Published in final edited form as:

Adv Anat Pathol. 2012 November ; 19(6): 351–362. doi:10.1097/PAP.0b013e318273f998.

Human Correlates of Provocative Questions in Pancreatic Pathology

Oliver G. McDonald, M.D., Ph.D.¹, Anirban Maitra, M.B.B.S.^{1,2,3}, and Ralph H. Hruban, M.D.^{1,2}

¹Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD

²Department of Oncology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD

³Institute for Genetic Medicine, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

Studies of cell lines and of animal models of pancreatic cancer have raised a number of provocative questions about the nature and origins of human pancreatic cancer and have provided several leads into exciting new approaches for the treatment of this deadly cancer. In addition, clinicians with little or no contact with human pathology have challenged the way that pancreatic pathology is practiced, suggesting that “genetic signals” may be more accurate than today’s multi-modal approach to diagnoses. In this review we consider eight provocative issues in pancreas pathology, with an emphasis on “the evidence derived from man.”

“We would make a plea that so far as any conclusions are drawn as to the mechanism of human disease, the evidence derived from man should at least be considered”

Sir George W. Pickering And Robert H. Heptinstall, The
Lancet, Volume II, 1952, page 1081

Introduction

The development of a genetically engineered mouse model (GEMM) of pancreatic cancer by Hingorani and Tuveson in 2003 that recapitulates many of the histological features of the cognate human disease provided unique opportunities to manipulate and study the fundamental biology of pancreatic neoplasia¹. Models such as this one have been used to study precursor lesions, tumor-stroma interactions, and the delivery of drugs to neoplastic cells^{2–4}. All too often however, the results generated studying animals are not directly correlated back to human pathology.

We would argue that from beginning to end, those working with animal models and *in vitro* systems should always keep human pathobiology in mind. For example, if one is going to introduce genetic alterations into cells in the pancreata of mice, we would argue that it would be preferential to select genes that are known to be altered in human disease over genes that have not been shown to play a role in humans. Similarly, when experiments conducted on GEMM are analyzed, the results should be interpreted in light of human

pathology⁵. It is our belief that in this way experiments conducted in animal models can have the biggest impact on alleviating human suffering.

We would also argue that a firm grasp of human pancreatic pathology and the way it is practiced forms a solid foundation for clinicians wishing to help patients suffering from pancreatic cancer through their clinical work.

In this review we will discuss eight provocative issues in pancreatic pathology. Our goal is to discuss these issues in the framework of our understanding of human pathology. We should make it absolutely clear that we are in no way denigrating our clinical colleagues or experiments performed using animals; indeed we are co-authors on many of the manuscripts we will discuss in this review. Instead, we wish to emphasize the value gained by considering “the evidence derived from man.” Studies in animals can open our eyes to fundamental mechanisms underlying disease and to what can happen in living tissues in ways we never would have imagined. These studies need to be balanced with what does happen in humans.

Provocative Issue #1 Epithelial-mesenchymal transition is important for invasion and metastasis

Epithelial to mesenchymal transition (EMT) is a paradigm of cell plasticity, thought to be crucial for embryogenesis, injury repair, organ fibrosis, and malignant progression^{6, 7}. EMT is characterized by reversible loss of epithelial characteristics coupled with gain of mesenchymal properties, including transition from epithelial to stellate morphology, loss of cell polarity, enhanced motility, and synthesis of extracellular matrix remodeling proteins. EMT is thought to be reversible under most circumstances, a process called mesenchymal to epithelial transition (MET).

Although it has been appreciated for decades that EMT is crucial for proper development of various embryonic tissues, over the past few years numerous publications have also highlighted the importance of EMT in cancer, and have suggested that EMT may be a driving force behind invasion, metastasis, and chemoresistance in many carcinomas including pancreatic ductal adenocarcinoma (PDAC)^{8, 9}.

Much of what we know regarding the signaling pathways and molecules involved in regulating EMT are derived from *in vitro* cell culture studies, due to the ease of studying pure populations of epithelial cells undergoing synchronous EMT. In the decades since the first *in vitro* description of EMT by Elizabeth Hay,¹⁰ much of our current understanding of EMT has been obtained by treatment of cultured epithelial cells with various extracellular signaling molecules that trigger EMT, such as transforming growth factor-beta (TGF- β). These signaling molecules activate pathways that up-regulate expression of certain “master regulatory” transcription factors, including Snail, Slug, Zeb1, Twist, and SIP1, that converge upon the promoter of E-cadherin (and likely other epithelial-specific genes) to repress transcription^{6, 7}. E-cadherin is a vital component of epithelial desmosomes, and loss of E-cadherin triggers the disassembly of adherens junctions. This results in loss of cell contacts that normally maintain epithelial differentiation and inhibit induction of EMT. Indeed, overexpression of master regulatory factors or knockdown of E-cadherin expression by RNAi is sufficient to induce EMT in the absence of exogenous signaling molecules¹¹. In addition to master regulatory factors, several recent studies in cell culture have also implicated other molecular processes that regulate EMT, including chromatin remodeling and microRNAs, many of which also regulate expression of E-cadherin via interplay with the master transcription factors.

The *in vivo* study of EMT in mice is hampered by the lack of known biological situations where homogenous populations of cells undergo synchronous EMT. Many studies to date have focused on whether or not the EMT process and the factors that control it are active in human cancers transplanted into mouse models. Several lines of evidence from these models support a role for EMT in malignant progression. First, single cells that break apart from the bulk of well-differentiated tumors at their invasive fronts undergo EMT in models of breast cancer¹². Second, many human carcinomas (including PDAC) with poorly differentiated and/or spindled morphology often display features of EMT and are usually (although not always) more aggressive than their well-differentiated counterparts, and this behavior is often associated with loss of E-cadherin and changes in EMT master regulatory transcription factors^{13–15}. Third, there is accumulating evidence that cells undergoing EMT may constitute the fraction of tumor initiating cells (TICs) in some carcinomas. Isolation of cells with properties ascribed to TICs from bulk tumors has shown that these cells display features of EMT¹⁶, and differentiated cells induced to undergo EMT acquire TIC properties in culture and in mice^{17, 18}. Indeed, compounds that eradicate cells undergoing EMT also eradicate TIC subpopulations¹⁸. Finally, PDAC cells with properties of EMT are highly chemoresistant (e.g. to gemcitabine) when compared to differentiated cells¹⁹. Chemoresistance is a property often ascribed to TICs, and chemoresistant TICs are thought to be present in human PDAC^{20, 21}. Indeed, treatment of PDAC cells with gemcitabine seems to greatly enrich the fraction of TIC-like cells, presumably via selecting for EMT^{20, 22–24}.

Thus, experimental studies suggest that EMT or some EMT-like process does play a crucial role in propagation, malignant progression, and chemoresistance of many cancers including PDAC, at least in cell culture and in mouse models. Does the same hold true for PDAC in human patients, where tumor evolves within its native human host rather than an immunodeficient murine host environment?

A broad range of degrees of differentiation can be observed for human invasive PDAC in histologic sections. Poorly and undifferentiated carcinomas, as the names suggest, feebly recapitulate the glandular differentiation of non-neoplastic ductal cells²⁵. The neoplastic cells, instead, are mononuclear or spindle shaped with aggressive clinical behavior. These mononuclear and spindle-shaped cells often display immunolabeling patterns consistent with EMT, including loss of E-cadherin¹⁴. For these types of poorly differentiated tumors, it has been hypothesized that these neoplastic cells are genetically driven into an irreversible EMT state while retaining the ability to proliferate²⁶, via bypassing EMT-driven growth arrest.

While these observations in humans would at first appear to support the hypothesis that EMT occurs in human PDAC and other cancers, pathologic examination of those PDACs with well-differentiated morphology reveal two observations that appear to run contrary to this²⁵.

First, not all aggressive PDACs are poorly differentiated. Despite the dismal prognosis associated with pancreatic cancer, many PDACs, including those that metastasize and kill patients, are remarkably well-differentiated (Figure 1A). This is one of the great paradoxes of pancreatic pathology- that such an aggressive neoplasm is often so well-differentiated. Second, the most invasive components of some pancreatic cancers still form nearly perfect glands. This includes adenocarcinoma that has colonized perineural spaces (Figure 1B), blood vessels, and even in distant metastatic sites (e.g. “lepidic growth” in lungs). Simply put, in many cases, the overtly malignant PDAC cells in nerves, vessels and metastatic sites are not poorly differentiated or spindle-shaped mesenchymal appearing cells. They are the opposite. They are so well-differentiated that they are often even impossible to distinguish

histologically from non-neoplastic glands except for their abnormal location²⁵. In addition, expression of E-cadherin is nearly always intact in these cells (Figure 1C).

How then can we put these two observations together? How can we reconcile the importance of EMT for malignant behavior in mouse models and poorly differentiated human PDAC, with the finding that many highly aggressive pancreatic adenocarcinomas in humans can be extremely well-differentiated? Why aren't these cells undergoing EMT and/or poorly-differentiated or spindled upon invasion and metastasis? One explanation is that EMT is simply not required for aggressive behavior for all carcinomas in human microenvironments, well-differentiated ones in particular. In some mouse models and human cancers, carcinomas may invade collectively without infiltration of single EMT cells at the invasive tumor front²⁷, and retention of their epithelial cell adhesion molecules may be required for invasion and growth in vessels, nerves, and sites of distant metastasis. One could even envision a scenario that once vascular invasion is achieved, these well-differentiated cells may utilize adhesion molecules to "hitch a ride" to distant metastatic sites by attaching to blood cells or platelets. Even simpler, a metastatic process that depends on tightly adherent malignant cells circulating in the bloodstream as a neoplastic bolus may offer a much more efficient means by which carcinoma can lodge and colonize a metastatic site than a process based on EMT and single cell dissemination. Thus, at first glance it would appear that EMT does not explain malignant behavior in well-differentiated PDAC in humans.

However, in a recent elegant review, Thomas Brabletz outlined recent evidence that EMT coupled with the *reverse* process MET, may play a crucial role in malignant progression of well-differentiated carcinomas²⁶. First, although most, if not all, neoplastic cells in well-differentiated carcinomas appear epithelial, rare individual cells that appear to be undergoing EMT can be observed in histologic sections and even isolated from these tumors (Figure 1D). As in mouse models, it is conceivable that these rare EMT-like cells may underlie single cell invasion into stroma, nerves, and vessels, and are also required for dissemination into the bloodstream. However, efficient colonization and growth within locally invaded tissues and sites of distant metastasis may require reversal of the process, mesenchymal to epithelial transition (MET), thereby producing well-differentiated cells that are more efficient at populating a site once EMT has allowed access. Indeed, it is well-known that cells undergoing EMT are typically growth arrested and therefore incapable dividing to form a mass, whereas fully differentiated carcinoma cells readily enter the cell cycle. Also, cell surface adhesion molecules required for epithelial attachments to each other and other cells are lost during EMT, which could also inhibit growth within stroma, nerves, and vessels, as well as disrupt attachments to other tumor cells required to produce mass lesions. Careful examination of histologic sections of locally invasive and/or metastatic well-differentiated PDACs for rare cells undergoing EMT and MET could help resolve these questions.

An understanding of the role of EMT and MET in humans has implications for therapies targeting EMT, since these treatments would only eradicate a small number of cells at the invasive front of tumors and those that are disseminating in the bloodstream. They would not treat the bulk of the invasive primary pancreatic carcinoma or well-differentiated cells in established metastasis. Obviously, if any malignant cell in a well-differentiated PDAC is capable of undergoing EMT, then combination therapy targeted toward both EMT and well-differentiated cells would be required. Clearly, EMT raises many provocative questions for pancreatic cancer pathology that will need to be addressed with studies in model systems and in man.

Provocative Issue #2 Pancreatic neoplasia metastasizes or “delaminates” before it invades

One of the more controversial questions pertaining to pancreatic cancer is the timing of onset of metastatic disease. Do pancreatic cancer cells disseminate very early during disease evolution (potentially even at the step of precursor lesions), or does this process occur only once the primary tumor has become bulky? Although the answer to this question has direct implications for screening and treatment, it could not be addressed directly until appropriate animal models became available. Recently, Rhim and colleagues utilized the credentialed genetically engineered mouse model of pancreatic cancer (“KPC” mice) to study the timing of onset of metastatic disease²⁸. The “KPC” mice were genetically modified such that any cell expressing mutant *Kras* and *p53* allele was also labeled with a fluorescent reporter. The authors noted the presence of fluorescently labeled cells in the periductal stroma of non-invasive murine pancreatic intraepithelial neoplasia (mPanIN) lesions, a process they named “delamination”, as a more equivocal surrogate terminology for invasion. Delamination was observed in pancreata where the presence of a primary mass-forming neoplasm was excluded by rigorous histological analysis and only mPanIN lesions were present. The delaminated cells had features of EMT (see provocative issue #1), including loss of E-cadherin and expression of mesenchymal antigens. Notably, the delaminated cells were indistinguishable by morphology from surrounding stromal cells, and were only identifiable due to the expression of the reporter gene.

Remarkably the investigators were also able to detect circulating fluorescently labeled cells in mice that had no histologically demonstrable invasive primary in the pancreas, suggesting that the delaminated cells arising from precursor lesions have vascular access within the pancreatic milieu²⁸. In mice harboring mPanIN lesions, the circulating labeled cells had surface antigenic markers demonstrable in tumor initiating populations (CD24+, CD44+), and demonstrated clonogenic growth properties when passaged *in vitro*. Based on the findings in this mouse model, Rhim et al postulated that stromal invasion by neoplastic cells which have not yet formed a mass but which harbor properties of EMT precedes the onset of a demonstrable primary tumor in the pancreas²⁸. At least theoretically, therefore, delaminated cells arising from the pancreas that have gained access to the circulation could seed distant sites like the liver and lung, and form the origins for clonally independent metastatic foci even in the setting of “curative” resection of an apparently non-invasive primary tumor.

What do the data in humans suggest? Is there any evidence that apparently non-invasive precursor lesions in the pancreas delaminate and metastasize before an invasive component is identifiable? While Rhim and colleagues suggest that metastatic carcinomas of unknown primary provide evidence for the process of delamination, in fact, the vast majority of patients with metastatic carcinoma of unknown primary have bulky disease involving multiple organs. The problem isn't that there isn't an organ that could be the primary, the problem in most cases is that there are too many candidates!

In addition, there have been a number of clinical studies in humans in which patients with non-invasive intraductal papillary mucinous neoplasms (IPMNs) of the pancreas have been followed without surgery, and these patients don't develop metastatic disease without first developing a solid component or some other finding to suggest local invasion^{29–31}. The same is true for almost every other solid organ. Pap smears are effective in reducing cervical cancer precisely because intraepithelial neoplasia goes through a predictable histologic progression before it invades. One could argue that the smaller precursor lesions in the pancreas, the pancreatic intraepithelial neoplasia (PanIN) lesions, are in some way unique among the epithelial neoplasms; that they fundamentally differ from cervical intraepithelial

neoplasia (CIN), ductal carcinoma *in situ* of the breast (DCIS), adenomas of the colon, and *in situ* neoplasms of the skin. Here again, although the data in humans are spotty because PanINs are hard to detect and follow clinically, the data do not support the hypothesis that non-invasive PanIN lesions delaminate and metastasize before an invasive component is identifiable. For example, M. Canto et al. have screened a large number of patients with a strong family history of pancreatic cancer and most of these patients have been found to have multi-focal PanIN lesions, and some have multi-focal high-grade PanIN lesions^{32, 33}. Although the follow-up is limited, these patients do not appear to succumb to metastatic disease in the absence of a recognizable invasive lesion in the pancreas. Larger studies of patients without a family history of pancreatic cancer suggest that PanINs are, in fact, extremely common in the general population. A study of 1,174 autopsied patients by Kozuka et al. found that 36% of patients without pancreatic cancer had a PanIN lesion³⁴. By contrast, invasive pancreatic cancer is diagnosed in only 9 per 100,000 Americans per year, and virtually all patients with metastatic pancreatic cancer have a bulky primary lesion. If PanINs are so prevalent and invasive pancreatic cancers so rare, the rate of “delamination,” should it occur, must be extraordinarily low in humans.

Additional studies in GEMM, particularly those designed to rule out “leakiness” of the reporter (expression of the reporter in non-neoplastic stromal cells), and additional studies in humans, particularly the long-term follow-up of patients with non-invasive precursor lesions, will be needed to determine why even minimally invasive pancreatic cancer is so lethal.

Provocative Issue #3 Stroma is a barrier to the treatment of pancreatic cancer and accounts for pancreatic cancer’s chemoresistance

One of the enigmatic questions vis-à-vis pancreatic cancer that has plagued clinicians and researchers alike is its unique recalcitrance to chemotherapy. This is underscored by the excitement generated when gemcitabine was first approved as therapy of choice for pancreatic cancer in 1997 with a median improvement in survival of few weeks over 5-fluorouracil (5-FU)³⁵. More recently, a multidrug combination of oxaliplatin, irinotecan, and 5-FU with leucovorin rescue (FOLFIRINOX) improved median survival of metastatic pancreatic cancer to 11 months, which meant a net gain of 5.5 months in median survival despite 14 years of intense clinical and basic research³⁶. Clearly the resistance of pancreatic cancer to treatment has been a frustrating problem.

A potential insight into the generally refractory nature of pancreatic cancer was obtained using the “KPC” mouse model, which not only recapitulates the epithelial progression of the cognate human disease, but also mimics the intense stromal reaction (a.k.a. desmoplasia) characteristically observed in the peritumoral milieu of human adenocarcinomas^{1, 2}. The intense stromal response was once considered a “passive” sheathing response by the host to contain the invading cancer cells; over time, the stroma has come to be recognized as an active partner in the growth and dissemination of pancreatic cancer, with a robust paracrine communication between neoplastic and host stromal compartments^{4, 37, 38} (*see provocative issue #4*).

Using the “KPC” model, Tuveson and colleagues recently uncovered another potential role for the stroma in pancreatic cancer, that of a barrier to effective drug delivery³. In this model, the delivery of the chemotherapeutic gemcitabine was impeded in the presence of the desmoplastic stromal barrier, while depletion of the stroma using a Hedgehog antagonist allowed robust gemcitabine delivery into the peritumoral milieu and increased therapeutic efficacy³. More recently, this paradigm has been validated in the “KPC” model by two groups that targeted stromal hyaluronan, using a PEGylated hyaluronidase^{39, 40}. These

studies identified striking interstitial hydrostatic pressure within the stromal compartment that constricts blood vessels and prevents the passive efflux of delivered chemotherapeutics from the vasculature into the peritumoral milieu^{39,40}. Elimination of the stroma diminishes this hydrostatic pressure, allowing efflux of the administered chemotherapeutic agents. The preclinical success of these stromal depletion efforts in the “KPC” model has engendered enthusiasm that a similar approach would also enable improved chemotherapeutic delivery to human pancreatic cancers and improve the stubbornly dismal outcome observed with this disease.

The stroma clearly plays an important role in invasive ductal adenocarcinomas. Indeed, one could argue that pancreatic cancers are among the most desmoplastic of the epithelial malignancies (Figure 1E). It is important to note, however, that while primary and metastatic pancreatic cancers in humans are both poorly responsive to therapy, the primaries tend to have a much more intense desmoplastic response than the metastases. For example, as illustrated in Figure 1F, pancreatic cancer metastatic to the lung can grow in a lipidic pattern; growing as a thin layer along pre-existing alveolar walls and mimicking a bronchoalveolar carcinoma. These cells sit right on the alveolar walls with little to no intervening stroma between the small vessels and the neoplastic cells. Despite the absence of stroma, these metastatic lesions also do not respond to systemic chemotherapy. The stroma, while it may contribute, cannot be the sole explanation for the chemoresistance of pancreatic cancer.

Provocative Issue #4: Interactions between neoplastic cells and stroma can be targeted as a treatment for pancreatic cancer

As outlined above, a hallmark feature of PDAC is the non-neoplastic desmoplastic stroma that accompanies the malignant epithelial cells (Figure 1E). This stroma often comprises the bulk of the tumor, and is composed of reactive “cancer-associated” fibroblasts (CAFs), activated pancreatic stellate cells (PSCs), scattered inflammatory cells, partially collapsed microvasculature, nerve fibers, dense connective tissue, interstitial fluid, and numerous cytokines and growth factors that are embedded within the stromal matrix. Even preinvasive PanIN precursor lesions are surrounded by a ring of similar reactive stroma, both in human tissues and genetic mouse models⁴¹. It is thought by many that desmoplastic tumor stroma may promote growth, survival, and malignant progression of invasive PDAC and perhaps even PanINs. Because of this, it has been proposed that targeting the various non-neoplastic stromal elements that accompanies PDAC may be a new therapeutic approach for the treatment of PDAC, via abolishing crucial cancer:stromal interactions that are essential for growth and survival of invasive PDAC and its precursors.

Studies based largely on *in vitro* co-culture experiments of PDAC cell lines with PSCs and CAFs have suggested that interactions of neoplastic cells with stromal cells and their secreted signaling molecules are important for the growth and survival of invasive PDAC. These studies have shown that media conditioned by PSCs isolated from PDAC stroma improve growth, survival, invasion, and chemoradioresistance of PDAC cell lines *in vitro*³⁷, and that the presence PSCs and/or CAFs with PDAC cells results in production of a desmoplastic stroma accompanied by enhanced tumor incidence, growth, and metastasis in various mouse xenograft models^{37, 42, 43}. Further studies have shown that these effects may be due, at least in part, to activation of the hedgehog (Hh) pathway in PSCs³⁸, secondary to secretion of Hh ligands by PDAC cells^{44–48}. Platelet derived growth factor (PDGF), transforming growth factor- β (TGF- β), Notch, cyclooxygenase-2, and other pathways also appear to play a role⁴³. There is also preliminary evidence that PSCs can promote EMT of PDAC cells, either directly⁴⁹ or indirectly via creating a hypoxic microenvironment through

stimulation of dense stroma fibrosis coupled with suppression of intratumoral microvasculature⁵⁰. Hypoxia is a well-known inducer of EMT⁵¹.

Experiments in culture and in mice examining tumor infiltrating macrophages also support a role for the stroma in promoting PDAC progression, and offer a therapeutic target⁵². For example, CD40 agonists have been utilized to activate tumoricidal macrophages that infiltrate the tumor and cause degradation of surrounding fibrotic tumor stromal elements. This approach produced regression of primary and metastatic PDAC in a subset of patients and mice genetically engineered to develop spontaneous PDAC, and this effect was independent of chemotherapy with gemcitabine. One caveat to this study is that it was unclear (at least to us) whether stromal depletion was the cause or an after-effect of PDAC cell death.

Finally, most if not all PDAC are accompanied by chronic pancreatitis in the surrounding pancreatic parenchyma. Although this may often be a secondary effect to obstructive changes caused by the tumor, it is clear that patients with chronic pancreatitis are at much higher risk of developing PDAC^{53, 54}, and repeated bouts of pancreatitis in mice engineered with highly oncogenic mutations stimulates PDAC progression from low grade PanIN lesions that in the absence of pancreatitis fail to progress⁵⁵. The stroma that characterizes chronic pancreatitis is thought to be quite similar to the stroma of PDAC⁵⁶. As such, this stroma may also promote malignant progression. Thus, treatment of pancreatitis and/or its associated stromal constituents could offer an avenue to prevent PanIN lesions from progressing to PDAC. Indeed, there is some preliminary evidence in mouse models that supports this notion^{57–59}.

However, if tumor-stromal interactions were required for pancreatic cancer cells to grow and survive, one might anticipate that pancreatic cancers would not grow in the absence of stromal cells and/or their secreted molecules (Figure 1F). A search of the ATCC library, however, reveals 20 human pancreatic cancer cell lines, none of which require stromal cells to grow. Many of these cell lines can grow *in vitro* on plastic even when cells are maintained in serum-free media which is presumably free of growth factors that might substitute for stromal secretions. Although the ability of these cell lines to seed and grow *in vivo* xenotransplantation and/or tail vein injections within immunodeficient mice is enhanced by stromal elements (PSCs, CAFs), these cells are perfectly capable of accomplishing this feat on their own, in the absence of stromal constituents. Furthermore, many metastatic lesions in humans remain chemoresistant despite lack of a stromal response at the metastatic site (e.g. lepidic growth in lung, Figure 1F). Collectively, these observations suggest that efforts to simply target the non-neoplastic stroma may slow progression, but will not ultimately eradicate those PDAC that harbor the ability to grow and invade independently of stromal elements.

One could further argue that the human PDAC stroma may actually represent a host response meant to generate a barrier against growth and/or metastasis. The PDAC desmoplastic stroma is densely fibrotic, hypoxic, nutrient-poor, and highly pressurized. One would think that even highly malignant cancer cells would find it tough-going to grow, survive, and invade within such a microenvironment. Consistent with this notion, matrix metalloproteinases, which function to degrade stromal elements, are thought to be essential for invasive growth of PDAC^{60, 61}. SPARC (also known as osteonectin) is a protein that plays an important role in turnover of stroma extracellular matrix (ECM) and modulating cancer cell:stroma ECM adhesive contacts for many tumors⁶². In patients with aggressive PDAC, SPARC is overexpressed by PSCs in the PDAC stroma^{63, 64}, and is thought to strongly promote or inhibit cancer cell invasion and metastasis, depending on the experimental context^{65–67}. If PDAC stroma does function to inhibit invasion and metastasis

of PDAC, therapies aimed at eradicating the stroma could actually result in a worse outcome for patients.

Although we do believe that targeting stromal elements does represent a potentially exciting adjuvant therapy for PDAC, we caution that significant questions remain regarding to what extent this approach will be beneficial versus detrimental to patient outcomes. We foresee much progress in the coming years delineating the necessary balance between ablating versus preserving tumor stroma for treating patients with PDAC.

Provocative Issue #5: Ductal adenocarcinomas of the pancreas arise from acinar or islet cells

One of the vexing questions in the cancer research arena pertains to the so-called “cell(s) of origin” for any given cancer type. This concept of hierarchical evolution from an adult stem or progenitor cell population bearing one or more critical driver mutations is probably best established in the field of hematopoietic malignancies^{68, 69}. In solid tumors, experimental models have provided notable insights into the putative cells of origin for various cancers (for example, the finding that murine intestinal adenomas and cancers arise from Lgr5+ crypt stem cells)^{70, 71}, but the human tissue correlates remain to be conclusively established. While it is clear that invasive pancreatic cancer, and the non-invasive precursor lesions from which it arises, including PanINs and intraductal papillary mucinous neoplasms (IPMNs), show ductal differentiation, including recapitulating duct formation histologically and the expression of ductal markers (including cytokeratin 19), one cannot presume that the “cell of origin” of these lesions is the same as their direction of differentiation. Indeed, two attempts at expressing mutant *Kras* from a cytokeratin CK19 ductal promoter in the mouse have resulted in a disappointingly minimal phenotype^{72, 73}.

In the “KPC” mouse model, *Cre*-mediated recombination and expression mutant *Kras* and *Trp53* occurs during development in the entire pancreatic anlage (characterized by Pdx1 expression), precluding precise assessment of the compartment of origin for the resulting murine PanIN and adenocarcinomas^{1, 2}. Subsequent studies have therefore focused on expressing mutant *Kras*, with or without additional cooperating elements like mutant *Trp53* or *Ink4a/Arf*, in specific pancreatic compartments, using either acinar-specific^{55, 74–76}, or islet specific *Cre* “driver” lines⁷⁵. These experiments have found remarkable “plasticity” in mature differentiated pancreatic acinar and islet compartments, with the ability of cells in these compartments to form precursor lesions of ductal adenocarcinoma upon mutant *Kras* expression, a phenotype that is greatly accelerated by concomitant exocrine injury. Thus, at least in the murine models, one postulates that there is no one “cell of origin” for pancreatic cancer, with most compartments in the mature pancreas retaining the ability to transdifferentiate along ductal lineages in the appropriate oncogenic context. In both the developmental Pdx1-*Cre* based and acinar models of mutant *Kras* expression, there is a preponderance of transitional structures characterized by acinar and ductal markers, referred to as acinar-to-ductal metaplasia (ADM)^{74, 77}. It has been suggested that ADMs form the *via media* through which acinar cells transdifferentiate into ductal elements, eventually culminating in histologically discernible mPanIN lesions.

The origin of human pancreatic cancer is difficult, if not impossible, to define because in most instances we see only advanced disease, decades after the earliest events in the development of that neoplasm have occurred. Furthermore, it is likely that invasive cancers obliterate the precursors from which they arose. These challenges can be addressed in two ways. One is a “birds of a feather flock together” approach, the other is to carefully examine pancreata surgically resected early in the course of disease.

The evidence linking PanIN lesions as a precursor to invasive ductal adenocarcinoma is strong. First, PanINs are more common in pancreata with an invasive cancer than they are in pancreata without cancer^{34, 78}. Second, PanINs harbor many of the genetic alterations found in invasive adenocarcinoma of the pancreas⁷⁹. Third, rare cases have been reported in which patients with histologically documented PanIN lesions later develop an invasive adenocarcinoma⁸⁰. Studies of the precursors to PanINs are, however, significantly more challenging in humans.

Eshleman et al used *KRAS* gene mutations to study acinar cells, PanINs and areas of acinar to ductal metaplasia in surgically resected human pancreata⁸¹. All foci of acinar cells studied were *KRAS* gene wild-type, as were all foci of acinar-ductal metaplasia not associated with a PanIN lesion⁸¹. By contrast, all foci of acinar to ductal metaplasia with a *KRAS* gene mutation harbored the exact same mutation as found in their associated PanIN lesions. These results suggest that ductal neoplasms in the human pancreas, as defined by *KRAS* gene mutations, do not appear to arise from acinar cells, and that the mutations found in areas of acinar to ductal metaplasia may simply represent retrograde growth of the PanIN lesions⁸¹. These studies, however, cannot rule out the possibility that *KRAS* mutations do occur in acinar cells and when they do the mutations quickly induce ductal differentiation.

Thus, a growing body of evidence in animal models suggests that pancreatic cancers may not arise in ductal cells. The evidence in humans is sketchy, and if origin outside of a ductal cell does occur the cell must quickly take on a ductal phenotype, making the clinical importance of cell of origin in humans, prior to the development of a PanIN lesion, questionable.

Provocative Issue #6 Specific genes drive pancreatic cancer metastasis

Mouse xenograft models utilizing cells isolated from late-stage, bulky primary human tumors indicate that these lesions are composed of heterogeneous subpopulations of cells. A portion of these subpopulations (possibly clones) are fully capable of metastasis with divergent metastatic site specificities and gene expression signatures. Elegant studies from the Massagué laboratory have identified genes that may control site-specific metastases to particular organs^{82–86}. In many of these studies, neoplastic cells are isolated from advanced primary lesions in patients, and injected into the circulation of immunodeficient mice. Those neoplastic cell subpopulations that metastasize to distant sites in mice are harvested, expanded in culture, re-injected back into mouse circulation, and those that metastasize to the same distant site are harvested again, cultured, and characterized^{82–86}. It is thought that this method selects for subpopulations of cells present in the primary tumor that have a strong tropism for a particular organ^{83–85, 87}. Gene expression profiles are then compared among these selected cell populations and metastatic lesions from the same site in patients, and overlapping genes are then functionally characterized to determine their role in metastatic tropism. Using this approach, genes important for mammary carcinoma metastases to bone, lung, and brain have been characterized^{82–86}. These mouse models therefore suggest that certain genes are misregulated in subpopulations of neoplastic cells at the primary site, which then facilitate homing and colonization of these cells to specific organs once these cells have disseminated into the bloodstream. The fact that heterogeneous subpopulations of cells with divergent metastatic potential are present in advanced primary lesions seems to support a linear progression model of cancer metastasis as originally proposed by Halstead. However, there is an alternative possibility whereby a parallel progression model could explain these findings, which involves a concept referred to as “self-seeding” as described below⁸⁸.

This same group has demonstrated that metastatic-competent subpopulations isolated from the primary tumor described above are also prone to metastasize back to the *primary lesion* once they have colonized a metastatic site, a process termed self-seeding⁸⁸. These cells seem to have the ability to home back to the primary lesion, expand within it, and even stimulate growth of other neoplastic cells within the primary site to generate bulky tumors. Thus, it could be argued that the presence of metastatic-competent subpopulations within late-stage, large human tumors could reflect re-seeding of the primary by neoplastic cells that disseminated and metastasized to other organs early during neoplastic progression. During this “parallel progression”, it could be argued that the evolution of these disseminated cells might be “instructed” by the microenvironment of whatever distant site they encounter, thereby giving rise to metastatic populations of cells with gene expression profiles fit for thriving in this foreign environment. Over time, these cells would generate macrometastases. If they retained an ability to re-seed the original primary tumor, then late-stage lesions would appear to harbor metastasis-competent subpopulations that evolved within the primary tumors as the linear progression model predicts. As outlined below, the possibility of self-seeding raises very significant issues for interpretation of data from human studies.

The identification of specific genes driving metastases in human pancreatic cancer is more challenging than in mouse models. Several “rapid autopsy” programs have been developed to study metastases, and correlation of patterns of spread with genetic alterations in the cancers suggest that PDACs with *SMAD4* inactivation are more likely to disseminate, while PDACs with intact *SMAD4* are more likely to be locally aggressive⁸⁹. To date, more extensive (whole exome) sequencing has failed to identify genetic alterations specific for metastases, and instead have suggested that metastases are late events and that clonal populations that give rise to distant metastases are represented within the primary carcinoma^{90, 91}.

Even more challenging to answer in human tissues is the question of metastases self-seeding the primary. Indeed, if this occurs in man, such a process would greatly cloud the interpretation of studies (e.g. rapid autopsy) comparing the findings in metastases with those in primary carcinomas, since both, in essence, would be metastases. One way the issue of metastases self-seeding the primary could be addressed in man would be serial sampling of a patient’s cancer (such as a resected primary, a local recurrence and then a distant metastasis). Clearly, biosamples will have to be collected with this important issue in mind.

Provocative Issue #7 How many genes are targeted in pancreatic cancer?

Recent technological advances have allowed for unprecedented studies of the genetics of both mouse models of pancreatic cancer and human pancreatic cancer^{92–99}. For example, Pedro Perez-Manchara used Sleeping Beauty transposon-mediated insertional mutagenesis in a mouse model to identify 1150 significant candidate genes which, when mutated, have the potential to contribute to the development of pancreatic cancer⁹³. By contrast, fewer genes are targeted in human PDAC. Jones et al sequenced the exomes of 24 clinically well-characterized PDACs, and Wu et al the exomes of all four types of cystic neoplasms in the pancreas (intraductal papillary mucinous neoplasms, mucinous cystic neoplasms, serous cystadenomas, and solid-pseudopapillary neoplasms)^{94, 96, 97}. In contrast to the large number of genes identified by sleeping beauty mutagenesis, Jones et al found an average of only 63 somatic mutations per tumor in the exomes of the human PDACs⁹⁴. Similarly, Wu et al found an average of 27 mutations in the intraductal papillary mucinous neoplasms, 16 in the mucinous cystic neoplasms, 10 in the serous cystadenomas, and 2.9 in the solid-pseudopapillary neoplasms^{94, 96, 97}.

The numbers of genes altered in mouse models and in human cancers highlight the difference between what can happen in mice and what does happen in humans. Sleeping beauty mutagenesis identified one to two logs more genes than actually appear to be targeted in human pancreatic cancer, and the known driver genes (or “mountains”) in human pancreatic cancer, including *TP53*, *SMAD4* and *p16/CDKN2A* were either not targeted at all in the mice (as in the case of *TP53*), or were targeted at a lower frequency than observed in humans (as seen in *SMAD4* and *p16/CDKN2A*)⁹⁴. Indeed, overall only 14% of the genes found in one study using the Sleeping Beauty mice are known to be mutated in human pancreatic cancer⁹³. These findings in mice can therefore be misleading unless they are validated in studies of human disease.

None the less, the study by Perez-Manchara does highlight the significant power of carefully integrating the findings in mouse models with human disease; as such an integration in this case identified the deubiquitinase *USP9XA* as a potential tumor suppressor gene in human pancreatic cancer⁹³. Sleeping beauty mutagenesis can help identify genes important to a cancer type that might be missed by simple sequencing, particularly if these genes are epigenetically targeted.

Provocative Issue #8 Mouse models of cancer driven by a single gene underestimate the complexity of human disease

Translating pancreatic cancer therapies from the preclinical arena to the clinic has often been fraught with challenges, with striking results in mouse models not reaching commensurate success in humans. Examples include earlier failures using farnesyltransferase inhibitors that targeted post-translational modification of Ras¹⁰⁰, and more recently, with small molecule antagonists of the Hedgehog signaling pathway³. While the underlying reasons for this “benchside to bedside” discordance vary, it does underscore the fallacies of extrapolating from animal model data to predict efficacy in patients.

One advantage that some genetically engineered models harbor is the ability to “turn on” or “turn off” the principal driver oncogene (e.g., *Kras*, *Myc*, etc.) with an extraneously inducible (tetracycline dependent) promoter. In these models, the expression of the driver mutation within tissues of interest can be temporally regulated in order to address the issue of oncogene dependence or “addiction” of neoplastic cells. The concept of oncogene “addiction”, first proposed by Weinstein¹⁰¹, suggests that even brief inactivation of a critical driver may be sufficient to revert the malignant phenotype of the resulting cancer. Indeed, in animal models of Myc-driven sarcomas, transient inactivation of Myc expression alone leads to either regression or differentiation of tumor cells¹⁰². Similar striking tumor regressions have been shown with inducible Ras-dependent models of murine melanoma upon transient downregulation of Ras expression within established lesions¹⁰³. Recently, two groups have extended this strategy to genetically engineered models of pancreatic cancer, by temporally regulated expression of an “inducible Kras” (*iKras*)^{104, 105}. In both of these models, the sustained expression of mutant *Kras* was required not only for the development of non-invasive precursor lesions (i.e., mPanINs), but also for the maintenance of established cancers. Downregulation of mutant *Kras* expression was sufficient in inducing spectacular regression of murine cancers, suggesting an ongoing dependence of pancreatic tumor cells on Ras effector mechanisms. The common thread running through all of these studies is the observation that blocking expression of a single driver oncogene, even transiently, might have a profound impact on the natural history of a cancer that is dependent on that oncogene.

How do these studies measure up to in humans? The results vary based on the particular entity in question. In some neoplasms that are characterized by a single overarching driver

mutation, such as the *bcr-abl* translocation observed in chronic myelogenous leukemia, pharmacological blockade of oncogene function using Imatinib and second-generation inhibitors have, in fact, led to prolonged, albeit not lifelong, cures^{106, 107}. The data in most other solid tumors, however, is far more nuanced. Most human carcinomas are clearly very heterogeneous histologically and genetically^{108–114}. This heterogeneity appears to impact response to therapy. For example, in non-small cell lung carcinomas that have the classic epidermal growth factor receptor (EGFR) activating mutations associated with an oncogene addiction phenotype in lung cancer cell lines^{115, 116}, pharmacological blockade using small molecule EGFR tyrosine kinase inhibitors as a first line therapy does significantly extend the patient's median progression free survival and overall survival compared to conventional chemotherapy, but only by a matter of few months^{117, 118}. Similar nuanced results are observed in *BRAF*-mutant melanomas treated with the recently approved BRAF small molecule inhibitor (vemurafenib) *versus* dacarbazine-based regimens^{119, 120}. In pancreatic cancer, agents that directly block Kras function have not been evaluated beyond the previously cited farnesyltransferase inhibitor trials, as this small GTPase remains incredibly challenging to antagonize at the pharmacological level¹²¹. Nonetheless, one can speculate that because of the significant heterogeneity within pancreatic cancers, the rather spectacular near-tumor regressions observed in inducible *iKras* models is unlikely to be recapitulated in the clinic.

We hasten to add that the point of this discussion is not to undermine the considerable inroads into improving cancer outcomes that molecularly targeted therapies have delivered, but to emphasize the caveats that come with extrapolating “single gene” blocking strategies from mouse to man. There are many reasons why transient oncogene blockade is unlikely to yield results comparable to what is observed in oncogene addicted models, and some of these insights have come from human tumors that have become refractory to targeted therapies. These include the expansion of cell populations with secondary mutations that cause resistance to first-line targeted therapies, as well as upregulation of compensatory oncogenic / growth factor pathways^{122, 123}.

Closing Thoughts

While it is clear that an understanding of human pathology is important for the interpretation of animal models, we believe that an understanding of human pathology is equally important for our clinical colleagues. At a TEDMED conference in 2012, Dr. Otis Brawley, the Chief Medical Officer of the American Cancer Society called for a “21st century” approach to cancer diagnosis, and stated that “pathologists still use drawings made in 1840 to determine which cells in a biopsy are cancerous.” (<http://www.technologyreview.com/blog/editors/27742/?p1=blogs>, accessed 4/18/2012)¹²⁴. In fact, this is not an uncommon belief among clinicians unfamiliar with the complexities of modern surgical pathology. We therefore decided to research this question and went to the Institute of the History of Medicine at the Johns Hopkins University School of Medicine, the oldest history of medicine department in the United States.

We found that the great Italian morbid anatomist Giovanni Battista Morgagni vividly described the symptoms caused by pancreatic cancer when he described the pain a patient of his suffered as “as if he were being torn to pieces by dogs,” the descriptions of the microscopic appearance of pancreatic cancer in the middle of the nineteenth century were atrocious. For example, Julius Vogel in his 1843 book “*Pathologischen Histologie*” described a 38 year old patient with pancreatic cancer and ascites¹²⁵. The illustration of the histologic appearance of her/his cancer is however, not diagnosable (Figure 2). None of the six histologic features commonly used to establish a diagnosis of pancreatic cancer-1) haphazard arrangement of glands, 2) perineural invasion, 3) vascular invasion, 4) nuclear

variation greater than 4 to 1, 5) incomplete lumina, and 6) a gland next to a muscular vessel-are present²⁵. In fact, the illustration focuses on the fibers in the desmoplastic stroma and looks more like a fern than a pancreatic cancer.

What about the tools that are commonly used in diagnostic surgical pathology today? Are the tools used today the same as those used in the 1840s as Dr. Brawley suggests? The routine stain used in the vast majority of surgical pathology laboratories today is the hematoxylin and eosin, or “H and E,” stain. Hematoxylin, a dye derived from the *Haematoxylon campechianum* tree, was first applied to tissue sections by Bohmer in 1865, and eosin was synthesized by Baeyer in 1871 and first used to stain tissue sections in 1876 by Dreschfeld and Fischer¹²⁶. Neither stain was used in the 1840s. Immunohistochemistry (IHC) is another tool used in the daily practice of diagnostic surgical pathology. While the detection of antigens in tissue sections dates back to 1941 when Coons and colleagues labeled an antibody with fluorescent dye and used it to identify an antigen in tissue sections, it really wasn't until the 1970s that IHC began to be applied more routinely to tissue diagnoses. The third arm of modern surgical pathology, that of molecular genetics, is even more recent than IHC. For example, Smad4 loss can be used to suggest that a neoplasm arose in the pancreas, but the *SMAD4* gene wasn't discovered until 1996 and IHC was first applied to detect loss of the protein, Smad4, in tissues in 2000^{127, 128}. Clearly, the foundations on which modern surgical pathology laboratories are built, H and E staining, IHC and molecular genetic analyses, were not available in the 1840s.

All joking aside, this is a serious issue. Microscopy remains at the center of diagnostic pathology not because diagnostic pathologists are stuck in the 19th Century, instead, microscopy remains at the center of modern pathology because extensive evidence-based medicine has established that in the vast majority of instances, microscopy remains the fastest, most sensitive, and most specific way to establish accurate diagnoses. We should not, as Dr. Brawley suggests, simply adopt “molecular” approaches simply because they are novel. Instead, each new approach should be examined carefully side-by-side with existing methodologies. Those found to be superior should be adopted, those novel but of no added benefit should not.

This is not a case of John Henry versus the steam powered hammer. Instead it is a balanced evidence-based approach to the practice of modern medicine.

Acknowledgments

Supported by: NIH grant P50CA062924 and the Ruth Kirschstein National Research Service Award

References

1. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM, Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell*. 2003; 4:437–450. [PubMed: 14706336]
2. Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell*. 2005; 7:469–483. [PubMed: 15894267]
3. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Ruckert F, Grutzmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition

- of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science*. 2009; 324:1457–1461. [PubMed: 19460966]
4. Feig C, Gopinathan SK, Neesse A, Chan D, Cook N, Tuveson DA. The pancreas cancer microenvironment. *Clinical Cancer Research*. 2012; 18:xx–xx.
 5. Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, Boivin GP, Furth EE, Furukawa T, Klein AP, Klimstra DS, Klöppel G, Lauwers GY, Longnecker DS, Lüttges J, Maitra A, Offerhaus GJ, Perez-Gallego L, Redston M, Tuveson DA. Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. *Cancer Research*. 2006; 66:95–106. [PubMed: 16397221]
 6. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009; 139:871–890. [PubMed: 19945376]
 7. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *The Journal of clinical investigation*. 2009; 119:1420–1428. [PubMed: 19487818]
 8. Krantz SB, Shields MA, Dangi-Garimella S, Munshi HG, Bentrem DJ. Contribution of epithelial-to-mesenchymal transition and cancer stem cells to pancreatic cancer progression. *J Surg Res*. 2012; 173:105–112. [PubMed: 22099597]
 9. Wang Z, Li Y, Ahmad A, Banerjee S, Azmi AS, Kong D, Sarkar FH. Pancreatic cancer: understanding and overcoming chemoresistance. *Nature reviews Gastroenterology & hepatology*. 2011; 8:27–33.
 10. Greenburg G, Hay ED. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J Cell Biol*. 1982; 95:333–339. [PubMed: 7142291]
 11. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res*. 2008; 68:3645–3654. [PubMed: 18483246]
 12. Trimboli AJ, Fukino K, de Bruin A, Wei G, Shen L, Tanner SM, Creasap N, Rosol TJ, Robinson ML, Eng C, Ostrowski MC, Leone G. Direct evidence for epithelial-mesenchymal transitions in breast cancer. *Cancer Res*. 2008; 68:937–945. [PubMed: 18245497]
 13. Rasheed ZA, Yang J, Wang Q, Kowalski J, Freed I, Murter C, Hong SM, Koorstra JB, Rajeshkumar NV, He X, Goggins M, Iacobuzio-Donahue C, Berman DM, Laheru D, Jimeno A, Hidalgo M, Maitra A, Matsui W. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. *J Natl Cancer Inst*. 2010; 102:340–351. [PubMed: 20164446]
 14. Masugi Y, Yamazaki K, Hibi T, Aiura K, Kitagawa Y, Sakamoto M. Solitary cell infiltration is a novel indicator of poor prognosis and epithelial-mesenchymal transition in pancreatic cancer. *Hum Pathol*. 2010; 41:1061–1068. [PubMed: 20413143]
 15. Winter JM, Ting AH, Vilardeell F, Gallmeier E, Baylin SB, Hruban RH, Kern SE, Iacobuzio-Donahue CA. Absence of E-cadherin expression distinguishes noncohesive from cohesive pancreatic cancer. *Clin Cancer Res*. 2008; 14:412–418. [PubMed: 18223216]
 16. Scheel C, Weinberg RA. Cancer stem cells and epithelial-mesenchymal transition: Concepts and molecular links. *Semin Cancer Biol*. 2012
 17. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Briskin C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008; 133:704–715. [PubMed: 18485877]
 18. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, Lander ES. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell*. 2009; 138:645–659. [PubMed: 19682730]
 19. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and characterization of gemcitabine-resistant pancreatic tumor cells. *Ann Surg Oncol*. 2007; 14:3629–3637. [PubMed: 17909916]
 20. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell stem cell*. 2007; 1:313–323. [PubMed: 18371365]

21. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res.* 2007; 67:1030–1037. [PubMed: 17283135]
22. Arumugam T, Ramachandran V, Fournier KF, Wang H, Marquis L, Abbruzzese JL, Gallick GE, Logsdon CD, McConkey DJ, Choi W. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. *Cancer Res.* 2009; 69:5820–5828. [PubMed: 19584296]
23. Wang Z, Li Y, Kong D, Banerjee S, Ahmad A, Azmi AS, Ali S, Abbruzzese JL, Gallick GE, Sarkar FH. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. *Cancer Res.* 2009; 69:2400–2407. [PubMed: 19276344]
24. Hong SP, Wen J, Bang S, Park S, Song SY. CD44-positive cells are responsible for gemcitabine resistance in pancreatic cancer cells. *International journal of cancer Journal international du cancer.* 2009; 125:2323–2331. [PubMed: 19598259]
25. Hruban, RH.; Pitman, MB.; Klimstra, DS. Tumors of the pancreas. Atlas of tumor pathology. Washington, DC: American Registry of Pathology and Armed Forces Institute of Pathology; 2007.
26. Brabletz T, Hlubek F, Spaderna S, Schmalhofer O, Hiendlmeyer E, Jung A, Kirchner T. Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. *Cells, tissues organs.* 2005; 179:56–65. [PubMed: 15942193]
27. Wicki A, Lehenbre F, Wick N, Hantusch B, Kerjaschki D, Christofori G. Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell.* 2006; 9:261–272. [PubMed: 16616332]
28. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. *Cell.* 2012; 148:349–361. [PubMed: 22265420]
29. Chari ST, Yadav D, Smyrk TC, DiMagno EP, Miller LJ, Raimondo M, Clain JE, Norton IA, Pearson RK, Petersen BT, Wiersema MJ, Farnell MB, Sarr MG. Study of recurrence after surgical resection of intraductal papillary mucinous neoplasm of the pancreas. *Gastroenterology.* 2002; 123:1500–1507. [PubMed: 12404225]
30. Traverso LW, Moriya T, Hashimoto Y. Intraductal Papillary Mucinous Neoplasms of the Pancreas: Making a Disposition Using the Natural History. *Curr Gastroenterol Rep.* 2012
31. Tanaka M, Fernandez-Del Castillo C, Adsay V, Chari S, Falconi M, Jang JY, Kimura W, Levy P, Pitman MB, Schmidt CM, Shimizu M, Wolfgang CL, Yamaguchi K, Yamao K. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology.* 2012; 12:183–197. [PubMed: 22687371]
32. Canto MI, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, Fishman EK, Brune K, Axilbund J, Griffin C, Ali S, Richman J, Jagannath S, Kantsevoy SV, Kalloo AN. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2006; 4:766–781. quiz 665. [PubMed: 16682259]
33. Canto MI, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, Topazian M, Takahashi N, Fletcher J, Petersen G, Klein AP, Axilbund J, Griffin C, Syngal S, Saltzman JR, Morteale KJ, Lee J, Tamm E, Vikram R, Bhosale P, Margolis D, Farrell J, Goggins M. Frequent Detection of Pancreatic Lesions in Asymptomatic High-Risk Individuals. *Gastroenterology.* 2012
34. Kozuka S, Sassa R, Taki T, Masamoto K, Nagasawa S, Saga S, Hasegawa K, Takeuchi M. Relation of pancreatic duct hyperplasia to carcinoma. *Cancer.* 1979; 43:1418–1428. [PubMed: 445339]
35. Burris HA III, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol.* 1997; 15:2403–2413. [PubMed: 9196156]
36. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardiere C, Bennouna J, Bachet JB, Khemissa-Akouz F, Pere-Verge D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M.

- FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *The New England journal of medicine*. 2011; 364:1817–1825. [PubMed: 21561347]
37. Hwang RF, Moore T, Arumugam T, Ramachandran V, Amos KD, Rivera A, Ji B, Evans DB, Logsdon CD. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res*. 2008; 68:918–926. [PubMed: 18245495]
 38. Hwang RF, Moore TT, Mertens Hattersley M, Scarpitti M, Yang B, Devereaux E, Ramachandran V, Arumugam T, Ji B, Logsdon CD, Brown JL, Godin R. Inhibition of the Hedgehog Pathway Targets the Tumor-Associated Stroma in Pancreatic Cancer. *Molecular cancer research : MCR*. 2012
 39. Jacobetz MA, Chan DS, Neesse A, Bapiro TE, Cook N, Frese KK, Feig C, Nakagawa T, Caldwell ME, Zecchini HI, Lolkema MP, Jiang P, Kultti A, Thompson CB, Maneval DC, Jodrell DI, Frost GI, Shepard HM, Skepper JN, Tuveson DA. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut*. 2012
 40. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell*. 2012; 21:418–429. [PubMed: 22439937]
 41. Brune KA, Abe T, Canto MI, O'Malley L, Klein AP, Maitra A, Adsay NV, Fishman EK, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH. Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. *American Journal of Surgical Pathology*. 2006; 30:1067–1076. [PubMed: 16931950]
 42. Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA, Fearon DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein- α . *Science*. 2010; 330:827–830. [PubMed: 21051638]
 43. Apte MV, Wilson JS. Dangerous liaisons: pancreatic stellate cells and pancreatic cancer cells. *J Gastroenterol Hepatol*. 2012; 27 (Suppl 2):69–74. [PubMed: 22320920]
 44. Berman DM, Karhadkar SS, Maitra A, Montes DO, Gerstenblith MR, Briggs K, Parker AR, Shimada Y, Eshleman JR, Watkins DN, Beachy PA. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature*. 2003; 425:846–851. [PubMed: 14520411]
 45. Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernandez-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature*. 2003; 425:851–856. [PubMed: 14520413]
 46. Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, Karikari C, Alvarez H, Iacobuzio-Donahue C, Jimeno A, Gabrielson KL, Matsui W, Maitra A. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res*. 2007; 67:2187–2196. [PubMed: 17332349]
 47. Yauch RL, Gould SE, Scales SJ, Tang T, Tian H, Ahn CP, Marshall D, Fu L, Januario T, Kallop D, Nannini-Pepe M, Kotkow K, Marsters JC, Rubin LL, de Sauvage FJ. A paracrine requirement for hedgehog signalling in cancer. *Nature*. 2008; 455:406–410. [PubMed: 18754008]
 48. Tian H, Callahan CA, DuPree KJ, Darbonne WC, Ahn CP, Scales SJ, de Sauvage FJ. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. *Proc Natl Acad Sci U S A*. 2009; 106:4254–4259. [PubMed: 19246386]
 49. Kikuta K, Masamune A, Watanabe T, Ariga H, Itoh H, Hamada S, Satoh K, Egawa S, Unno M, Shimosegawa T. Pancreatic stellate cells promote epithelial-mesenchymal transition in pancreatic cancer cells. *Biochem Biophys Res Commun*. 2010; 403:380–384. [PubMed: 21081113]
 50. Erkan M, Reiser-Erkan C, Michalski CW, Deucker S, Sauliunaite D, Streit S, Esposito I, Friess H, Kleeff J. Cancer-stellate cell interactions perpetuate the hypoxia-fibrosis cycle in pancreatic ductal adenocarcinoma. *Neoplasia*. 2009; 11:497–508. [PubMed: 19412434]
 51. Nieto MA. The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annual review of cell and developmental biology*. 2011; 27:347–376.
 52. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, Sharp LL, Torigian DA, O'Dwyer PJ, Vonderheide RH. CD40 agonists alter tumor stroma and

- show efficacy against pancreatic carcinoma in mice and humans. *Science*. 2011; 331:1612–1616. [PubMed: 21436454]
53. Lowenfels AB, Maisonneuve P, Whitcomb DC. Risk factors for cancer in hereditary pancreatitis. International Hereditary Pancreatitis Study Group. *Med Clin North Am*. 2000; 84:565–573. [PubMed: 10872414]
 54. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. *BestPractResClin Gastroenterol*. 2006; 20:197–209.
 55. Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, Dubus P, Sandgren EP, Barbacid M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by k-ras oncogenes in adult mice. *Cancer Cell*. 2007; 11:291–302. [PubMed: 17349585]
 56. Binkley CE, Zhang L, Greenson JK, Giordano TJ, Kuick R, Misek D, Hanash S, Logsdon CD, Simeone DM. The molecular basis of pancreatic fibrosis: common stromal gene expression in chronic pancreatitis and pancreatic adenocarcinoma. *Pancreas*. 2004; 29:254–263. [PubMed: 15502640]
 57. Guerra C, Collado M, Navas C, Schuhmacher AJ, Hernandez-Porras I, Canamero M, Rodriguez-Justo M, Serrano M, Barbacid M. Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. *Cancer Cell*. 2011; 19:728–739. [PubMed: 21665147]
 58. Ijichi H, Chytil A, Gorska AE, Aakre ME, Bierie B, Tada M, Mohri D, Miyabayashi K, Asaoka Y, Maeda S, Ikenoue T, Tateishi K, Wright CV, Koike K, Omata M, Moses HL. Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. *The Journal of clinical investigation*. 2011; 121:4106–4117. [PubMed: 21926469]
 59. Daniluk J, Liu Y, Deng D, Chu J, Huang H, Gaiser S, Cruz-Monserrate Z, Wang H, Ji B, Logsdon CD. An NF-kappaB pathway-mediated positive feedback loop amplifies Ras activity to pathological levels in mice. *The Journal of clinical investigation*. 2012; 122:1519–1528. [PubMed: 22406536]
 60. Bloomston M, Zervos EE, Rosemurgy AS 2nd. Matrix metalloproteinases and their role in pancreatic cancer: a review of preclinical studies and clinical trials. *Ann Surg Oncol*. 2002; 9:668–674. [PubMed: 12167581]
 61. Mahadevan D, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. *Molecular cancer therapeutics*. 2007; 6:1186–1197. [PubMed: 17406031]
 62. Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *The Journal of clinical investigation*. 2001; 107:1049–1054. [PubMed: 11342565]
 63. Infante JR, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, Yeo C, Iacobuzio-Donahue C, Goggins M. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2007; 25:319–325. [PubMed: 17235047]
 64. Mantoni TS, Schendel RR, Rodel F, Niedobitek G, Al-Assar O, Masamune A, Brunner TB. Stromal SPARC expression and patient survival after chemoradiation for non-resectable pancreatic adenocarcinoma. *Cancer Biol Ther*. 2008; 7:1806–1815. [PubMed: 18787407]
 65. Guweidhi A, Kleeff J, Adwan H, Giese NA, Wente MN, Giese T, Buchler MW, Berger MR, Friess H. Osteonectin influences growth and invasion of pancreatic cancer cells. *Ann Surg*. 2005; 242:224–234. [PubMed: 16041213]
 66. Puolakkainen PA, Brekken RA, Muneer S, Sage EH. Enhanced growth of pancreatic tumors in SPARC-null mice is associated with decreased deposition of extracellular matrix and reduced tumor cell apoptosis. *Molecular cancer research : MCR*. 2004; 2:215–224. [PubMed: 15140943]
 67. Seux M, Peugeot S, Montero MP, Siret C, Rigot V, Clerc P, Gigoux V, Pellegrino E, Pouyet L, N'Guessan P, Garcia S, Dufresne M, Iovanna JL, Carrier A, Andre F, Dusetti NJ. TP53INP1 decreases pancreatic cancer cell migration by regulating SPARC expression. *Oncogene*. 2011; 30:3049–3061. [PubMed: 21339733]

68. Notta F, Mullighan CG, Wang JC, Poepl A, Doulatov S, Phillips LA, Ma J, Minden MD, Downing JR, Dick JE. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. *Nature*. 2011; 469:362–367. [PubMed: 21248843]
69. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. 1994; 367:645–648. [PubMed: 7509044]
70. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature*. 2009; 457:608–611. [PubMed: 19092804]
71. Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, Clevers H. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. *Science*. 2012; 337:730–735. [PubMed: 22855427]
72. Brembeck FH, Schreiber FS, Deramaudt TB, Craig L, Rhoades B, Swain G, Grippo P, Stoffers DA, Silberg DG, Rustgi AK. The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. *Cancer Research*. 2003; 63:2005–2009. [PubMed: 12727809]
73. Ray KC, Bell KM, Yan J, Gu G, Chung CH, Washington MK, Means AL. Epithelial tissues have varying degrees of susceptibility to Kras(G12D)-initiated tumorigenesis in a mouse model. *PloS one*. 2011; 6:e16786. [PubMed: 21311774]
74. Habbe N, Shi G, Meguid RA, Fendrich V, Esni F, Chen H, Feldmann G, Stoffers DA, Konieczny SF, Leach SD, Maitra A. 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; 105:18913–18918. [PubMed: 19028870]
75. Gidekel Friedlander SY, Chu GC, Snyder EL, Girnius N, Dibelius G, Crowley D, Vasile E, DePinho RA, Jacks T. Context-dependent transformation of adult pancreatic cells by oncogenic K-Ras. *Cancer Cell*. 2009; 16:379–389. [PubMed: 19878870]
76. De La OJ, Emerson LL, Goodman JL, Froebe SC, Illum BE, Curtis AB, Murtaugh LC. Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. *Proc Natl Acad Sci U S A*. 2008; 105:18907–18912. [PubMed: 19028876]
77. Shi G, Zhu L, Sun Y, Bettencourt R, Damsz B, Hruban RH, Konieczny SF. Loss of the Acinar-Restricted Transcription Factor Mist1 Accelerates Kras-Induced Pancreatic Intraepithelial Neoplasia. *Gastroenterology*. 2009
78. Cubilla AL, Fitzgerald PJ. Morphological lesions associated with human primary invasive nonendocrine pancreas cancer. *Cancer Research*. 1976; 36:2690–2698. [PubMed: 1277176]
79. Iacobuzio-Donahue CA, Velculescu VE, Wolfgang CL, Hruban RH. Genetic basis of pancreas cancer development and progression: insights from whole-exome and whole-genome sequencing. *Clin Cancer Res*. 2012; 18:4257–4265. [PubMed: 22896692]
80. Brat DJ, Lillemoe KD, Yeo CJ, Warfield PB, Hruban RH. Progression of pancreatic intraductal neoplasias to infiltrating adenocarcinoma of the pancreas. *Am J Surg Pathol*. 1998; 22:163–169. [PubMed: 9500216]
81. Shi C, Hong SM, Lim P, Kamiyama H, Khan M, Anders RA, Goggins M, Hruban RH, Eshleman JR. KRAS2 mutations in human pancreatic acinar-ductal metaplastic lesions are limited to those with PanIN: implications for the human pancreatic cancer cell of origin. *MolCancer Res*. 2009; 7:230–236.
82. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell*. 2003; 3:537–549. [PubMed: 12842083]
83. Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. Genes that mediate breast cancer metastasis to lung. *Nature*. 2005; 436:518–524. [PubMed: 16049480]
84. Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R, Massague J. Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *The Journal of clinical investigation*. 2005; 115:44–55. [PubMed: 15630443]

85. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, Minn AJ, van de Vijver MJ, Gerald WL, Foekens JA, Massague J. Genes that mediate breast cancer metastasis to the brain. *Nature*. 2009; 459:1005–1009. [PubMed: 19421193]
86. Zhang XH, Wang Q, Gerald W, Hudis CA, Norton L, Smid M, Foekens JA, Massague J. Latent bone metastasis in breast cancer tied to Src-dependent survival signals. *Cancer Cell*. 2009; 16:67–78. [PubMed: 19573813]
87. Gupta GP, Minn AJ, Kang Y, Siegel PM, Serganova I, Cordon-Cardo C, Olshen AB, Gerald WL, Massague J. Identifying site-specific metastasis genes and functions. *Cold Spring Harbor symposia on quantitative biology*. 2005; 70:149–158.
88. Comen E, Norton L, Massague J. Clinical implications of cancer self-seeding. *Nature reviews Clinical oncology*. 2011; 8:369–377.
89. Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, Herman JM, Cameron JL, Yeo CJ, Halushka MK, Eshleman JR, Raben M, Klein AP, Hruban RH, Hidalgo M, Laheru D. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009; 27:1806–1813. [PubMed: 19273710]
90. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature*. 2010; 467:1114–1117. [PubMed: 20981102]
91. Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, Morsberger LA, Latimer C, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal SA, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Griffin CA, Burton J, Swerdlow H, Quail MA, Stratton MR, Iacobuzio-Donahue C, Futreal PA. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature*. 2010; 467:1109–1113. [PubMed: 20981101]
92. Mann KM, Ward JM, Yew CC, Kovochich A, Dawson DW, Black MA, Brett BT, Sheetz TE, Dupuy AJ, Chang DK, Biankin AV, Waddell N, Kassahn KS, Grimmond SM, Rust AG, Adams DJ, Jenkins NA, Copeland NG. Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma. *Proc Natl Acad Sci U S A*. 2012; 109:5934–5941. [PubMed: 22421440]
93. Perez-Mancera PA, Rust AG, van der Weyden L, Kristiansen G, Li A, Sarver AL, Silverstein KA, Grutzmann R, Aust D, Rummele P, Knosel T, Herd C, Stemple DL, Kettleborough R, Brosnan JA, Morgan R, Knight S, Yu J, Stegeman S, Collier LS, ten Hoeve JJ, de Ridder J, Klein AP, Goggins M, Hruban RH, Chang DK, Biankin AV, Grimmond SM, Wessels LF, Wood SA, Iacobuzio-Donahue CA, Pilarsky C, Largaespada DA, Adams DJ, Tuveson DA. The deubiquitinase USP9X suppresses pancreatic ductal adenocarcinoma. *Nature*. 2012; 486:266–270. [PubMed: 22699621]
94. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*. 2008; 321:1801–1806. [PubMed: 18772397]
95. Jones S, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, Lin JC, Palmisano E, Brune K, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Parmigiani G, Kern SE, Velculescu VE, Kinzler KW, Vogelstein B, Eshleman JR, Goggins M, Klein AP. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science*. 2009; 324:217. [PubMed: 19264984]
96. Wu J, Jiao Y, Dal Molin M, Maitra A, de Wilde RF, Wood LD, Eshleman JR, Goggins MG, Wolfgang CL, Canto MI, Schulick RD, Edil BH, Choti MA, Adsay V, Klimstra DS, Offerhaus GJ, Klein AP, Kopelovich L, Carter H, Karchin R, Allen PJ, Schmidt CM, Naito Y, Diaz LA Jr, Kinzler KW, Papadopoulos N, Hruban RH, Vogelstein B. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. *Proc Natl Acad Sci U S A*. 2011; 108:21188–21193. [PubMed: 22158988]

97. Wu J, Matthaei H, Maitra A, Dal Molin M, Wood LD, Eshleman JR, Goggins M, Canto MI, Schulick RD, Edil BH, Wolfgang CL, Klein AP, Diaz LA Jr, Allen PJ, Schmidt CM, Kinzler KW, Papadopoulos N, Hruban RH, Vogelstein B. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. *Science translational medicine*. 2011; 3:92ra66.
98. Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, Schulick RD, Tang LH, Wolfgang CL, Choti MA, Velculescu VE, Diaz LA Jr, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N. DAXX/ATRAX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science*. 2011; 331:1199–1203. [PubMed: 21252315]
99. Roberts NJ, Jiao Y, Yu J, Kopelovich L, Petersen GM, Bondy ML, Gallinger S, Schwartz AG, Syngal S, Cote ML, Axilbund J, Schulick R, Ali SZ, Eshleman JR, Velculescu VE, Goggins M, Vogelstein B, Papadopoulos N, Hruban RH, Kinzler KW, Klein AP. ATM mutations in patients with hereditary pancreatic cancer. *Cancer Discov*. 2012; 2:41–46. [PubMed: 22585167]
100. Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2004; 22:1430–1438. [PubMed: 15084616]
101. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heel of cancer. *Science*. 2002; 297:63–64. [PubMed: 12098689]
102. Jain M, Arvanitis C, Chu K, Dewey W, Leonhardt E, Trinh M, Sundberg CD, Bishop JM, Felsher DW. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science*. 2002; 297:102–104. [PubMed: 12098700]
103. Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou H, Horner JW 2nd, Cordon-Cardo C, Yancopoulos GD, DePinho RA. Essential role for oncogenic Ras in tumour maintenance. *Nature*. 1999; 400:468–472. [PubMed: 10440378]
104. Collins MA, Bednar F, Zhang Y, Brisset JC, Galban S, Galban CJ, Rakshit S, Flannagan KS, Adsay NV, Pasca di Magliano M. Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *The Journal of clinical investigation*. 2012; 122:639–653. [PubMed: 22232209]
105. Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E, Locasale JW, Son J, Zhang H, Colloff JL, Yan H, Wang W, Chen S, Viale A, Zheng H, Paik JH, Lim C, Guimaraes AR, Martin ES, Chang J, Hezel AF, Perry SR, Hu J, Gan B, Xiao Y, Asara JM, Weissleder R, Wang YA, Chin L, Cantley LC, DePinho RA. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell*. 2012; 149:656–670. [PubMed: 22541435]
106. Sherbenou DW, Druker BJ. Applying the discovery of the Philadelphia chromosome. *The Journal of clinical investigation*. 2007; 117:2067–2074. [PubMed: 17671641]
107. Sherbenou DW, Wong MJ, Humayun A, McGreevey LS, Harrell P, Yang R, Mauro M, Heinrich MC, Press RD, Druker BJ, Deininger MW. Mutations of the BCR-ABL-kinase domain occur in a minority of patients with stable complete cytogenetic response to imatinib. *Leukemia*. 2007; 21:489–493. [PubMed: 17252009]
108. Diaz LA Jr, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, Allen B, Bozic I, Reiter JG, Nowak MA, Kinzler KW, Oliner KS, Vogelstein B. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*. 2012; 486:537–540. [PubMed: 22722843]
109. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K. Molecular definition of breast tumor heterogeneity. *Cancer Cell*. 2007; 11:259–273. [PubMed: 17349583]
110. Park SY, Lee HE, Li H, Shipitsin M, Gelman R, Polyak K. Heterogeneity for stem cell-related markers according to tumor subtype and histologic stage in breast cancer. *Clin Cancer Res*. 2010; 16:876–887. [PubMed: 20103682]
111. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. *Biochim Biophys Acta*. 2010; 1805:105–117. [PubMed: 19931353]
112. Polyak K. Heterogeneity in breast cancer. *The Journal of clinical investigation*. 2011; 121:3786–3788. [PubMed: 21965334]

113. Bhatia S, Frangioni JV, Hoffman RM, Iafrate AJ, Polyak K. The challenges posed by cancer heterogeneity. *Nat Biotechnol.* 2012; 30:604–610. [PubMed: 22781679]
114. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? *Nature reviews Cancer.* 2012; 12:323–334.
115. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *The New England journal of medicine.* 2004; 350:2129–2139. [PubMed: 15118073]
116. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science.* 2004; 304:1497–1500. [PubMed: 15118125]
117. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P, Longo F, Moran T, Insa A, De Marinis F, Corre R, Bover I, Illiano A, Dansin E, de Castro J, Milella M, Reguart N, Altavilla G, Jimenez U, Provencio M, Moreno MA, Terrasa J, Munoz-Langa J, Valdivia J, Isla D, Domine M, Molinier O, Mazieres J, Baize N, Garcia-Campelo R, Robinet G, Rodriguez-Abreu D, Lopez-Vivanco G, Gebbia V, Ferrera-Delgado L, Bombaron P, Bernabe R, Bearz A, Artal A, Cortesi E, Rolfo C, Sanchez-Ronco M, Drozdowskyj A, Queralto C, de Aguirre I, Ramirez JL, Sanchez JJ, Molina MA, Taron M, Paz-Ares L. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EORTC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* 2012; 13:239–246. [PubMed: 22285168]
118. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S, Nukiwa T. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *The New England journal of medicine.* 2010; 362:2380–2388. [PubMed: 20573926]
119. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *The New England journal of medicine.* 2011; 364:2507–2516. [PubMed: 21639808]
120. Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *The New England journal of medicine.* 2012; 366:707–714. [PubMed: 22356324]
121. Gysin S, Salt M, Young A, McCormick F. Therapeutic strategies for targeting ras proteins. *Genes & cancer.* 2011; 2:359–372. [PubMed: 21779505]
122. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science.* 2007; 316:1039–1043. [PubMed: 17463250]
123. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature.* 2012; 483:100–103. [PubMed: 22281684]
124. Young, S. MIT, editor. [Accessed September 11, 2012] Cancer Medicine is Stuck in the Past. The chief medical officer of the American Cancer Society calls for a genomics-based approach. 2012. Available at: <http://www.technologyreview.com/blog/editors/27742/?p1=blogs>
125. Vogel, J. Erläuterungstafeln zur Pathologischen Histologie: mit Vorzüglicher Rücksicht auf sein Handbuch der Pathologischen Anatomie. Leipzig, Germany: Leopold Boss; 1843.

126. King DF, King LA. A brief historical note on staining by hematoxylin and eosin. *Am J Dermatopathol.* 1986; 8:168. [PubMed: 2424331]
127. Wilentz RE, Su GH, Dai JL, Sparks AB, Argani P, Sohn TA, Yeo CJ, Kern SE, Hruban RH. Immunohistochemical labeling for Dpc4 mirrors genetic status in pancreatic adenocarcinomas: a new marker of DPC4 inactivation. *American Journal of Pathology.* 2000; 156:37–43. [PubMed: 10623651]
128. Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science.* 1996; 271:350–353. [PubMed: 8553070]

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.

Infiltrating ductal adenocarcinoma of the pancreas. Well-differentiated carcinoma in the muscularis propria of the duodenum (A) and around nerves (B). Note how the neoplastic cells closely recapitulate normal ducts. C) Immunolabeling of for e-cadherin demonstrating intact membranous expression. D) Several individual infiltrating neoplastic cells (top left) associated with a better-differentiated neoplastic gland (lower right). E) As demonstrated in this image, most pancreatic cancers illicit a desmoplastic stroma. F) “Lipidic” growth pattern of a pancreatic cancer metastatic to the lungs. Note how the neoplastic cells are immediately adjacent to capillaries.

Figure 2. Illustration of a pancreatic cancer from *Pathologischen Histologie* by Julius Vogel published in 1843¹²⁵. The original figure legends for this illustration describe the “fibers which made up the pancreatic tumor,” and interspersed “fat globules.”