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***In vitro* models of pancreatic cancer for translational oncology research**

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Abstract

Background—Pancreatic cancer is a disease of near uniform fatality and the overwhelming majority of patients succumb to their advanced malignancy within a few months of diagnosis. Despite considerable advances in our understanding of molecular mechanisms underlying pancreatic carcinogenesis, this knowledge has not yet been fully translated into clinically available treatment strategies that yield significant improvements in disease free or overall survival.

Objective—Cell line-based *in vitro* model systems provide powerful tools to identify potential molecular targets for therapeutic intervention as well as for initial pre-clinical evaluation of novel drug candidates. Here we provide a brief overview of recent literature on cell line-based model systems of pancreatic cancer and their application in the search for novel therapeutics against this vicious disease.

Conclusion—While *in vitro* models of pancreatic cancer are of tremendous value for genetic studies and initial functional screenings in drug discovery, they carry several immanent drawbacks and are often poor in predicting therapeutic response in humans. Therefore, in most instances they are successfully exploited to generate hypothesis and identify molecular targets for novel therapeutics, which are subsequently subject to further in-depth characterization using more advanced *in vivo* model systems and clinical trials.

Keywords

pancreatic cancer; cell line models; mouse models of pancreatic cancer; translational research; drug discovery

1. Introduction

Ductal adenocarcinoma of the pancreas (a.k.a. pancreatic cancer; the terms pancreatic cancer and pancreatic ductal adenocarcinoma will be used synonymously in the text) is an almost uniformly lethal malignancy, which accounts for an estimated 213,000 annual deaths worldwide and is the fourth most common cause of cancer-related mortality in the United States, with an overall median survival of less than 6 months ^{1, 2}. The five year survival rate is below 5% when combined for all stages, and <2% for the majority of cases that present at with advanced (metastatic) disease, which are amongst the most dismal seen in any human malignancy ¹.

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Ongoing research efforts over the past decade have led to significant advances in our understanding of the underlying etiological and molecular mechanisms facilitating pancreatic carcinogenesis. Nevertheless, this increase in knowledge has not yet been translated into clinically tangible advances in terms of novel therapeutic strategies with marked improvement of overall patient survival³.

Cell-line based *in vitro* model systems of pancreatic cancer provide powerful tools for discovery of molecular targets for novel therapeutics as well as for preclinical evaluation of drug candidates. A brief review of available *in vitro* models and its use and limitations in drug research is given in the following text.

2. *In vitro* culture of non-neoplastic pancreatic cells

The human pancreas is a complex organ consisting of several tissue compartments and as of to date we are still far from fully understanding all of the physiologic interactions underlying regulation of organ development and homeostasis, as well as those governing the development of malignant neoplasia. The question of the ‘cell of origin’ of pancreatic cancer has long been a controversial issue. While the traditional model, which is mostly based on morphologic similarities observed by light microscopy on histological specimens, suggests that pancreatic cancer arises from the ductal cell compartment, there is an opposing hypothesis suggesting that pancreatic cancer arises from transdifferentiated acinar cells^{4–6}. A variation of the latter theory is the concept of pancreatic cancer possibly arising from a yet to be defined population of pancreatic stem/progenitor cells, which some schools of thought believe might reside within the acinar cell compartment, or in centro-acinar cells^{7–9}. Establishment and *in vitro* culture of non-neoplastic pancreatic cells is of interest with regard to pancreatic cancer research in at least two aspects: firstly, it allows distinct examination of conditions regulating growth and differentiation of the respective distinct cell compartments in an isolated *in vitro* setting, as well as determination of the genetic alterations required for malignant transformation of these cells. Secondly, such non-neoplastic cells provide valuable controls in functional studies using novel experimental therapeutic approaches, specifically identifying therapeutic targets that cancer cells depend on in order to maintain a fully malignant phenotype, while exerting little, or ideally, no effects on these non-malignant cells.

2.1 Pancreatic ductal cell culture

Despite the immense relevance for pancreatic cancer research, surprisingly few cases of long-term propagated *in vitro* cultures of pancreatic ductal cells have been reported. This may be due to several factors, such as the relative scarcity of ductal cells in the human pancreas (<5% of the total pancreatic volume), a general lack of knowledge regarding physiologic properties regulating their growth and differentiation, thus hampering establishment of appropriate culture conditions, and the frequent occurrence of senescence in *in vitro* cultures of ductal cells, which often prevent successful long-term culture^{10–12}. Therefore, initially it has proven to be a challenge to propagate human pancreatic ductal cells in culture for more than 1–2 months^{13–15}.

Generation of two distinct models of epithelial cell lines that could readily be maintained in long term culture has been described, and these were generated either through immortalization by introduction of the human papillomavirus 16 gene E6E7 proteins, or by stable transfection with human telomerase reverse transcriptase (hTERT) and growth in a special culture medium containing epidermal growth factor (EGF)^{16–18}. Unfortunately, these immortalized lines do not represent ideal *in vitro* models of genuinely ‘normal’ human pancreatic ductal cells, since introduction of viral proteins abrogates the function of key tumor suppressor pathways like p53 or Rb, while the presence of EGF activates several oncogenic pathways, e.g. downstream of EGFR. Nevertheless, both of these cell lines lack key functional features of fully malignant

pancreatic cancer cells, e.g. anchorage independent growth in soft agar or the ability to engraft as tumors in athymic mice, and have thus been of tremendous value as controls in functional *in vitro* assays. Moreover, they have been successfully used in various *in vitro*-transformation models^{17, 19, 20} (see example in Figure 1).

2.2 Culture of acinar cells

While isolation and *in vitro* propagation of rat pancreatic acinar cells under special conditions has been described^{21, 22}, long-term culture of human or mouse pancreatic acinar cells has proven to be challenging²³. Short term (primary) explants of mouse acinar cells have been established and have been successfully utilized for a variety of *ex vivo* functional assays over their limited period (<7 days) of viability²⁴.

2.3 Culture of islet cells

Isolation of human islet cells has so far proved to be difficult and long-term survival under *in vitro* culture conditions has been limited^{25–31}. As observed with ductal cells, cultured islet cells undergo senescence in *in vitro* culture. However, as opposed to duct cells, immortalization by stable transfection techniques has so far been mostly unsuccessful in the case of islet cells²³.

3. Generation of pancreatic cancer cell lines

There are two general approaches for the generation of cell lines from human pancreatic cancers that are frequently used: Cell lines can either be directly established from primary patient-derived tumor tissue samples or from murine xenografts of human pancreatic cancers in athymic nude or SCID mice^{32–35}. The latter method carries the advantage that *ex vivo* passaging in mice is a relatively innocuous way to enrich the neoplastic cell compartment, while at the same time eliminating stromal components of human origin, thus enhancing the probability for successful establishment of a cancer cell line^{36, 37}. Addition of matrigel can further increase the take rate of xenografted tumor tissue samples³⁸. Disadvantages of this method include the requirement of significantly more time (propagation of a subcutaneous xenograft from primary human pancreatic cancer tissue samples typically requires some weeks up to several months), the chance of contamination with murine fibroblasts and the risk of acquiring additional genetic alterations during the period of *in vivo* growth as a murine xenograft.

Success rates for direct establishment of pancreatic cancer cell lines from primary tissues are generally relatively low, and appear to be only slightly higher when metastatic tumor tissues are used as starting material as opposed to primary tumor tissue samples^{35, 39–47}. Jaffee et al. systematically optimized a protocol for establishment of cell lines from primary pancreatic cancer tissues⁴⁸. In this study, the authors describe four key factors as being critical for successful *in vitro* establishment of pancreatic cancer cell lines: firstly, freshly harvested tissues have to be cut into fragments of one to five mm in diameter and digested by collagenase (300 units/mL) and hyaluronidase (200 units/mL) overnight on a shaker at 37 °C. Secondly, before initial plating stromal cells have to be removed from the digested samples as far as possible by two 20 min centrifugation steps at gravity. Plating of resuspended cells is then done at a density of 1–2 million viable cells per milliliter of fresh Panc media. Thirdly, for optimum growth of primary carcinoma cells RPMI-1640 base medium containing 15% of fetal bovine serum (FBS), 200 µM of L-glutamine, 1× non-essential amino acids solution and 1% sodium pyruvate has to be supplemented with human insulin (0.2 units/mL) and insulin-like growth factors (IGF) I and II (0.01 µg/mL each). Lastly, during the first weeks of *in vitro* culture stromal overgrowth has to be avoided and contaminating fibroblasts have to be eliminated by repeated differential

trypsinization. Using this optimized protocol, the authors report a success rate of ~30% for the establishment of pancreatic cancer cell lines from primary tissue samples.

In our experience complete purification of a primary cancer cell line from contaminating human (in the case of primary human tumor samples as starting material) or murine fibroblasts (in the case of xenograft tissues) can often be challenging despite repetitive differential trypsinization. It is therefore tempting to speculate, whether novel, improved techniques might become available in the near future that allow for reliable and more rapid purification of primary cell lines.

4. *In vitro* methods for translational research in pancreatic cancer

Pancreatic cancer is a complex genetic disease and the underlying genetic and functional alterations required during the multistep pancreatic cancer progression cascade are still not fully understood⁴⁹. Isolation of patient derived pancreatic cancer cell lines provides the only means available to date to accurately mimic the entirety of genomic alterations involved in the human disease in an *in vitro* model system that can be maintained in culture for more than a few days⁵⁰ (see⁵¹ for a review on molecular genetics of pancreatic cancer).

The use of cell line-based *in vitro* approaches for drug discovery in pancreatic cancer carries some obvious immanent shortcomings which often limit the applicability of such techniques. For example, while direct drug effects on the neoplastic cells themselves can generally be studied on cell lines in a relatively straightforward manner, the vast majority of known or supposed tumor-host interactions are difficult to impossible to accurately mimic in a purely cell culture-based system. These include - to various degrees - provision of a specific growth environment formed by the extracellular matrix of tumor stromal cells, tumor-associated neo-angiogenesis, secretion of cytokines and chemokines and possibly other growth-modulating factors by the tumor microenvironment as well as anti-neoplastic defense mechanisms conferred by the host immune system^{51, 52}.

A good example for possible shortcomings of cell culture based *in vitro* models as compared to animal models was given in a recent report by Curran and co-workers. While spontaneously developing medulloblastomas in *Ptch*^{+/-}; *p53*^{-/-} mice were dependent on Hedgehog-signaling and thus showed dramatic response to pharmacological Hedgehog blockade *in vivo*, no signs of Hedgehog pathway-activity or therapeutic response to Hedgehog-inhibition was observed by the same group in medulloblastoma cells after establishment as cell lines *in vitro*^{53, 54}. This was almost certainly as a result of the absence of tumor-stroma interactions in tissue culture models. Some of the key advantages and disadvantages of different *in vitro* and *in vivo* model systems of pancreatic cancer with respect to their application in drug discovery are summarized in Table 1.

4.1 *In vitro* models of tumor-stroma interactions

In humans, pancreatic cancer is characterized by a pronounced deposition of extracellular matrix components and proliferation of stromal cells, namely surrounding fibroblasts, commonly referred to as “desmoplastic reaction”⁵⁵. An increasing body of evidence suggests that this process is centrally involved in regulating neoplastic cell growth, invasiveness and metastatic spread and is thus of pivotal interest both for a deeper understanding of pathophysiological mechanisms governing pancreatic cancer progression as well as in order to identify processes that might be exploited as molecular targets for novel therapeutic approaches (see⁵⁶⁻⁵⁸ for more comprehensive recent reviews on the topic of tumor-stromal cell interactions in pancreatic cancer). A striking example for the importance of the tumor microenvironment in mediating cancer progression was recently given by studies reporting somatic mutations and copy number alterations within cancer-associated fibroblasts which

might be involved in promoting enhanced cancer cell growth^{59–66}. An ever growing number of secreted growth factors, cytokines and chemokines that are produced by stromal cells have been suggested to be pathophysiologically involved in pancreatic cancer progression, among them transforming growth factor-beta (TGF-beta), connective tissue growth factor (CTGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), nerve growth factor (NGF), leukemia inhibitory factor (LIF), oncostatin M, interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), chemokine (C-C motif) ligand 2 (CCL2), C-X-C chemokines CXCL1, CXCL2, CXCL8, CXCL12 and wingless-type MMTV integration site family members 1 and 3 (WNT1 and WNT3)^{56, 67–69}. Moreover, a growing body of evidence suggests that extracellular matrix components expressed by stromal cells can stimulate membrane-bound receptors on pancreatic cancer cells and mediate cell survival and motility⁵⁶.

A more recent finding is the observation that pancreatic stellate cells (PSC) are activated in pancreatic cancers and are potentially involved in modulating the malignant phenotype and inducing desmoplastic reaction^{70–74}. Cell culture models aimed at studying potential synergistic effects on *in vitro* growth and motility of pancreatic cancer cells – by either using co-culture techniques or conditioned media – have been tested, but their value and applicability for functional studies in translational research is only beginning to be well understood at present^{68, 75–77}.

4.2 Functional *in vitro* assays

Finally, and especially in the case of pancreatic cancer, metastatic dissemination is a major predictor of therapeutic success and overall survival in solid tumors. The complex cascade of metastatic spread involves several steps which a neoplastic cell has to overcome in order to form a metastatic tumor, including proliferation of neoplastic cells at the primary tumor site, active migration and invasion into surrounding tissue, intravasation into lymph or blood vessels, anchorage independent survival in the blood stream, accumulation and extravasation and autonomous growth at a distant organ site, and induction of neo-angiogenesis at the metastatic tumor site (Figure 2). To date, most of these steps are far from being sufficiently well understood on a pathophysiological level and are therefore difficult to simulate in an *in vitro* setting in a way that would allow to accurately mirror each and every of the biological aspects involved in regulating these events *in situ*^{78, 79}.

Of note, an ever growing number of *in vitro* assays has been developed, attempting to mimic some of the aspects of neoplastic tumor growth and dissemination named above. For example, net tumor cell growth can readily be assessed *in vitro* by direct cell counting or by colorimetric 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assays, proliferation can clearly be quantified by assays such as BrdU or tritium incorporation, immunolabeling for Ki67 and other surrogate markers of proliferation, CFSE assays etc., while a significant number of assays exists to accurately quantify different stages of apoptosis *in vitro* (e.g. DNA fragmentation, TUNEL, annexin binding, caspase activation assays, etc.)^{80–83}. Also, a number of *in vitro* assays exist which simulate and quantify some of the more complex steps pointed out above. Cell motility can readily be assessed *in vitro* by time-lapse observations under the microscope, wound healing or Boyden chamber assays; introduction of a layer of matrigel or other coating material can be used to mimic tumor cell invasion and breach through the epithelial basement membrane⁸⁴. Anchorage independent growth can be simulated *in vitro* by means of soft agar, methylcellulose or other three-dimensional culturing techniques⁸⁵, and re-plating of highly diluted cell suspensions and subsequent ability to form colonies has been used as a means to determine clonogenicity *in vitro*. Finally, various *in vitro* models of tube formation and angiogenesis are commonly used⁸⁶.

All of the above-mentioned assays have been utilized for assessing *in vitro*-effects of novel candidate drugs on these distinct central cell functions, and in many cases, a reasonable correlation between these *in vitro* assays and the *in vivo* scenario has been documented. Moreover, these *in vitro* assays enable scientists to specifically examine functional effects of manipulating single proteins by means of genetic techniques. Gene function can be specifically abrogated through gene knockout, RNAi-mediated knockdown of gene expression or introduction of dominant negative protein. Enhanced gene function on the other hand is readily achieved by forced overexpression or introduction of constitutively active mutant gene products. This approach can be applied to link specific genes and signaling pathways to distinct cellular functions involved in pancreatic carcinogenesis and metastatic spread, with the final goal being the identification of potential targets for the development of novel therapeutics⁸⁷. One promising *in vitro* screening method to emerge in recent times has been pharmacological synthetic lethal screening, wherein isogenic cell lines differing in a single gene product are screened against large scale chemical libraries to identify small molecules that are lethal only to that line in the isogenic pair which has a defective gene⁸⁸. The use of synthetic lethal screen was first employed in yeast to identify genetic pathways that are lethal to survival when mutated in concert, and its extrapolation to cancer cells as a tool for drug discovery was first proposed by Hartwell and colleagues in 1997⁸⁹. Recently, using such synthetic lethal screening of isogenic pancreatic cancer cell lines differing only in Dpc4 function, von Hoff and colleagues were able to isolate compounds that are specifically toxic to Dpc4- null cells, a finding with immediate translational significance given that 55% of pancreatic cancer lack Dpc4 function⁹⁰.

4.3 *In vitro* models for genetic studies

While these techniques are undoubtedly of immense value as screening tests to identify potential new drug targets, there are some obvious limitations of these cell culture-based *in vitro* model systems and the *in vivo* relevance of obtained *in vitro* results is often unclear⁹¹. Firstly, as mentioned above, it is almost impossible to accurately mimic the complex network of tumor-host interactions in its entirety. This crosstalk includes direct effects conferred by the physical interaction of neoplastic cells with the extracellular matrix of surrounding stromal tissue, humoral factors, e.g. secretion of cytokines and growth factors by host cells, and effects mediated by the host immune system. Secondly, and possibly more importantly, pancreatic cancer cells might progressively alter genetically over time with increasing duration of *in vitro* culture.

There are various reports demonstrating that pancreatic cancer cells cultured *ex vivo* as xenografts or as cell lines *in vitro* accurately mirror the genetic setup and dependence on known oncogenic signaling pathways of their parent tumors and remain fairly stable over time^{36, 92}. However, there are also several lines of evidence suggesting that additional genetic alterations might in fact occur during long-term *in vitro* propagation of pancreatic cancer cells.

Firstly, from a theoretical point of view it seems likely that pancreatic cancer cell lines might not have the ability to maintain their genetic fidelity over longer periods of time. Genomic instability is a key feature observed in almost all cases of pancreatic cancer and is among the earliest aberrations found during pancreatic carcinogenesis^{93–96}. Therefore, under cell culture conditions optimized for maximum survival of neoplastic cells and in the absence of other selection mechanisms usually found in the *in vivo* situation (i.e. lack of immune-surveillance, absence of anatomical barriers etc.) there might likely be a greater tendency towards acquiring additional, heterogeneous genetic alterations.

Secondly, there are also various lines of empirical evidence suggesting that accumulation of additional genetic changes might in fact occur in pancreatic cancer cell lines with increasing passage numbers. In a recent global sequence analysis study on 24 cases of pancreatic cancer

covering the majority of genes represented in the RefSeq database, Jones et. al found an average of 48 somatic mutations per pancreatic cancer sample (ranging from less than 30 to over 140 mutations in individual samples) ⁴⁹. While this number is significantly lower than the average number of mutations found in breast (on average 101 per tumor) and colorectal cancers (77 per tumor) using a similar approach ⁹⁷, it is still by far higher than what might have been expected based on *in vitro* and *in vivo* transformation experiments (Table 2).

For example, in a recently described genetically engineered mouse model of pancreatic cancer it was found, that introduction of one single genetic alteration, i.e. pancreas-specific overexpression of an oncogenic Kras^{G12D} allele, was sufficient to induce formation of murine pancreatic intraepithelial neoplasia (mPanIN) lesions. In two of 29 mice followed longitudinally (i.e. less than 10%) these precursor lesions progressed into frank malignancy after 6 and 8 months, respectively ⁹⁸. In this particular model, the long latency and low frequency of actual cancers suggest, that additional mutations must occur in order to result in a fully malignant phenotype, whereas the majority of Kras^{G12D} expressing cells might undergo ras-induced senescence and be eliminated, thereby failing to accumulate additional genetic alterations required for complete malignant transformation ⁹⁹. These observations are in line with data from our own group showing that expression of Kras^{G12D} is sufficient to generate mPanIN lesions in adult mouse pancreata when targeted under the control of tamoxifen-inducible Ela-Cre^{ERT2} or Mist1-Cre^{ERT2} constructs ⁷. However, progression to fully malignant pancreatic cancer was not observed in this model during 12 months of follow-up.

A later report by Hingorani et al. showed that additional expression of a dominant negative form of the tumor suppressor gene p53 (Trp53^{R172H}) under the control of Pdx1-Cre led to development of moderately to well-differentiated metastatic pancreatic cancers with a median survival of 5 months and 100% lethality after 12 months of follow-up ¹⁰⁰. As observed in pancreata overexpressing Kras^{G12D} alone, development of pancreatic cancer in this latter model was preceded by the appearance occurrence of mPanIN lesions. Of note, LOH of the p53 gene locus was reproducibly observed in cell lines derived from murine pancreatic cancers in this model system, but expression analysis and direct sequencing did not show any evidence for alterations in the Cdkn2/Ink4a, Cdk4 or Smad4 signaling pathways ¹⁰⁰.

These data are in line with another report of specific expression of Kras^{G12D} and concomitant abrogation of p53 expression by Cre-mediated excision under the control of Pdx1-Cre ¹⁰¹. In this model, pancreatic adenocarcinomas occurred with an average latency of 6.2 weeks. In yet another example, DePinho and colleagues reported that pancreas-specific targeting of oncogenic Kras^{G12D} expression in combination with abrogated Ink4a/Arf (murine p16 and p19) function by crossing on a Pdx1-Cre genetic background led to the rapid formation of poorly differentiated pancreatic cancers ¹⁰². These mice reproducibly die of metastatic disease between 7 to 11 weeks of age ¹⁰³. Similarly, pancreas-specific Kras^{G12D} expression in combination with targeted silencing of transforming growth factor beta-signaling by specific Tgfr2- knockout led to the development of well-differentiated ductal adenocarcinomas in the murine pancreata with 100% penetrance and a short median survival of only 59 days ¹⁰⁴.

While all of the above mentioned transformation models were done in mice, i.e. in a different species and conclusions drawn from these models can therefore be applied to the human disease only with some caution ^{105, 106}, there are nevertheless also some *in vitro* transformation models of human cells suggesting that the number of genetic alterations required for malignant transformation might actually be considerably smaller than the average of 48 alterations observed by global direct sequencing ⁴⁹.

In a recent study, Campbell and colleagues found that only 4 genetic alterations are necessary for *in vitro* transformation of immortalized human pancreatic epithelial cells (HPNE) 20•

107. hTERT-HPNE cells are immortalized through ectopic expression of the catalytic subunit of telomerase (hTERT) 108–110. Upon subsequent stable transfection of the human papillomavirus 16-derived genes E6 and E7 (E6/E7), constitutively active Kras^{G12D} and SV40 small t (st) antigen, HPNE cells readily showed colony formation and anchorage independent growth, as well as spontaneous migration and invasion into a matrigel layer in modified Boyden chamber assays, in line with acquisition of a malignant phenotype.

Using a similar experimental approach, Tsao and colleagues found that isolated non-neoplastic human pancreatic ductal epithelial cells, which had previously been immortalized by transfection with the human papillomavirus 16 genes E6 and E7 (E6/E7) 16–17, showed xenograft tumor formation upon subcutaneous injection of one million cells subcutaneously into SCID mice in 4/7 cases (57%) and in 2/5 cases (40%) upon orthotopic (intrapancratic) injection of two million cells 19. Curiously, these cells did not shown signs of *in vitro* transformation, specifically no colony formation was observed in soft agar assays.

Taken together, these observations suggest that the actual number of genetic alterations required for pancreatic carcinogenesis in humans might be considerably smaller than the average of 48 alterations found in the global sequencing approach by Jones et. al 49. Not only is this of immense interest for our understanding of underlying pathophysiological mechanisms leading to the development of pancreatic cancer, but this idea also holds extremely promising implications for the identification of molecular targets for therapeutic intervention. Despite the lack of clinically tangible progress in the development of novel therapeutic strategies for pancreatic cancer over the last decades, it seems likely that in the end pancreatic cancer is caused by only a limited number of defined genetic alterations, many or most of which are already known to date and hence readily accessible for the development of novel therapeutics.

5. Expert opinion

From the discussion above, it is fair to conclude that panels of freshly generated low-passage pancreatic cancer cell lines mirror genetic features and response to therapeutic interventions with better fidelity than cell lines which have been cultured *in vitro* for longer periods of time 36, 47, 48.

In the case of pancreatic cancer, a significant limitation for the use of *in vitro* models for translational research and drug discovery is the lack of cell line models for pancreatic cancer precursor lesions. In recent years it has increasingly become commonly accepted that virtually all cases of pancreatic cancer arise via the development of tangible and genetically defined precursor lesions, including pancreatic intraepithelial neoplasia (PanIN), mucinous cystic neoplasms (MCN) and intraductal papillary mucinous neoplasms (IPMN) 111–112. Better understanding of the genetics of these precursor lesions has direct implications for early detection as well as for secondary prophylaxis. Moreover, studying the genetics of precursor lesions allows us to separate “early” (disease initiating) alterations from “late” (disease promoting) changes. While nearly all or majority of genetic changes in precursors are likely to be “driver” lesions, many of the genetic alterations in frank malignancies are likely to be “passenger” mutations, acquired as a result of progressive genetic instability.

While genetically engineered mouse models mimicking mPanINs 98, MCNs 113, 114 and IPMNs 115 could be successfully developed in recent years, long term *in vitro* cultures of the human counterparts of these precursor lesions are currently not available. The lack of such cell line models seems to be most likely due to technical difficulties. For example, PanIN lesions are, by definition, microscopic in nature and nearly impossible to localize macroscopically, such that enrichment for these cells through *ex vivo* passage in mice is not an option with currently available techniques. Moreover, due to their likely limited growth potential, the ability of PanINs to engraft and be serially propagated in immunocompromised mice is also

questionable. It remains an interesting point of speculation, whether 17 or not improvements of *ex vivo* and *in vitro* culturing techniques will enable generation of such cell line models of pancreatic cancer precursor lesions in the near future.

One can conclude from the mentioned points that although cell line models are undoubtedly of immense value for drug discovery in translational research related to pancreatic cancer, the use of animal models is nevertheless indispensable for a more concise preclinical evaluation of novel drug candidates. This is even more so, since acquisition of toxicity data and estimation of adverse effect profiles can only be done using test animals in a preclinical setting. Estimation of toxicity profiles based on cell line data alone is almost uniformly less useful.

Therefore, the main scope for the use of cell line based models remains in the early phase of drug discovery, wherein rapid, repetitive screening of large numbers of candidate substances, often in an automated or semi-automated manner, is required. As opposed to the use of experimental animal models, cell lines enable to cheaply and rapidly obtain virtually unlimited amounts of cancer cells and are therefore often ideal for such screening setups.

Also, at an early phase of drug discovery, where a direct molecular target or oncogenic signaling pathway has already been identified, genetically manipulated cell line models can often be used successfully for high-throughput efficacy screening *in vitro*. Candidate small molecules with satisfactory efficacy can then undergo further biological testing, including assessment of toxicity profiles, *in vivo* pharmacokinetics and pharmacodynamics in animal models.

A recent example for this approach is the search for small-molecule Hedgehog inhibitors as novel cancer drug candidates. Aberrantly re-activated Hedgehog signaling has been described in basal cell carcinomas¹¹⁶, medulloblastomas^{117, 118} as well as in various cancers of the gastrointestinal¹¹⁹ and respiratory tract^{120, 121}, including pancreatic cancer^{119, 122}. Pharmacological blockade of Hedgehog signaling by means of the plant alkaloid cyclopamine^{123, 124} has been identified as valid experimental therapeutic approach in preclinical *in vivo* and *in vitro* model systems^{117, 121}. However, cyclopamine itself is not an ideal drug candidate for clinical application due to its poor water solubility and bioavailability as well as a relatively low affinity to Smo. Therefore, cell based high-throughput screens have been used to identify other small molecule Hedgehog inhibitors with more favorable pharmacokinetics and – dynamics that are more suitable as drug candidates¹²⁵. The *in vitro* model system for this screen consisted of C3H.10T1/2 cells stably transfected with a Hh-responsive (Gli_{8x}-luciferase) reporter construct, and a lipid-modified form of Hedgehog-ligand with enhanced potency which had an octyl moiety attached to its N-terminus (Shh^{OCT})¹²⁶ was used for basal induction of Hedgehog pathway activity. This reporter cell line was assayed against a library of ~100,000 small synthetic organic molecules. One small molecule Hedgehog-inhibitor thus identified, designated Hh-Antag691¹²⁷, inhibited pathway activity at ~10fold lower concentrations as compared to cyclopamine and was subsequently shown to inhibit medulloblastoma growth in a genetically engineered Ptch1^{+/-} p53^{+/-} mouse model⁵⁴. Similar reporter systems used in other studies included stably transfected NIH-3T3 cells and a palmitoyl- and cholesteryl-modified ShhN polypeptide (ShhN)^{128, 129}. Of note, these examples also unmasked some of the inherent drawbacks of this approach for drug discovery, namely that pharmacokinetics, *in vivo* efficacy and toxicity profiles in humans are often difficult to estimate based on the initial preclinical data alone. Moreover, potential off-target-effects of a given substance in the *in vivo* situation can greatly affect its suitability as a drug candidate – either by conferring undesirable adverse effects or by contributing additional but unexpected favorable effects on tumor cell survival. Therefore, in scenarios like the one mentioned here, it is quite common that drug candidates which performed well in the initial discovery screens and early preclinical characterization phases fail at later stages during preclinical and clinical development, so that they need to be modified in chemical composition

and reevaluated or completely abandoned from further development towards clinical application. Only a minority of candidate substances identified in discovery screens can be developed all the way towards successful application as novel drugs in the clinical arena. In the case of Hedgehog-Antagonists described here follow-up drug candidate small molecule Hedgehog-inhibitors are currently evaluated in clinical phase I and II trials in various malignant solid tumors including pancreatic cancer (www.clinicaltrials.gov) and others are likely to follow in the near future.

Another example demonstrating the amount of tedious work and time-consuming steps involved in translating an identified molecular target into clinical application as novel therapeutic strategy is given by attempts to target tumor-neoangiogenesis in pancreatic cancer as well as other solid tumors over recent years. While various studies reported striking successes of such strategies in mouse models of various cancers beginning over a decade ago ^{130–135}, drugs resulting from these efforts have only recently been introduced into the clinical arena and have so far shown overall disappointing results in terms of improving overall survival of pancreatic cancer patients, although additional evaluations are still ongoing ¹³⁶ (www.clinicaltrials.gov).

Additional examples of signaling pathways recently identified to be pathogenetically involved in genesis and maintenance of pancreatic cancer and thus representing potential targets for the development of novel therapeutics, for which similar screening and preclinical evaluation strategies are being followed include the Notch ^{137–140}, Wnt ^{141, 142}, TGF-beta ¹⁴³, EGF ^{144–146}, Raf/Mek/Erk ^{147, 148}, PI3K/Akt ¹⁴⁹ and mTOR ^{150–153} signaling pathways.

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Declaration of interests

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Bibliography

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008 Mar–Apr;58(2):71–96. [PubMed: 18287387]
2. ACS. Cancer Facts & Figures 2008. Atlanta: American Cancer Society; 2008.
3. Carpelan-Holmstrom M, Nordling S, Pukkala E, Sankila R, Luttges J, Kloppel G, et al. Does anyone survive pancreatic ductal adenocarcinoma? A nationwide study re-evaluating the data of the Finnish Cancer Registry. *Gut* 2005 Mar;54(3):385–7. [PubMed: 15710987]
4. Jamieson JD. Prospectives for cell and organ culture systems in the study of pancreatic carcinoma. *J Surg Oncol* 1975;7(2):139–41. [PubMed: 1097831]
5. Longnecker DS, Wiebkin P, Schaeffer BK, Roebuck BD. Experimental carcinogenesis in the pancreas. *Int Rev Exp Pathol* 1984;26:177–229. [PubMed: 6400513]
6. Hall PA, Lemoine NR. Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas. *J Pathol* 1992 Feb;166(2):97–103. [PubMed: 1373187]
7. Habbe N, Shi G, Meguid RA, Fendrich V, Esni F, Chen H, et al. Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc Natl Acad Sci U S A* 2008 Dec 2;105(48):18913–8. [PubMed: 19028870]
8. Park SW, Davison JM, Rhee J, Hruban RH, Maitra A, Leach SD. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. *Gastroenterology* 2008 Jun;134(7):2080–90. [PubMed: 18549880]
9. Murtaugh LC, Leach SD. A case of mistaken identity? Noductal origins of pancreatic “ductal” cancers. *Cancer Cell* 2007 Mar;11(3):211–3. [PubMed: 17349578]

10. Githens S. Pancreatic duct cell cultures. *Annu Rev Physiol* 1994;56:419–43. [PubMed: 8010746]
11. Githens S. The pancreatic duct cell: proliferative capabilities, specific characteristics, metaplasia, isolation, and culture. *J Pediatr Gastroenterol Nutr* 1988 Jul–Aug;7(4):486–506. [PubMed: 2456383]
12. Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY, Aye T, et al. The pancreatic ductal epithelium serves as a potential pool of progenitor cells. *Pediatric diabetes* 2004;5 Suppl 2:16–22. [PubMed: 15601370]
13. Jones RT, Barrett LA, van Haaften C, Harris CC, Trump BF. Carcinogenesis in the pancreas. I. Long-term explant culture of human and bovine pancreatic ducts. *J Natl Cancer Inst* 1977 Mar;58(3):557–65. [PubMed: 557115]
14. Oda D, Savard CE, Nguyen TD, Swenson ER, Lee SP. Culture of human main pancreatic duct epithelial cells. *In Vitro Cell Dev Biol Anim* 1998 Mar;34(3):211–6. [PubMed: 9557938]
15. Trautmann B, Schlitt HJ, Hahn EG, Lohr M. Isolation, culture, and characterization of human pancreatic duct cells. *Pancreas* 1993 Mar;8(2):248–54. [PubMed: 8460098]
16. Furukawa T, Duguid WP, Rosenberg L, Viallet J, Galloway DA, Tsao MS. Long-term culture and immortalization of epithelial cells from normal adult human pancreatic ducts transfected by the E6E7 gene of human papilloma virus 16. *Am J Pathol* 1996 Jun;148(6):1763–70. [PubMed: 8669463]
17. Ouyang H, Mou L, Luk C, Liu N, Karaskova J, Squire J, et al. Immortal human pancreatic duct epithelial cell lines with near normal genotype and phenotype. *Am J Pathol* 2000 Nov;157(5):1623–31. [PubMed: 11073822]
18. Lee KM, Yasuda H, Hollingsworth MA, Ouellette MM. Notch 2-positive progenitors with the intrinsic ability to give rise to pancreatic ductal cells. *Lab Invest* 2005 Aug;85(8):1003–12. [PubMed: 15924149]
19. Qian J, Niu J, Li M, Chiao PJ, Tsao MS. In vitro modeling of human pancreatic duct epithelial cell transformation defines gene expression changes induced by K-ras oncogenic activation in pancreatic carcinogenesis. *Cancer Res* 2005 Jun 15;65(12):5045–53. [PubMed: 15958547]
20. Campbell PM, Lee KM, Ouellette MM, Kim HJ, Groehler AL, Khazak V, et al. Ras-driven transformation of human nestin-positive pancreatic epithelial cells. *Methods Enzymol* 2008;439:451–65. [PubMed: 18374182]
21. Bendayan M, Dühr MA, Gingras D. Studies on pancreatic acinar cells in tissue culture: basal lamina (basement membrane matrix promotes three-dimensional reorganization. *Eur J Cell Biol* 1986 Oct;42(1):60–7. [PubMed: 3792342]
22. Longnecker DS, Lilja HS, French J, Kuhlmann E, Noll W. Transplantation of azaserine-induced carcinomas of pancreas in rats. *Cancer Lett* 1979 Aug;7(4):197–202. [PubMed: 509403]
23. Ulrich AB, Schmied BM, Standop J, Schneider MB, Pour PM. Pancreatic cell lines: a review. *Pancreas* 2002 Mar;24(2):111–20. [PubMed: 11854615]
24. Esni F, Miyamoto Y, Leach SD, Ghosh B. Primary explant cultures of adult and embryonic pancreas. *Methods Mol Med* 2005;103:259–71. [PubMed: 15542912]
25. Hober C, Benhamou PY, Watt PC, Watanabe Y, Nomura Y, Stein E, et al. A new culture method for human pancreatic islets using a biopore membrane insert. *Pancreas* 1997 Mar;14(2):199–204. [PubMed: 9057194]
26. Kenmochi T, Miyamoto M, Une S, Nakagawa Y, Moldovan S, Navarro RA, et al. Improved quality and yield of islets isolated from human pancreata using a two-step digestion method. *Pancreas* 2000 Mar;20(2):184–90. [PubMed: 10707935]
27. Lucas-Clerc C, Massart C, Campion JP, Launois B, Nicol M. Long-term culture of human pancreatic islets in an extracellular matrix: morphological and metabolic effects. *Molecular and cellular endocrinology* 1993 Jul;94(1):9–20. [PubMed: 8375579]
28. Yuan S, Rosenberg L, Paraskevas S, Agapitos D, Duguid WP. Transdifferentiation of human islets to pancreatic ductal cells in collagen matrix culture. *Differentiation* 1996 Oct;61(1):67–75. [PubMed: 8921586]
29. Beattie GM, Itkin-Ansari P, Cirulli V, Leibowitz G, Lopez AD, Bossie S, et al. Sustained proliferation of PDX-1+ cells derived from human islets. *Diabetes* 1999 May;48(5):1013–9. [PubMed: 10331405]
30. Lu J, Gu YP, Xu X, Liu ML, Xie P, Song HP. Adult islets cultured in collagen gel transdifferentiate into duct-like cells. *World J Gastroenterol* 2005 Jun 14;11(22):3426–30. [PubMed: 15948249]

31. Murray HE, Paget MB, Bailey CJ, Downing R. Sustained insulin secretory response in human islets co-cultured with pancreatic duct-derived epithelial cells within a rotational cell culture system. *Diabetologia*. 2009 Jan 8;
32. Mueller BM, Reisfeld RA. Potential of the scid mouse as a host for human tumors. *Cancer Metastasis Rev* 1991 Oct;10(3):193–200. [PubMed: 1764764]
33. Pantelouris EM. Absence of thymus in a mouse mutant. *Nature* 1968 Jan 27;217(5126):370–1. [PubMed: 5639157]
34. van Weerden WM, Romijn JC. Use of nude mouse xenograft models in prostate cancer research. *Prostate* 2000 Jun 1;43(4):263–71. [PubMed: 10861745]
35. Ikeda Y, Ezaki M, Hayashi I, Yasuda D, Nakayama K, Kono A. Establishment and characterization of human pancreatic cancer cell lines in tissue culture and in nude mice. *Jpn J Cancer Res* 1990 Oct; 81(10):987–93. [PubMed: 2172194]
36. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, et al. An in vivo platform for translational drug development in pancreatic cancer. *Clin Cancer Res* 2006 Aug 1;12(15):4652–61. [PubMed: 16899615]
37. Walter K, Eshleman J, Goggins M. Xenografting and harvesting human ductal pancreatic adenocarcinomas for DNA analysis. *Methods Mol Med* 2005;103:103–11. [PubMed: 15542900]
38. Pretlow TG, Delmoro CM, Dilley GG, Spadafora CG, Pretlow TP. Transplantation of human prostatic carcinoma into nude mice in Matrigel. *Cancer Res* 1991 Jul 15;51(14):3814–7. [PubMed: 2065335]
39. Elsasser HP, Lehr U, Agricola B, Kern HF. Establishment and characterisation of two cell lines with different grade of differentiation derived from one primary human pancreatic adenocarcinoma. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1992;61(5):295–306. [PubMed: 1348891]
40. Kobari M, Hisano H, Matsuno S, Sato T, Kan M, Tachibana T. Establishment of six human pancreatic cancer cell lines and their sensitivities to anti-tumor drugs. *Tohoku J Exp Med* 1986 Nov;150(3): 231–48. [PubMed: 3547771]
41. Bleday R, Tzanakakis GN, Schwalke MA, Wanebo HJ, Vezeridis MP. Epidermal growth factor stimulation and metastatic rate in human pancreatic carcinoma cell lines. *J Surg Res* 1990 Sep;49(3): 276–9. [PubMed: 2395372]
42. Silberberg JM, Gordon S, Zucker S. Identification of tissue factor in two human pancreatic cancer cell lines. *Cancer Res* 1989 Oct 1;49(19):5443–7. [PubMed: 2548721]
43. Kyriazis AP, McCombs WB 3rd, Sandberg AA, Kyriazis AA, Sloane NH, Lepera R. Establishment and characterization of human pancreatic adenocarcinoma cell line SW-1990 in tissue culture and the nude mouse. *Cancer Res* 1983 Sep;43(9):4393–401. [PubMed: 6871872]
44. Chang BK, Gutman R, Chou TC. Schedule-dependent interaction of alphasdifluoromethylornithine and cis-diamminedichloroplatinum(II) against human and hamster pancreatic cancer cell lines. *Cancer Res* 1987 May 1;47(9):2247–50. [PubMed: 3105873]
45. Elsasser HP, MacDonald R, Dienst M, Kern HF. Characterization of a transglutaminase expressed in human pancreatic adenocarcinoma cells. *Eur J Cell Biol* 1993 Aug;61(2):321–8. [PubMed: 7901019]
46. Suwa H, Yoshimura T, Yamaguchi N, Kanehira K, Manabe T, Imamura M, et al. K-ras and p53 alterations in genomic DNA and transcripts of human pancreatic adenocarcinoma cell lines. *Jpn J Cancer Res* 1994 Oct;85(10):1005–14. [PubMed: 7961102]
47. Lieber M, Mazzetta J, Nelson-Rees W, Kaplan M, Todaro G. Establishment of a continuous tumor-cell line (panc-1) from a human carcinoma of the exocrine pancreas. *Int J Cancer* 1975 May 15;15 (5):741–7. [PubMed: 1140870]
48. Jaffee EM, Schutte M, Gossett J, Morsberger LA, Adler AJ, Thomas M, et al. Development and characterization of a cytokine-secreting pancreatic adenocarcinoma vaccine from primary tumors for use in clinical trials. *Cancer J Sci Am* 1998 May–Jun;4(3):194–203. [PubMed: 9612602]
49. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008 Sep 26;321(5897): 1801–6. [PubMed: 18772397]
50. Moore PS, Sipos B, Orlandini S, Sorio C, Real FX, Lemoine NR, et al. Genetic profile of 22 pancreatic carcinoma cell lines. Analysis of K-ras, p53, p16 and DPC4/Smad4. *Virchows Arch* 2001 Dec;439 (6):798–802. [PubMed: 11787853]

51. Feldmann G, Maitra A. Molecular genetics of pancreatic ductal adenocarcinomas and recent implications for translational efforts. *J Mol Diagn* 2008 Mar;10(2):111–22. [PubMed: 18258927]
52. Grippo PJ, Sandgren EP. Modeling pancreatic cancer in animals to address specific hypotheses. *Methods Mol Med* 2005;103:217–43. [PubMed: 15542910]
53. Sasai K, Romer JT, Lee Y, Finkelstein D, Fuller C, McKinnon PJ, et al. Shh pathway activity is down-regulated in cultured medulloblastoma cells: implications for preclinical studies. *Cancer Res* 2006 Apr 15;66(8):4215–22. [PubMed: 16618744]
54. Romer JT, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, et al. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in *Ptc1(+/-)p53(-/-)* mice. *Cancer Cell* 2004 Sep;6(3):229–40. [PubMed: 15380514]
55. Maitra A, Hruban RH. Pancreatic cancer. *Annual review of pathology* 2008;3:157–88.
56. Mahadevan D, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. *Mol Cancer Ther* 2007 Apr;6(4):1186–97. [PubMed: 17406031]
57. Kleeff J, Beckhove P, Esposito I, Herzig S, Huber PE, Lohr JM, et al. Pancreatic cancer microenvironment. *Int J Cancer* 2007 Aug 15;121(4):699–705. [PubMed: 17534898]
58. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. *Annual review of pathology* 2006;1:119–50.
59. Qiu W, Hu M, Sridhar A, Opeskin K, Fox S, Shipitsin M, et al. No evidence of clonal somatic genetic alterations in cancer-associated fibroblasts from human breast and ovarian carcinomas. *Nat Genet* 2008 May;40(5):650–5. [PubMed: 18408720]
60. Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nat Genet* 2002 Nov;32(3):355–7. [PubMed: 12379854]
61. Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancers reveals genotypic heterogeneity. *Cancer Res* 1998 Jan 1;58(1):23–8. [PubMed: 9426051]
62. Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res* 2000 May 1;60(9):2562–6. [PubMed: 10811140]
63. Weber F, Shen L, Fukino K, Patocs A, Mutter GL, Caldes T, et al. Total-genome analysis of BRCA1/2-related invasive carcinomas of the breast identifies tumor stroma as potential landscaper for neoplastic initiation. *Am J Hum Genet* 2006 Jun;78(6):961–72. [PubMed: 16685647]
64. Fukino K, Shen L, Matsumoto S, Morrison CD, Mutter GL, Eng C. Combined total genome loss of heterozygosity scan of breast cancer stroma and epithelium reveals multiplicity of stromal targets. *Cancer Res* 2004 Oct 15;64(20):7231–6. [PubMed: 15492239]
65. Fukino K, Shen L, Patocs A, Mutter GL, Eng C. Genomic instability within tumor stroma and clinicopathological characteristics of sporadic primary invasive breast carcinoma. *Jama* 2007 May 16;297(19):2103–11. [PubMed: 17507346]
66. Tuhkanen H, Anttila M, Kosma VM, Heinonen S, Juhola M, Helisalmi S, et al. Frequent gene dosage alterations in stromal cells of epithelial ovarian carcinomas. *Int J Cancer* 2006 Sep 15;119(6):1345–53. [PubMed: 16642473]
67. Lohr M, Schmidt C, Ringel J, Kluth M, Muller P, Nizze H, et al. Transforming growth factor-beta1 induces desmoplasia in an experimental model of human pancreatic carcinoma. *Cancer Res* 2001 Jan 15;61(2):550–5. [PubMed: 11212248]
68. Shek FW, Benyon RC, Walker FM, McCrudden PR, Pender SL, Williams EJ, et al. Expression of transforming growth factor-beta 1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis. *Am J Pathol* 2002 May;160(5):1787–98. [PubMed: 12000730]
69. Sato N, Maehara N, Goggins M. Gene expression profiling of tumor-stromal interactions between pancreatic cancer cells and stromal fibroblasts. *Cancer Res* 2004 Oct 1;64(19):6950–6. [PubMed: 15466186]
70. Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Invest* 2007 Jan;117(1):50–9. [PubMed: 17200706]
71. Schneiderhan W, Diaz F, Fundel M, Zhou S, Siech M, Hasel C, et al. Pancreatic stellate cells are an important source of MMP-2 in human pancreatic cancer and accelerate tumor progression in a murine xenograft model and CAM assay. *J Cell Sci* 2007 Feb 1;120(Pt 3):512–9. [PubMed: 17227797]

72. Erkan M, Kleeff J, Gorbachevski A, Reiser C, Mitkus T, Esposito I, et al. Periostin creates a tumorsupportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. *Gastroenterology* 2007 Apr;132(4):1447–64. [PubMed: 17408641]
73. Masamune A, Kikuta K, Watanabe T, Satoh K, Hirota M, Hamada S, et al. Fibrinogen induces cytokine and collagen production in pancreatic stellate cells. *Gut*. 2008 Dec 3;
74. Vonlaufen A, Phillips PA, Xu Z, Goldstein D, Pirola RC, Wilson JS, et al. Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. *Cancer Res* 2008 Oct 1;68(19):7707–10. [PubMed: 18829522]
75. Manapov F, Muller P, Rychly J. Translocation of p21(Cip1/WAF1) from the nucleus to the cytoplasm correlates with pancreatic myofibroblast to fibroblast cell conversion. *Gut* 2005 Jun;54(6):814–22. [PubMed: 15888791]
76. Jiang HB, Xu M, Wang XP. Pancreatic stellate cells promote proliferation and invasiveness of human pancreatic cancer cells via galectin-3. *World J Gastroenterol* 2008 Apr 7;14(13):2023–8. [PubMed: 18395901]
77. Kanno A, Satoh K, Masamune A, Hirota M, Kimura K, Umino J, et al. Periostin, secreted from stromal cells, has biphasic effect on cell migration and correlates with the epithelial to mesenchymal transition of human pancreatic cancer cells. *Int J Cancer* 2008 Jun 15;122(12):2707–18. [PubMed: 18381746]
78. Bellahcene A, Castronovo V, Ogbureke KU, Fisher LW, Fedarko NS. Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nat Rev Cancer* 2008 Mar; 8(3):212–26. [PubMed: 18292776]
79. Pantel K, Brakenhoff RH. Dissecting the metastatic cascade. *Nat Rev Cancer* 2004 Jun;4(6):448–56. [PubMed: 15170447]
80. Lyons AB, Parish CR. Determination of lymphocyte division by flow cytometry. *J Immunol Methods* 1994 May 2;171(1):131–7. [PubMed: 8176234]
81. Nolte T, Kaufmann W, Schorsch F, Soames T, Weber E. Standardized assessment of cell proliferation: the approach of the RITA-CEPA working group. *Exp Toxicol Pathol* 2005 Nov;57(2):91–103. [PubMed: 16325520]
82. Heatwole VM. TUNEL assay for apoptotic cells. *Methods Mol Biol* 1999;115:141–8. [PubMed: 10098176]
83. Aitken RJ, De Iuliis GN. Value of DNA integrity assays for fertility evaluation. *Soc Reprod Fertil Suppl* 2007;65:81–92. [PubMed: 17644956]
84. Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, et al. A rapid in vitro assay for quantitating the invasive potential of tumor cells. *Cancer Res* 1987 Jun 15;47(12):3239–45. [PubMed: 2438036]
85. Lee J, Cuddihy MJ, Kotov NA. Three-dimensional cell culture matrices: state of the art. *Tissue Eng Part B Rev* 2008 Mar;14(1):61–86. [PubMed: 18454635]
86. Staton CA, Stribbling SM, Tazzyman S, Hughes R, Brown NJ, Lewis CE. Current methods for assaying angiogenesis in vitro and in vivo. *Int J Exp Pathol* 2004 Oct;85(5):233–48. [PubMed: 15379956]
87. Caldwell JS. Cancer cell-based genomic and small molecule screens. *Adv Cancer Res* 2007;96:145–73. [PubMed: 17161679]
88. Kaelin WG Jr. The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 2005 Sep;5(9):689–98. [PubMed: 16110319]
89. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic approaches into the discovery of anticancer drugs. *Science* 1997 Nov 7;278(5340):1064–8. [PubMed: 9353181]
90. Wang H, Han H, Von Hoff DD. Identification of an Agent Selectively Targeting DPC4 (Deleted in Pancreatic Cancer Locus 4)-Deficient Pancreatic Cancer Cells. *Cancer Res* 2006 Oct 1;66(19):9722–30. [PubMed: 17018631]
91. Romer J, Curran T. Targeting medulloblastoma: small-molecule inhibitors of the Sonic Hedgehog pathway as potential cancer therapeutics. *Cancer Res* 2005 Jun 15;65(12):4975–8. [PubMed: 15958535]
92. Jones S, Chen WD, Parmigiani G, Diehl F, Beerenwinkel N, Antal T, et al. Comparative lesion sequencing provides insights into tumor evolution. *Proc Natl Acad Sci U S A* 2008 Mar 18;105(11):4283–8. [PubMed: 18337506]

93. Hansel DE, Kern SE, Hruban RH. Molecular pathogenesis of pancreatic cancer. *Annu Rev Genomics Hum Genet* 2003;4:237–56. [PubMed: 14527303]
94. Yamano M, Fujii H, Takagaki T, Kadowaki N, Watanabe H, Shirai T. Genetic progression and divergence in pancreatic carcinoma. *Am J Pathol* 2000 Jun;156(6):2123–33. [PubMed: 10854233]
95. van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoe KD, Cameron JL, et al. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am J Pathol* 2002 Nov;161(5):1541–7. [PubMed: 12414502]
96. Luttges J, Gahldari H, Bocker V, Schwarte-Waldhoff I, Henne-Bruns D, Kloppel G, et al. Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis. *Am J Pathol* 2001 May;158(5):1677–83. [PubMed: 11337365]
97. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. *Science* 2007 Nov 16;318(5853):1108–13. [PubMed: 17932254]
98. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 2003 Dec;4(6):437–50. [PubMed: 14706336]
99. Bardeesy N, Sharpless NE. RAS unplugged: negative feedback and oncogene-induced senescence. *Cancer Cell* 2006 Dec;10(6):451–3. [PubMed: 17157783]
100. Hingorani SR, Wang L, Multani AS, Combs C, Deramaut TB, Hruban RH, et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* 2005 May;7(5):469–83. [PubMed: 15894267]
101. Bardeesy N, Aguirre AJ, Chu GC, Cheng KH, Lopez LV, Hezel AF, et al. Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. *Proc Natl Acad Sci U S A* 2006 Apr 11;103(15):5947–52. [PubMed: 16585505]
102. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev* 2003 Dec 15;17(24):3112–26. [PubMed: 14681207]
103. Feldmann G, Habbe N, Dhara S, Bisht S, Alvarez H, Fendrich V, et al. Hedgehog inhibition prolongs survival in a genetically engineered mouse model of pancreatic cancer. *Gut* 2008 Oct;57(10):1420–30. [PubMed: 18515410]
104. Ijichi H, Chytil A, Gorska AE, Aakre ME, Fujitani Y, Fujitani S, et al. 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* 2006 Nov 15;20(22):3147–60. [PubMed: 17114585]
105. Hahn WC, Weinberg RA. Rules for making human tumor cells. *N Engl J Med* 2002 Nov 14;347(20):1593–603. [PubMed: 12432047]
106. Renan MJ. How many mutations are required for tumorigenesis? Implications from human cancer data. *Mol Carcinog* 1993;7(3):139–46. [PubMed: 8489711]
107. Campbell PM, Groehler AL, Lee KM, Ouellette MM, Khazak V, Der CJ. K-Ras promotes growth transformation and invasion of immortalized human pancreatic cells by Raf and phosphatidylinositol 3-kinase signaling. *Cancer Res* 2007 Mar 1;67(5):2098–106. [PubMed: 17332339]
108. Lee KM, Nguyen C, Ulrich AB, Pour PM, Ouellette MM. Immortalization with telomerase of the Nestin-positive cells of the human pancreas. *Biochem Biophys Res Commun* 2003 Feb 21;301(4):1038–44. [PubMed: 12589817]
109. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. *Nature* 1999 Jul 29;400(6743):464–8. [PubMed: 10440377]
110. Counter CM, Hahn WC, Wei W, Caddle SD, Beijersbergen RL, Lansdorp PM, et al. Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. *Proc Natl Acad Sci U S A* 1998 Dec 8;95(25):14723–8. [PubMed: 9843956]
111. Feldmann G, Beaty R, Hruban RH, Maitra A. Molecular genetics of pancreatic intraepithelial neoplasia. *J Hepatobiliary Pancreat Surg* 2007;14(3):224–32. [PubMed: 17520196]
112. Feldmann, G.; Maitra, A. Molecular pathology of precursor lesions of pancreatic cancer: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous

- cystic neoplasm (MCN). In: Neoptolemos, J.; Abbruzzese, J.; Buchler, M.; Urrutia, R., editors. *Handbook of Pancreatic Cancer*. New York: Springer; 2009. (in press)
113. Mao J, Ligon KL, Rakhlin EY, Thayer SP, Bronson RT, Rowitch D, et al. A novel somatic mouse model to survey tumorigenic potential applied to the Hedgehog pathway. *Cancer Res* 2006 Oct 15;66(20):10171–8. [PubMed: 17047082]
 114. Izeradjene K, Combs C, Best M, Gopinathan A, Wagner A, Grady WM, et al. Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. *Cancer Cell* 2007 Mar;11(3):229–43. [PubMed: 17349581]
 115. Siveke JT, Einwachter H, Sipos B, Lubeseder-Martellato C, Kloppel G, Schmid RM. Concomitant pancreatic activation of Kras(G12D) and Tgfa results in cystic papillary neoplasms reminiscent of human IPMN. *Cancer Cell* 2007 Sep;12(3):266–79. [PubMed: 17785207]
 116. Hahn H, Wicking C, Zaphiropoulos PG, Gailani MR, Shanley S, Chidambaram A, et al. Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. *Cell* 1996 Jun 14;85(6):841–51. [PubMed: 8681379]
 117. Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, et al. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* 2002 Aug 30;297(5586):1559–61. [PubMed: 12202832]
 118. Kimura H, Stephen D, Joyner A, Curran T. Gli1 is important for medulloblastoma formation in *Ptc1* +/- mice. *Oncogene* 2005 Jun 9;24(25):4026–36. [PubMed: 15806168]
 119. Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* 2003 Oct 23;425(6960):846–51. [PubMed: 14520411]
 120. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* 2003 Mar 20;422(6929):313–7. [PubMed: 12629553]
 121. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov* 2006 Dec;5(12):1026–33. [PubMed: 17139287]
 122. Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* 2003 Oct 23;425(6960):851–6. [PubMed: 14520413]
 123. Binns W, James LF, Shupe JL, Everett G. A Congenital Cyclopian-Type Malformation in Lambs Induced by Maternal Ingestion of a Range Plant, *Veratrum Californicum*. *Am J Vet Res* 1963 Nov; 24:1164–75. [PubMed: 14081451]
 124. Keeler RF, Binns W. Teratogenic compounds of *Veratrum californicum* (Durand). V. Comparison of cyclopian effects of steroidal alkaloids from the plant and structurally related compounds from other sources. *Teratology* 1968 Feb;1(1):5–10. [PubMed: 5696817]
 125. Williams JA, Guicherit OM, Zaharian BI, Xu Y, Chai L, Wichterle H, et al. Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions. *Proc Natl Acad Sci U S A* 2003 Apr 15;100(8):4616–21. [PubMed: 12679522]
 126. Taylor FR, Wen D, Garber EA, Carmillo AN, Baker DP, Arduini RM, et al. Enhanced potency of human Sonic hedgehog by hydrophobic modification. *Biochemistry* 2001 Apr 10;40(14):4359–71. [PubMed: 11284692]
 127. Gabay L, Lowell S, Rubin LL, Anderson DJ. Deregulation of dorsoventral patterning by FGF confers trilineage differentiation capacity on CNS stem cells in vitro. *Neuron* 2003 Oct 30;40(3):485–99. [PubMed: 14642274]
 128. Taipale J, Chen JK, Cooper MK, Wang B, Mann RK, Milenkovic L, et al. Effects of oncogenic mutations in *Smoothed* and *Patched* can be reversed by cyclopamine. *Nature* 2000 Aug 31;406(6799):1005–9. [PubMed: 10984056]
 129. Pepinsky RB, Zeng C, Wen D, Rayhorn P, Baker DP, Williams KP, et al. Identification of a palmitic acid-modified form of human Sonic hedgehog. *J Biol Chem* 1998 May 29;273(22):14037–45. [PubMed: 9593755]
 130. Hotz HG, Reber HA, Hotz B, Sanghavi PC, Yu T, Foitzik T, et al. Angiogenesis inhibitor TNP-470 reduces human pancreatic cancer growth. *J Gastrointest Surg* 2001 Mar–Apr;5(2):131–8. [PubMed: 11331474]

131. Kawarada Y, Ishikura H, Kishimoto T, Saito K, Takahashi T, Kato H, et al. Inhibitory effects of the antiangiogenic agent TNP-470 on establishment and growth of hematogenous metastasis of human pancreatic carcinoma in SCID beige mice in vivo. *Pancreas* 1997 Oct;15(3):251–7. [PubMed: 9336788]
132. Kato H, Ishikura H, Kawarada Y, Furuya M, Kondo S, Yoshiki T. Anti-angiogenic treatment for peritoneal dissemination of pancreas adenocarcinoma: a study using TNP-470. *Jpn J Cancer Res* 2001 Jan;92(1):67–73. [PubMed: 11173546]
133. Solorzano CC, Baker CH, Bruns CJ, Killion JJ, Ellis LM, Wood J, et al. Inhibition of growth and metastasis of human pancreatic cancer growing in nude mice by PTK 787/ZK222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases. *Cancer Biother Radiopharm* 2001 Oct;16(5):359–70. [PubMed: 11776753]
134. Bruns CJ, Shrader M, Harbison MT, Portera C, Solorzano CC, Jauch KW, et al. Effect of the vascular endothelial growth factor receptor-2 antibody DC101 plus gemcitabine on growth, metastasis and angiogenesis of human pancreatic cancer growing orthotopically in nude mice. *Int J Cancer* 2002 Nov 10;102(2):101–8. [PubMed: 12385004]
135. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971 Nov 18;285(21):1182–6. [PubMed: 4938153]
136. Burris H 3rd, Rocha-Lima C. New therapeutic directions for advanced pancreatic cancer: targeting the epidermal growth factor and vascular endothelial growth factor pathways. *The oncologist* 2008 Mar;13(3):289–98. [PubMed: 18378539]
137. van Es JH, Clevers H. Notch and Wnt inhibitors as potential new drugs for intestinal neoplastic disease. *Trends Mol Med* 2005 Nov;11(11):496–502. [PubMed: 16214417]
138. Kopan R, Goate A. A common enzyme connects notch signaling and Alzheimer's disease. *Genes Dev* 2000 Nov 15;14(22):2799–806. [PubMed: 11090127]
139. van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, Begthel H, et al. Notch/gammasecretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 2005 Jun 16;435(7044):959–63. [PubMed: 15959515]
140. Mullendore M, Koorstra JB, Li Y, Offerhaus GJ, Fan X, Henderson C, et al. Ligand-dependent Notch signaling is involved in tumor initiation and tumor maintenance in pancreatic cancer. *Clin Cancer Res*. 2009 in press.
141. Dihlmann S, von Knebel Doeberitz M. Wnt/beta-catenin-pathway as a molecular target for future anti-cancer therapeutics. *Int J Cancer* 2005 Feb 10;113(4):515–24. [PubMed: 15472907]
142. Lepourcelet M, Chen YN, France DS, Wang H, Crews P, Petersen F, et al. Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex. *Cancer Cell* 2004 Jan;5(1):91–102. [PubMed: 14749129]
143. Lahn M, Kloeker S, Berry BS. TGF-beta inhibitors for the treatment of cancer. *Expert Opin Investig Drugs* 2005 Jun;14(6):629–43.
144. Jimeno A, Tan AC, Coffa J, Rajeshkumar NV, Kulesza P, Rubio-Viqueira B, et al. Coordinated epidermal growth factor receptor pathway gene overexpression predicts epidermal growth factor receptor inhibitor sensitivity in pancreatic cancer. *Cancer Res* 2008 Apr 15;68(8):2841–9. [PubMed: 18413752]
145. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007 May 20;25(15):1960–6. [PubMed: 17452677]
146. Laurent-Puig P, Taieb J. Lessons from Tarceva in pancreatic cancer: where are we now, and how should future trials be designed in pancreatic cancer? *Curr Opin Oncol* 2008 Jul;20(4):454–8. [PubMed: 18525343]
147. Ng R, Chen EX. Sorafenib (BAY 43–9006): review of clinical development. *Curr Clin Pharmacol* 2006 Sep;1(3):223–8. [PubMed: 18666747]
148. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther* 2008 Oct;7(10):3129–40. [PubMed: 18852116]

149. Cheng GZ, Park S, Shu S, He L, Kong W, Zhang W, et al. Advances of AKT pathway in human oncogenesis and as a target for anti-cancer drug discovery. *Curr Cancer Drug Targets* 2008 Feb;8(1):2–6. [PubMed: 18288938]
150. Mita M, Sankhala K, Abdel-Karim I, Mita A, Giles F. Deforolimus (AP23573) a novel mTOR inhibitor in clinical development. *Expert Opin Investig Drugs* 2008 Dec;17(12):1947–54.
151. Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. *Clin Cancer Res* 2008 Mar 1;14(5):1286–90. [PubMed: 18316545]
152. Asano T, Yao Y, Zhu J, Li D, Abbruzzese JL, Reddy SA. The rapamycin analog CCI-779 is a potent inhibitor of pancreatic cancer cell proliferation. *Biochem Biophys Res Commun* 2005 May 27;331(1):295–302. [PubMed: 15845392]
153. Wolpin BM, Hezel AF, Abrams T, Blaszkowsky LS, Meyerhardt JA, Chan JA, et al. Oral mTOR Inhibitor Everolimus in Patients With Gemcitabine-Refractory Metastatic Pancreatic Cancer. *J Clin Oncol* 2009 Jan 10;27(2):193–8. [PubMed: 19047305]

Figure 1.

(top) Non-transformed hTERT-immortalized HPNE cells grow as a monolayer in tissue culture. (middle and bottom) Examples of transformed HPNE cells with multi-layering (“focus” formation). In this example, the gene(s) responsible for this neoplastic phenotype are not known, as transformation was induced by transient activation of a Sleeping Beauty transposon, leading to disruption and/or activation of cancer-associated genes in the HPNE genome. In vitro models of pancreatic epithelial cells can be used for such “forward” genetics experiments, as well as for more traditional reverse genetics using known combination of oncogenes or tumor suppressor genes, as discussed in the text.

Figure 2.

Pancreatic cancer does not occur 'de novo' but via development of clearly defined tangible precursor lesions that correlate with step-wise accumulation of genetic alterations. In order to develop systemic metastases, an invasive clone must overcome several additional selection barriers, including invasion into surrounding tissue, anchorage-independent survival in the blood stream, active migration and invasion at a distant organ site and establishment of a metastatic tumor lesion. While a variety of in vitro methods exist trying to mimic single aspects of this complex process, it is nevertheless impossible to faithfully mimic in its entirety by means of cell line-based in vitro models alone as of to date.

Table 1
Key advantages and disadvantages of different in vitro and in vivo model systems of pancreatic cancer in drug discovery

Model system	Advantages	Disadvantages
Patient-derived cell lines	<p>Accurately mirror entire spectrum of genetic alterations of human pancreatic cancers</p> <p>Cell-line based studies are comparably cheap and less time- and labor-consuming than studies on animal models</p> <p>Easily accessible</p> <p>Virtually unlimited amounts of cancer cells can be generated with relatively little required technical equipment</p>	<p>Functional studies generally highly prone to artificial results</p> <p>Purely cell-line based treatment studies aimed at determining efficacy of novel drug candidates are commonly extremely poor in predicting actual therapeutic response or adverse effects in humans</p> <p>Do not allow to study important in vivo factors such as pharmacokinetics and toxicity profiles</p> <p>Do not reflect effects conferred by tumor-stroma interactions</p> <p>May genetically change over time</p>
Co-cultures	<p>Can to some degree mimic effects of secreted growth-modulating stimuli and direct physical contact with the ECM conferred by stromal cells</p>	<p>Difficult to co-culture more than two different cell compartments in one experiment</p> <p>Difficult to accurately control for the physiological ratio of neoplastic to stromal cells</p> <p>Most of the other disadvantages listed above for "patient-derived cell lines" apply here as well</p>
Patient-derived xenografts	<p>Accurately mirror entire spectrum of genetic alterations of human pancreatic cancers</p> <p>Can be used to enrich for neoplastic cell compartment and eliminate contaminating human stromal cells</p> <p>Testing of candidate drugs or other novel experimental therapeutic strategies by using xenografts usually faster and cheaper as compared to transgenic models</p> <p>Orthotopic xenograft models allow to study effects of locally invasive growth and systemic metastasis</p>	<p>Xenogenic chimera between human and murine cells</p> <p>Do not accurately mirror effects conferred by host immune system</p> <p>Not suitable to accurately mimic tumor-stroma interactions in humans</p> <p>Different anatomies in mice versus humans</p> <p>May genetically change over time</p> <p>Require experimental exploitation of and potentially inflicting harm on mammals</p>
Genetically engineered mouse models	<p>Overall reliable, usually highly reproducible results</p> <p>Allow for distinct studies of virtually any desired combination of genetic alterations in an in vivo setting</p> <p>More recently described models enable to mirror most of the key features of the human disease surprisingly realistically, including local intrapancreatic growth, invasion into surrounding structures, systemic metastasis, development via mPanIN precursor lesions, histological microarchitecture resembling aspects of human pancreatic cancers</p> <p>Readily available for pre-clinical evaluation of novel experimental therapeutic approaches</p>	<p>Do not reflect the entire spectrum of genetic alterations found in human cancer tissues</p> <p>Xenogenic model systems</p> <p>Different anatomies in mice versus humans</p> <p>Require experimental exploitation of and potentially inflicting harm on mammals</p> <p>Usually highly cost-, labor- and time-intensive studies</p> <p>Though usually providing valuable data on efficacy, pharmacokinetics and toxicity profiles of novel experimental therapeutic interventions, results from these studies nevertheless often fail to accurately predict response in humans, i.e. can not substitute subsequent clinical evaluation</p>

Table 2
Studies estimating numbers of genetic alterations required in pancreatic carcinogenesis

Reference	Summary	Species	Genetic model used	Number of genetic alterations	Comments
49	direct sequencing of 24 human pancreatic cancer samples; DNA isolated from cell lines or murine xenografts	human	Genuine human pancreatic ductal adenocarcinoma cells	average of 48 per tumor sample	first and so far only global sequencing study of human pancreatic cancer samples
91	pancreas-specific expression of an oncogenic $Kras^{G12D}$, which is achieved by combination to a lox-stop-lox (LsL) cassette and expression of Cre-recombinase under control of Pdx1 or P48 promoters, led to development of mPanIN precursor lesions, which eventually progressed to fully invasive cancers at low frequency	mouse	LsL-Kras ^{G12D} ; Pdx1-Cre LsL-Kras ^{G12D} ; P48 ^{Cre}	1	
7	targeting of oncogenic Kras ^{G12D} expression to the adult pancreas by means of an inducible $Ela-Cre^{ERT2}$ construct leads to formation of mPanIN lesions but not fully malignant pancreatic cancer	mouse	LsL-Kras ^{G12D} ; $Ela-Cre^{ERT2}$	1	
93	pancreas-specific targeting of combined Kras ^{G12D} and $Trp53^{R172H}$ expression led to development of metastatic pancreatic carcinomas with median survival of 5 months	mouse	LsL-Kras ^{G12D} ; LsL- $Trp53^{R172H}$; Pdx1-Cre	2	no acquired mutations found in Cdkn2/Ink4a, Cdk4 or Smad4
94	development of pancreatic adenocarcinomas with average latency of 6.2 weeks in mice expressing Kras ^{G12D} in the absence of p53 und control of Pdx1-Cre construct	mouse	LsL-Kras ^{G12D} ; p53 ^{lox/lox} ; Pdx1-Cre	2	
95	rapid development of poorly differentiated metastasizing cancers in murine pancreata expressing oncogenic Kras ^{G12D} in combination with abrogation of Ink4a/Arf expression	mouse	LsL-Kras ^{G12D} ; Ink4a/Arf ^{lox/lox} ; Pdx1-Cre	3	
97	development of well-differentiated pancreatic ductal adenocarcinomas with high penetrance and short median survival of only 59 days upon concomitant oncogenic Kras-signaling and Tgfb β 2-knockout	mouse	LsL-Kras ^{G12D} ; Tgfb β 2 ^{lox/lox} ; Ptf1a ^{cre/+}	2	
100	Immortalization and in vitro transformation of cultured non-neoplastic human pancreatic ductal cells (hTERT-HPNE)	human	hTERT-HPNE; E6/E7; Kras ^{G12D} ; SV40st	5	
19	Expression of oncogenic Kras in cultured immortalized non-neoplastic human pancreatic ductal cells (HPDE) leads to tumorigenicity in SCID mice in 50% of cases	human	HPDE; E6/E7; Kras4B ^{G12V}	3	absence of in vitro clonogenicity