models have been extensively studied in the last decade, which include transgenic mouse models, knockin and knockout models, and conditional/inducible models. The transgenic mice provide the tool to probe gene function and allow monitoring tumor initiation and progression in a manner not applicable to human subjects (Table 1). Through the recognition of tissue-specific promoter/enhancer elements in the mouse elastase I locus, pancreas was one of the first organs in which transgenic tumor induction was achieved [46, 47]. Richmond and colleagues [48] employed the elastase promoter to investigate the effect of oncogene K-Ras, and found that these Ela-Kras^{G12D} mice displayed precancerous lesions without exhibiting signs of PDAC. Later mouse models confirmed that K-Ras plays a pivotal role in tumor initiation and requires other genetic aberrations for PDAC development. When C-MYC gene is inserted under the regulation of elastase promoter (Ela-1-myc), carcinomas of acinar and mixed type acinar/ductal cells phenotypes were induced with high percentage of ductal metaplasia [49, 50]. Grippo and colleagues [51]

created an elastase-1-TGF- α construct, the pancreas of these transgenic mice develops extensive fibrosis accompanied by tubular complexes which are formed by cells with phenotype in between ductal and acinar cells. In mice older than one year, these transitional cells lose their acinar phenotype and subsequently become more duct-like, implying the progression of acinar cell metaplasia to pancreatic ductal cancer [52].

Contrary to the above-mentioned method where transgene was introduced via SV40-Tag, Lewis and colleagues [53] somatically delivered the oncogene-bearing avian retroviruses to the mouse model that expressed the receptor TVA for avian leukosis sarcoma virus subgroup A (ALSV-A) under the control of the elastase. The formation of pancreatic tumors resulted when either mouse polyoma virus middle Y antigen (PyMT) encoding ALSV-A-based RCAS vectors or C-MYC were inserted to elastase-tv-a transgenic INK4a/ARF null mice. Because the induced pancreatic lesions express PDX1, a marker for pancreas progenitor cells, it was highly suggested the tumor may arise from progenitor cells. This advantageous delivery method not only prevents the expression of transforming oncogenes in the embryonic stage but also targets a specific subset of cells within the pancreas, rendering the neighboring cells unaffected. Although the loss of INK4a/ARF locus alone doesn't cause metastasis, additional TP53 deletion results in pancreatic tumor metastasis to the liver when inserting avian retrovirus encoding PyMT to elastase-tv-a transgenic mice via somatic and sporadic delivery [54]. Crossbreeding transgenic mice overexpressing transforming growth factor alpha (TGF- α) under the control of the elastase promoter with p53 null mice further accelerate the pancreatic cancer progression [55].

In addition to the elastase promoter, PDX1 promoter was also targeted for genetic manipulation [56, 57]. Pancreatic agenesis resulted from homozygous deletion of PDX1 demonstrate the critical role of PDX1 in pancreatic cell fate determination [31]. Regulation of Sonic Hedgehog (SHH) signaling pathway was studied with the transgenic mouse model utilizing the PDX1 promoter [58]. The PDX-Shh mice developed tubular structures of both PanIN-1 and PanIN-2 and mutations of K-Ras and HER-2/neu typically found in early stage of pancreatic cancer were observed, implying the Hedgehog signaling pathway plays a pivotal role in both aberrant cell proliferation and tumorigenesis.

One of the disadvantages of the transgenic mouse model is the low efficiency of microinjection in inserting foreign DNA into the host genome [59]. Accidental transgene insertion into sensitive genomic sites often results in unexpected phenotype due to secondary effect. The fundamental flaw of transgenic mice lies in the fact that typically two wild-type alleles are positioned at the transgene integration site in contrast with one wild-type and one mutant allele in real human tumor genome. However, transgenic mouse model had been a significant leap forward before other gene-targeting approaches developed. Recently, Tasic and colleagues [60] described an integrase-mediated method for site-specific transgenesis in mice via pronuclear microinjection that successfully inserted an intact single-copy transgene into predetermined chromosomal loci with up to 40% efficiency, which might be a useful technique in creating transgenic mouse models for pancreatic cancer.

5.2. Knockin and Knockout Mouse Models

In contrast to the transgenic mouse model, the knockin and knockout mice are created by targeted insertion or deletion of the gene at a desired locus, modifying the endogenous genomic sequences (Table 1). The most widely targeted oncogene in pancreatic cancer is K-Ras. The activation of K-Ras in GEM often utilizes the knockin technology. Tuveson and colleagues [61] targeted the expression of Kras^{G12D} to the MIST1 locus, a gene expressed at early stages of pancreatic exocrine development. These Mist1-Kras^{G12D/+} mice developed acinar-ductal metaplasia and hyperplasia, and when concomitant Trp53^{+/-} mutation was introduced, advance pathologic features and parenchymal liver metastases were observed.

However, due to the dependence of Kras^{G12D} on Mist1 promoter activity, it is difficult to distinguish the contribution of acinar-to-ductal metaplasia from confounding pancreatic neoplasia promoted by the expansion of Mist1⁺ progenitor cell.

In contrast to the transgenic mouse model where pronuclear injection is employed to introduce multiple copies of transgenes, both knockin and knockout mouse models utilize embryonic stem (ES) cell-based method so that the transgene can be either inserted or deleted in its native chromosomal location in a locus-specific manner. However, oftentimes gene knockout can lead to embryonic lethality if the targeted deletion is responsible for early development without proper spatial or temporal control. Although conventional knockin and knockout mouse models provide more specificity in terms of gene-targeting transgenesis compared to the transgenic mouse models, lack of spatial and temporal leads to the design of conditional and inducible models.

5.3. Conditional Knockin and Knockout Models

To overcome the limitations of universal expression, a transcriptional silencing element is placed between a promoter and the gene of interest so the mutated genes are silent during embryogenesis and early postnatal development. Conditional gene knockin provides a more sophisticated animal model in which the gene is activated in a tissue-specific manner (Table 1). The most widely used technique for conditional gene knockin is the Cre-Lox recombination system [62]. Regulation of transgene expression can be programmed in specific temporal patterns depending on the chosen Cre driver. Conditional knockin mouse models are used heavily on examining the role K-Ras plays in pancreatic cancer. In contrast to the basic knockin strategy seen in the Mist1-kras^{G12D/+} mouse model, conditional knockin models allow constitutive K-Ras expression under both temporal and spatial control and ensure the transcription of mutant K-Ras allele is controlled by the endogenous K-Ras promoter. Hingorani and colleagues [63] created the endogenous Kras^{G12D} expression model by crossing mice expressing a Cre-activated Kras^{G12D} allele with mice expressing Cre recombinase in pancreatic tissue. This Cre-induced activation of LSL-Kras^{G12D} resulted in rapid development of PanIN lesions in the first few weeks of life. Mice with a pancreas-specific deletion of INK4a/ARF or TP53 alone do not result in pancreatic neoplasia, but when combined with K-Ras, not only did the mice developed invasive PDAC by 7–11 weeks, the tumor was developed in a more aggressive fashion with metastasis, shorter latency and complete penetrance [64]. This implicates the role for K-Ras in tumor initiation and INK4a/ARF in tumor development and progression. Carriere and colleagues [65] constructed a conditional knockin mouse model based on Nestin-Cre; LSL-Kras^{G12D} to study the cell compartment in which oncogenic mutation takes place in PDAC. PanINs observed in the Nestin-Cre; LSL-Kras^{G12D} model displays PanIN virtually indistinguishable from those observed in the Pdx1-Cre; LSL-Kras^{G12D} model, suggesting Nestin-expressing cells could be the cells of origin for PDAC.

Most conditional tumor suppressor gene knockouts in the pancreas do not result in pre-cancer or cancer unless K-Ras is activated; however, Cre-mediated deletion of PI3K signaling pathway antagonist PTEN results in ductal metaplasia from centroacinar cells [66]. Some acinar cells are replaced with pseudoducts and this pTENflox knockout model develops both PanINs and ductal adenocarcinoma. Ijichi and colleagues [67] established the Kras^{G12D} expression plus Tgfbr2 knockout model to investigate the growth inhibitory effect of tumor suppressor TGF- β . The TGF- β signaling pathway is blocked by Tgfbr2 knockout and this model displays PanIN-like lesions with abundant desmoplastic stroma formation. Almost complete loss of normal pancreas structure was observed by 6–7 weeks of age. However, when the blockage of TGF- β signaling pathway is introduced by knocking out SMAD4, slightly downstream of tgfbr2, the models showed histological resemblance to that of intraductal papillary mucinous neoplasm (IPMN) or mucinous cystic neoplasm (MCN),

which both are considered to be a less aggressive pre-cancer lesions than PanIN [68, 69]. Mice with homozygous deletion of SMAD4 alone didn't display any substantial anatomical or physiological differences when the tumor-initiating K-Ras gene was not activated. The role of Hedgehog signaling pathway has been investigated using Pdx1-Cre; CLEG2 conditional knockin mouse model [70] by crossing CLEG2 transgenic mice with Pdx1-Cre mice, which results in epithelium-specific activation of the Hedgehog signaling pathway. Although pancreatic tumors are developed in these mouse models, they do not resemble PDAC histologically. When intercrossing Pdx1-Cre; CLEG2 mouse model with LSL-Kras^{G12D} mouse model, PanIN2 and PanIN3 lesions were developed but none progressed to PDAC. Interestingly, the Pdx1-Cre; CLEG2; LSL-Kras^{G12D} model displayed aberrant Hedgehog ligand expression, implicating PDAC development might depend on Hedgehog ligand signaling [71]. In addition to SHH signaling, the relationship of Notch signaling and K-Ras activation was investigated by crossing Notch-1 transgene, Rosa26NIC, along into K-Ras and Pdx1-Cre^{ERT} transgenes [72]. The histological analysis showed that the Pdx1-Cre^{ERT}; LSL-Kras^{G12D}; R26-Notch mouse model displaying accelerated development of acinar-derived PanIN, suggesting the possible role Notch signaling plays in sensitizing the pancreatic progenitor cells to K-Ras.

The conditional knockin and knockout mouse model was also employed to elucidate the key role of the NF- κ B signaling pathway in Ras-driven proinflammatory signals as well as tumorigenesis. To investigate the role of NF- κ B activation plays in PDAC development, Ling and colleagues [73] constructed mouse models in which they inactivate the pancreas-target IKK2/ β known to inhibit NF- κ B activation in Kras^{G12D} and Kras^{G12D}; Ink4a/Arf^{F/F} mice, generating the following mouse models Pdx1-Cre;Kras^{LSL-G12D};IKK2/ β ^{F/F} and Pdx1-Cre;Kras^{LSL-G12D};Ink4a/Arf^{F/F};IKK2/ β ^{F/F}. They found these mutants with inactivated IKK2/ β failed to develop PDAC, suggesting a causative role IKK2/ β plays in PDAC development. Using the same mouse models, they also demonstrated IKK2/ β /NF- κ B is activated by Kras^{G12D} through dual feed-forward loops of IL-1 α /p62.

5.4. Inducible Transgenic Mouse Models

The underlying problem with transgenic techniques is that the expression of transgene is regulated by the transgenic promoter rather than its native promoter. When regulated by the transgenic promoter, the transgene is usually expressed early in the development in contrast to the late onset human cancer development. To avoid the undesired outcome caused by the lack of control for the transgene activation, the inducible promoter system is added so that the transgene can be turned on at any given time (Table 1). Two most commonly used "inducible" GEM models include the tamoxifen-inducible Cre-ER system and the tetracycline-inducible Tet-ON/OFF system [74, 75]. In this inducible system, the gene of interest is inactivated through the binding of the chimeric Cre protein to heat-shock protein Hsp90. When the ligand tamoxifen binds to the Cre protein, Hsp90 is disassociated, allowing the unbound Cre to migrate into the nucleus. An example of inducible format using tamoxifen is the Mist1-Cre^{ERT}; LSL-Kras mice that only develop low-grade PanINs without further progression to PDAC [76]. Another tamoxifen-inducing mouse model Pdx1-Cre^{ERT}; LSL-Kras displayed ductal metaplasia with PanIN formation after tamoxifen binding. When Notch is activated in this model, it sensitizes Pdx-1 cells to Kras-driven mPanIN formation and progression.

Another commonly used inducible transgene system is based on the tet operon of *E. Coli*. The tetracycline-response system consists of a tet-controlled transcriptional activator (tTA) from the fusion of tetR and the herpes simplex virus VP16 transcriptional activation domain [77]. The tet operon is regulated by the tet repressor (tetR) that binds to the tet operator (tetO). Depending whether the inducible system is tetON or tetOFF, the presence of tetracycline can either activate or repress transcription respectively. Guerra and colleagues

[78] crossed the conditional LSL-Kras^{G12V} knockin strain with bitransgenic Ela-tTA/tetO-Cre mice that express the Cre recombinase in a tet-off system so that in the absence of doxycycline, the Elastase-driven Cre recombinase converts the silent LSL-Kras^{G12V} allele into the transcriptionally active Kras^{G12V} allele. In these inducible Ela-tTA/tetO-Cre; LSL-Kras^{G12V} mice, the ductal component shows high rates of cell division and some PanINs and ductal carcinoma are also observed.

6. Ras Activity and PDAC

The average time span required for developing parental non-metastatic founder cells from the start of mutation is at least a decade in humans [79]. It will take five more years for these founder cells to obtain their metastatic capacity and the patients usually die an average of two years after the onset of metastasis. However, generating mouse models mimicking the time frame of human tumorigenesis seems impractical. Most of the currently proposed mouse models were created by activating K-Ras at endogenous level from its native promoter and PDAC is induced from its precursor pancreatic intraepithelial neoplasia (PanIN) in the period of 1–2 years. Recently Ji and colleagues presented a new concept that a high level of Ras activity must be reached in order for cellular transformation to take place [80]. Elevated level of Ras can be achieved either by extrinsic Ras activators such as co-expression of TGF- α or CCK treatment, or, by increased expression of mutant K-Ras. To further investigate the effects of higher Ras activity, Ji and colleagues constructed a mouse model by inserting K-Ras^{G12V} after the CAG promoter, which consists of cytomegalovirus (CMV) early enhancer element and chicken beta-actin promoter, and after a loxp-GFP-loxp cassette (cLGL-Kras^{G12V}). Not only they observed a higher level of Ras activity in these strategically constructed mutant K-Ras mice, they also noticed that the elevated levels of Ras activity in acinar cells leads to rapid development of PanIN, cystic papillary carcinoma and finally, PDAC. This important study suggests that high level of Ras activity is the key for pancreatic tumorigenesis. Another intriguing point made by Ji and colleagues is that higher level of Ras activity also generates inflammation and fibrosis resembling the histological feature of chronic pancreatitis (CP) that may promote genetic instability, contributing to the loss of tumor suppressor genes. Their findings implicate that regardless of the time course of tumorigenesis or promoter used, the mouse models can closely mimic the natural development of human pancreatic cancer as long as high Ras activity is achieved above a certain threshold.

7. Genetically Engineered Mouse Models vs. Xenograft Mouse Models

Numerous mouse models have been developed over the last few decades in order to study human pancreatic cancer progression and to test various therapeutic strategies. In the earlier days, xenograft model was one of the most commonly used mouse models for cancer research. The human tumor cells can be injected either subcutaneously or orthotopically into the immunocompromised mice. The subcutaneous model is a great tool for tumor biology studies to assess the gene functions *in vivo* due to its low cost and many other advantages, however it seems to least resemble human condition. In orthotopic xenograft, the human pancreatic tumor cells are injected directly to the pancreas, the same organ that it originates from, its vascular system will better mimic the human tumor microenvironment compared with the subcutaneous model. But because of the lack for stromal cells and other supporting cells, the orthotopic model also has limitations. More recently, the humanized NOD/SCID mouse model is adopted by injection of peripheral blood or bone marrow cells [81]. Although this provides a more realistic microenvironment for the tumor cells, it will not fully re-establish the immune response due to the challenge posed by restoring HLA class I and Class II-selecting elements in T-cell populations [82]. Recently, a “direct xenograft” model, tumorgraft, has been developed, in which resected human pancreatic

tumor tissues are implanted in immunodeficient mice. This model not only better recapitulates the histopathological feature and induces stromal formation but also preserves tumor heterogeneity, therefore, representing a much better model to study gene function and interactions between tumor cells and the microenvironment. However, similar to the indirect xenograft model, immunodeficient mice are still required for tumorgrafts. In contrast to the xenograft mouse model, the GEM model can be used to study the genetic variations responsible for tumor initiation and progression. The genetically engineered mice are immunocompetent, imparting a more realistic tumor microenvironment for genetic abnormalities studies. Because tumor in GEM arises spontaneously from normal cells, this more human-like tumorigenesis progression can be closely monitored and tissues samples at different stages can be obtained for any further genetic and histological analysis, which makes it superb tool for identifying new markers and therapeutic targets for pancreatic cancer (Table 2).

No models are perfect for everything. Numerous efforts have been made to develop a mouse model that closely mimics the microenvironment and progression of human pancreatic cancer, which can be used to identify new markers and test new therapeutic drugs. Although there are lots of limitations of the xenograft model, it is still a great tool for studying specific gene functions and testing pre-clinical drugs in a large scale. The orthotopic xenograft model also has a unique advantage in establishing a resectable pancreatic cancer model to test the efficacy of surgical resection (distal pancreatectomy) and surgery-based combinational therapy [39]. Previous studies have used the orthotopic xenograft models to examine the perineural invasion, tumor recurrence, and pancreatic cancer stem cells as well [83–85]. Compared to the xenograft mouse models, the use of the more sophisticated GEM model has been a significant breakthrough in studying the tumor initiation, early progression, microenvironment and immune regulation. However, due to the genetic discrepancies between human and mouse, many pathological differences have been observed between the GEM models and humans. In humans, most pancreatic carcinomas form a single neoplastic focus, whereas in GEM models, pancreatic carcinomas show a pattern multi-focal growth spread [61]. Desmoplasia, a common feature of invasive ductal adenocarcinoma observed in humans, is rarely seen in most carcinomas in the GEM models. Another major difference is the frequent appearance of acinar-ductal metaplasia in the GEM models constructed with the Pdx1 or Ptf1a promoter. This acinar-ductal metaplasia can further progress into PanIN-like ductal neoplasia and eventually results in invasive PDAC. However, acinar-ductal metaplasia is rarely observed and infrequently leads to PanIN-like ductal metaplasia or invasive PDAC in humans. In contrast to human pancreatic tumor progression where K-Ras mutation takes place in the early stage before the accumulation of tumor suppressor genes loss, mouse models are engineered in the way that K-Ras mutation and tumor suppressor genes loss take place simultaneously. Furthermore, many promising new markers and therapeutic targets identified using the GEM models do not have human counterparts, and many important genes in human pancreatic cancer are not expressed in mice, which raised serious concerns about the feasibility of the GEM models in predicting new targets for human pancreatic cancer (Table 2). Bearing the differences mentioned above, further refinement of GEM models is much needed. Although there are limitations and differences in these GEM models, they have contributed great understanding in disease etiology and future therapeutic design.

8. Conclusions

Pancreatic cancer is among the most lethal cancers due to its rapid onset and resistance to therapeutic treatment. There has yet been a single effective treatment that can significantly increase the survival rate of pancreatic cancer patients. Many different mouse models have helped to identify the molecular changes contributing to pancreatic cancer initiation and

progression despite the differences in the genetic background between humans and mice. Currently, the xenograft model is still widely used to study the gene functions and test the pre-clinical drugs, while GEM model better recapitulates human pancreatic cancer and has been used extensively to investigate tumor initiation, progression and tumor microenvironment. The combination of GEM models and xenograft models hold potential in finding the answer to pancreatic cancer etiology and providing novel strategies to screen, prevent and treat human pancreatic cancer.

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Figure 1. The Key Mutations and Signaling Pathways in Pancreatic Ductal Adenocarcinoma Initiation and Progression

Pdx1 and Ptf1a are responsible for early pancreatic development that is kept in check by the Hedgehog and Notch signaling pathways. Nestin is a marker for exocrine pancreas progenitor and Mist1 is a basic-loop-helix required for normal development of acinar cells. Pdx1, ptf1a, Nestin and Mist1 all have been used to construct the promoter region for gene manipulation in Genetically Engineered Mouse (GEM) models. The acinar cells and insulin-positive endocrine cells undergo ductal reprogramming before developing into pancreatic intraepithelial neoplasia (PanIN). Activating mutation of oncogene K-Ras is thought to initiate tumor development while the mutations in several tumor suppressor genes are thought to contribute to tumor progression. The stepwise tumor progression, mutations of oncogenes and tumor suppressor genes, and aberrant expression of cancer master switch genes such as ZIP4 gradually accumulate throughout the stages of PanIN, contributing to the development of a more advanced and more invasive pancreatic ductal adenocarcinoma (PDAC).

Table 1**Transgenic and Genetically Engineered Mouse models of Pancreatic Cancer**

Mouse model	Change in phenotype	Reference
Transgenic Mouse Model		
Ela-Kras ^{G12D}	Develop precancerous lesions without progressing to invasive cancer	Richmond 2008 [45]
Ela-TGF- α	Acinar cell carcinoma	Schmid 1999 [49]
Ela-myc	Mixed acinar-ductal tumor with ductal metaplasia	Sandgren 1991 [46]
Ela-SV40Tag	Acinar cell carcinoma	Ornitz 1985 [43]
Ela-PyMT (RCAS TVA)	Mixed acinar-ductal tumors in Ink4a/Arf-null mice	Lewis 2003 [50]
Ela-myc (RCAS TVA)	Pancreatic endocrine neoplasm in Ink4A/Arf-null	Lewis 2003 [50]
Pdx1-SHH	Ductal-intestinal metaplasia	Thayer 2003 [55]
Knockin Mouse Model		
Mist1-Kras ^{G12D/+}	Acinar-ductal metaplasia and hyperplasia	Tuveson 2006 [58]
Conditional Knockin Mouse Model		
Pdx1-Cre; LSL-Kras ^{G12D}	Rapid development of PanIN lesions	Hingorani 2003 [60]
Nestin-Cre; LSL-Kras ^{G12D}	Develop exocrine-derived PanIN	Carriere 2007 [62]
Pdx1-Cre; CLEG2	Develop pancreatic tumors different from PDAC	Pasca 2006 [67]
Pdx1-Cre; CLEG2; LSL-Kras ^{G12D}	Accelerated PanIN development without progressing to PDAC	Pasca 2006 [67]
cLGL-Kras ^{G12V}	Rapid development of PanIN, cystic Papillary Carcinoma and PDAC	Ji 2009 [77]
Conditional Knockout Mouse Model		
Pdx1-Cre; Pten ^{-/-}	Ductal metaplasia; some develop PDAC	Stanger 2005 [63]
Pdx1-Cre; Smad4 ^{-/-}	no evidence of any gross anatomic or physiological abnormalities	Bardeesy 2006[66]
Combination of Conditional Knockin & Knockout Mouse Model		
Pdx1-Cre; LSL-Kras ^{G12D} ; Ink4a ^{-/-}	Accelerated development of PanIN	Aguirre 2003 [61]
Pdx1-Cre; LSL-Kras ^{G12D} ; Trp53 ^{-/-}	Accelerated PanIN and PDAC development	Aguirre 2003 [61]
Pdx1-Cre; LSL-Kras ^{G12D} ; Smad4 ^{-/-}	Develop IPMN and MCN	Ijichi 2006 [64]
p48-Cre; LSL-Kras ^{G12D} ; Tgfb2 ^{-/-}	Develop PanIN-like lesions with and desmoplasia	Ijichi 2006 [64]
Pdx1-Cre; LSL-Kras ^{G12D} ; IKK2/ β ^{-/-}	Failed to develop PDAC	Ling 2012 [70]
Pdx1-Cre; LSL-Kras ^{G12D} ; Ink4a/Arf ^{-/-} ; IKK2/ β ^{-/-}	Failed to develop PDAC	Ling 2012 [70]
Inducible Mouse Model		
Pdx1-Cre ^{ERT} ; LSL-Kras	Ductal metaplasia with PanIN formation	De La O 2008 [69]
Pdx1-Cre ^{ERT} ; LSL-Kras ^{G12D} ; R26-	Accelerated development of acinar-derived PanIN	De La O 2008 [69]
Mist1-Cre ^{ERT} ; LSL-Kras	Develop low-grade PanINs without further progression to	Shi 2009 [73]
Ela-tTA/tetO-Cre; LSL-Kras ^{G12V}	Develop acinar and centroacinar derived PanIN and PDAC	Guerra 2007 [75]

Table 2

Comparison of xenograft and GEM pancreatic cancer mouse models.

	Xenograft	GEM
Tumor initiation	No	Yes
Early Progression	No	Yes
Genetic Interactions	No	Yes
Microenvironment	No	Yes (mouse stroma)
Immune Regulation	No	Yes (mouse)
Spontaneous	No	Yes
Metastasis	Yes	Yes
Test specific gene functions	Yes	Yes
Chemoresistance	Yes	No
Biomarkers	Yes	No
Therapeutic Testing	Yes	No
Surgical Resection	Yes	No
Relapse	Yes	No
Primary Tumorgraft	Yes	No