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Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development?

Ilse Rooman,[1] Francisco X Real[2,3]

[1]Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst-Sydney, NSW, Australia
[2]Programa de Patología Molecular, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain
[3]Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain

Correspondence to
Francisco X Real, CNIO, Melchor Fernandez Almagro, 3, Madrid 28029, Spain;
preal@cnio.es and
Ilse Rooman, Garvan Institute, 384 Victoria St, NSW 2010 Sydney, Australia;
i.rooman@garvan.org.au

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) has long been considered to arise from pancreatic ducts on the basis of its morphology, the occurrence of dysplasia in putative preneoplastic ductal lesions, and the absence of acinar dysplasia in the pancreas of patients with PDAC. However, evidence gathered through both in vitro studies and—more importantly—genetic mouse models of PDAC shows that ductal-type tumours can arise from acinar cells. These findings raise new important questions related to PDAC pathophysiology and call for in-depth studies of acinar cell differentiation in order to better understand PDAC biology. The authors review these issues and discuss how the novel findings should impact on future work aiming at early diagnosis and improved outcome of patients with PDAC.

PANCREATIC DUCTAL ADENOCARCINOMA (PDAC): THE PROBLEMS

The 5-year survival rate of patients with PDAC is <5%.[1] In patients whose tumour is resectable and who receive adjuvant chemotherapy (~15% of PDAC cases), the 5-year survival rate is up to 20%,[2] indicating that early detection can improve outcome. The overall outcomes for PDAC have in fact not changed for almost 50 years.[1]

PDAC preneoplastic lesions

A major advance in the pathological assessment of PDAC has been the consensus on the nomenclature and classification of ductal preneoplastic lesions.[3 4] A linear progression model for PDAC has been proposed according to which normal ductal cells evolve to a hyperplastic epithelium without dysplasia (pancreatic intraepithelial neoplasia (PanIN)-1A for a flat epithelium and PanIN-1B for papillary hyperplasia), then acquiring increasing levels of dysplasia (PanIN-2 and PanIN-3). PanIN-3 represents carcinoma in situ and it is the precursor to invasive carcinoma.

PanIN-1 lesions are common in older people and probably involve a low risk of developing PDAC, whereas high-risk PanIN-3 lesions are almost exclusively found in patients with invasive PDAC.[5] Therefore PanIN-2 lesions can be regarded as a turning point in this sequence of progression and as a major target for further study. Other mucinous precursor lesions, such as intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms, are subjects of increasing attention because of their raising incidence and they provide greater opportunities for improved clinical management.[6 7]

The PanIN sequence is paralleled genetically by telomere shortening and accumulation of activating point mutations in *K-RAS* codon 12 (~85% of cases), *p16/CDKN2A* inactivation (~100% of lesions), and—at later stages—*TP53* and *SMAD4/DPC4* inactivation (60–80% of tumours).[4 8] A study by Jones *et al* showed that PDAC harbours an average of 63 genetic abnormalities (mainly point mutations).[9] Genomic-scale data on tumour evolution are rapidly being generated using massive parallel sequencing.[10]

The linear progression model: up for review?

The linear model of PDAC progression is likely to be an oversimplification.[11] There is no formal evidence that PanIN-1 lesions are required for the development of PanIN-2 or PanIN-3 or PDAC. It is also conceivable that multiple roads may lead to PDAC and that different PanINs are fates determined by the cell of origin. Alternatively, the type or order of the genetic alterations may determine the final outcome.

Genomic data reveal the complexity of PDAC and the PanIN–PDAC model is limited by the correlative nature of the morphological–genetic associations. Genetic mouse models of PDAC should allow longitudinal and mechanistic analyses that cannot be carried out in humans. State-of-the-art genomic tools applied to large numbers of tumours should also provide a better understanding of the genetics of progression, the heterogeneity of PDAC, and the existence of tumour subtypes.

ON THE ORIGIN OF PDAC: "Don't ask what a cell can do, ask what it does do!" (quote from G Heppner[12])

Conditional mutant *K-Ras* knock-in mouse strains have allowed modelling of PDAC. In general, mutant K-Ras is activated in all pancreatic cells by targeting Cre recombinase to pancreatic progenitors at the time of pancreas formation (about E9) using the regulatory elements of pancreatic and duodenal homeobox 1 (*Pdx1*) or pancreatic transcription factor 1a (*Ptf1a*), two genes required for pancreas formation.[13 14] Most of the mouse models recapitulate the spectrum of PanIN lesions and PDAC. IPMNs, and to a much lesser extent mucinous cystic neoplasms, have been reported to be the most prevalent lesions upon mutant K-RAS

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expression and concomitant *SMAD4* deletion or transforming growth factor α (TGFα) overexpression.[15] Because all pancreatic epithelial cells develop from Pdx1$^+$ Ptf1a$^+$ precursors,[16] these studies are not informative regarding the cell of origin. Human PDAC has a ductal morphology, and it has long been assumed that PDAC originates in ductal cells. However, genetic mouse models have provided new paradigms on this issue.

Ductal cells

Ductal cells are poorly characterised at the molecular level: there is a paucity of embryonic and mature ductal differentiation markers, and little is known about ductal heterogeneity and the mechanisms involved therein. Keratin (Krt)7 and Krt19 are expressed in ducts.[17–19] *Krt19*—K-Ras^{G12V} transgenic mice develop hyperplastic ductal lesions with periductal lymphocytic infiltration but no tumours.[20] Mice in which mutant K-RAS is activated using *Krt19*—Cre^{ERT} develop PanIN-1A lesions.[21] *Hnf1b*—Cre^{ERT} [22] and *CAII*—Cre^{ERT} [23] strains are being used to activate mutant K-Ras in ductal cells at different stages. This will allow us to determine 'when and how' duct cells are sensitive to the oncogenic effects of K-Ras.

Acinar cells: mounting evidence for a role in PDAC

The notion that acinar cells can give rise to ductal-like cells is not new [24 25]: *Ela1*—TGFα and *Ela1*—c-myc transgenic mice show acinar to ductal metaplasia and develop acinar-derived ductal tumours.[26 27] Selective activation of mutant K-Ras in acinar cells and lineage tracing[28–30] has allowed us to conclude that: (1) acinar cells can give rise to PDAC in a K-Ras mutant context; (2) K-Ras activation in embryonic acinar cells leads to acinoductal metaplasia (ADM), abundant PanINs, and PDAC, whereas adult acinar cells are rather refractory; (3) chronic administration of caerulein, which induces a chronic pancreatitis (CP)-like lesion, promotes PanIN and PDAC development when K-Ras is activated in adult acinar cells.[29] Multiple episodes of acute pancreatitis have effects similar to those of a chronic insult.[31] Therefore recurrent tissue damage creates a permissive context for the oncogenic effects of mutant K-Ras in adult acini, in agreement with the increased risk of PDAC in patients with CP[32 33] (figure 1). Signalling events leading to Ras activation beyond a certain threshold (ie, mutation and pancreatitis) may modulate the differentiation state of adult acinar cells and render them more prone to malignant transformation.[33]

Most studies have reported on the acinar cell contribution to PanINs and PDAC. Concomitant overexpression of mutant K-RAS and *TGFα* results in IPMN formation when either the Ptf1a or Pdx1 promoter is used, but not when driven by the Ela1 promoter (ie, *Ela1*—Cre^{ERT}; LSL—K-Ras^{G12D}; *Ela1*—TGFα mice),[15] putting into question whether acinar cells can give rise to IPMNs as well. However, no attempts were made in this study to verify the susceptibility of stressed acinar cells (as in pancreatitis).

Centroacinar cells (CACs): somewhere in between?

CACs share many characteristics with ductal cells, including Krt19 expression, and are distinguished by the expression of the Notch target, hairy and enhancer of split 1 (Hes1).[34] CACs have recently been attributed progenitor stem cell features,[35] and *Hes1—Cre^{ERT2}* mice confirm CACs as the Hes1$^+$ population in the adult pancreas, but it is not clear whether they are true adult stem cells.[36] Pancreas-wide *Pten*-deficient mice develop metaplastic lesions and some PDAC, for which a CAC origin was proposed.[37] A definitive role of CACs in PDAC development has not been established, and their selective targeting with mutant genes is awaited.

Islet cells: me, too?

It was reported that targeting mutant K-Ras to insulin-expressing cells in combination with caerulein-induced damage results in PanINs.[38] However, their origin needs to be firmly established since the *RIP*—Cre^{ERT} transgene is also weakly active in acinar cells.[39]

Pancreatic stem cells: to be or not to be?

It has been hypothesised that normal stem/progenitor cells may serve as cancer stem cells.[40] Stem cells have been characterised mainly in tissues with self-renewing potential. In 'quiescent' tissues, such as the pancreas, the properties of cells with stem cell features may be fundamentally different. Most evidence supports self-replication as the mechanism involved in pancreatic homoeostasis and response to injury.[41]

Several pancreatic cancer stem cell markers have been proposed: CD44, CD24, epithelial-specific antigen, CD133 and the genes *Bmi1* and *Sonic Hedgehog (Shh)*.[40 42] However, all of these markers are also expressed in some differentiated cell types in the adult pancreas,[43–45] questioning the identity of cancer stem cells.

Overall, these studies indicate that oncogenic changes (such as K-Ras activation) in distinct differentiated cells, most convincingly acinar cells, can yield PanINs and PDAC. These findings underline the plasticity of acinar cells under experimental conditions ('what a cell can do') (figure 2). Pancreatic injury creates a permissive context favouring tumour development. Whether all that looks like PDAC is molecularly a uniform phenotype remains to be determined. The final question is 'what a cell does do'—that is, where does human PDAC arise? Massive parallel sequencing at the single cell level will allow clonal cell populations to be traced.

KEEPING ACINAR CELLS DIFFERENTIATED—DEVELOPMENT AND DISEASE

Because rodent models strongly support an acinar origin of PDAC, we focus on acinar cell differentiation and the pathways leading to the loss of their differentiated state.

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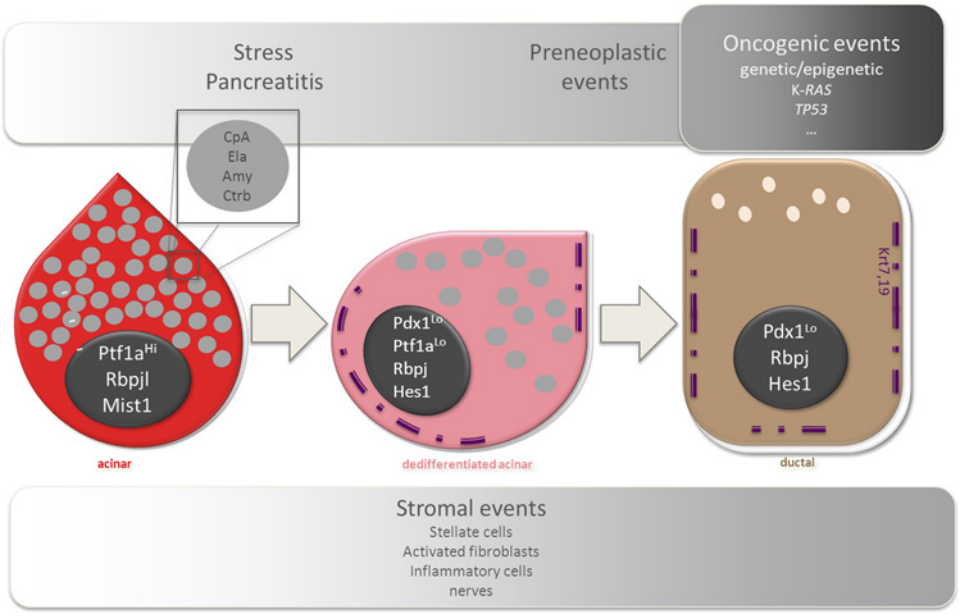

Figure 1 Acinar cells undergoing stress (eg, pancreatitis) dedifferentiate, which implies the loss of activity and expression of transcriptional regulators ($Ptf1a^{Hi}$, Hi refers to high level of expression, Rbpjl and Mist1), their target genes (eg, carboxypeptidase A (CpA), elastase (Ela), amylase (Amy) and chymotrypsin (Ctrb)) and as such the mature phenotype. The dedifferentiated acini have embryonic traits such as Hes1 and low Pdx1 ($Pdx1^{Lo}$), low Ptf1a levels ($Ptf1a^{Lo}$), and Rbpj in the PTF1 complex replacing Rbpjl. When they evolve into preneoplastic and tumour lesions, Ptf1a expression is lost and a more 'ductal' phenotype, with expression of Hes1 and Pdx1, is usually found in tumour lesions. Krt7 and Krt19 are also induced during the acinoductal metaplasia and remain expressed in tumour lesions. The dedifferentiation sensitises the cells to both genetic (ie, mutations, allele loss) and epigenetic (ie, methylation, histone modification) changes that promote PDAC development. Cues from the stroma may affect the acinar differentiation programme, and, vice versa, loss of differentiation may modulate stromal cell function, making it an essential player throughout the course of stress-induced, preneoplastic and neoplastic changes.

Differentiation during development

Mouse acinar cells are derived from multipotent precursors around day E14 and migrate outwards at the epithelial tips, leaving behind the trunk cells, which differentiate into islets and ducts.[16] The key transcription factors responsible for acinar differentiation are Ptf1a, which binds a ubiquitous basic helix–loop–helix transcription factor, and recombinant signal binding protein for immunoglobulin kappa J-region-like (Rbpjl). Together they constitute the adult-type PTF1 complex, which is the main activator of genes coding for digestive enzymes.[46] Before day E14, Rbpj is part of PTF1 instead of Rbpjl. Rbpj is a homologue of Rbpjl and is known as the DNA-binding transcriptional mediator of the canonical Notch signalling pathway. The embryonic PTF1 complex has distinct target gene specificity including the autoactivation of Ptf1a, of which a certain threshold of expression is needed for acinar cell differentiation. Another target of the PTF1 complex is Rbpjl, which gradually replaces Rbpj, leading to full differentiation of embryonic acinar cells.[47] High Ptf1a levels favour acinar differentiation, while low levels favour an endocrine fate during development.[48] Additional proteins probably help to refine the acinar transcriptional programme, including Mist1,[49] hepatic nuclear factor 1a (Hnf1a) (Molero *et al*, unpublished) and nuclear receptor subfamily five group A member 2 (Nr5a2).[16] Mist1 promotes terminal acinar differentiation by controlling the exocytosis and secretion programme[49] and by limiting proliferation through p21,[50] a function it has in common with Ptf1a.[51] The role of the other players is beginning to be explored. Extrinsic factors also contribute to full acinar differentiation, and the pathways thus activated are discussed later in this review.

Dedifferentiation: what's in a name?

Acinar-to-ductal metaplasia (ADM), transdifferentiation and dedifferentiation are terms with a mixed use.[44 52–56] 'Metaplasia' is a histological term referring to the replacement of one cell type (acinar) by another one (ductal) without any implication for the nature of the change (ie, selective cell death, selective expansion, transdifferentiation or dedifferentiation). 'Transdifferentiation' is a cell biology term referring to the switch from one differentiated (acinar) cell to another differentiated/functional (ductal) cell type.[57] 'Dedifferentiation' is the loss of mature, functional cell features with possible re-establishment of embryonic characteristics. As more acinar and ductal markers and distinct maturation stages are identified, more precision will be achieved in the molecular definition of these processes in acinar cells, especially when lineage tracing is not possible.

The current use of 'ductal markers' is not devoid of pitfalls: Krt20 is expressed by ductal cells in the rat but not in humans or mice; Krt19 and Krt7, Hnf1b and CD133 are expressed in both embryonic

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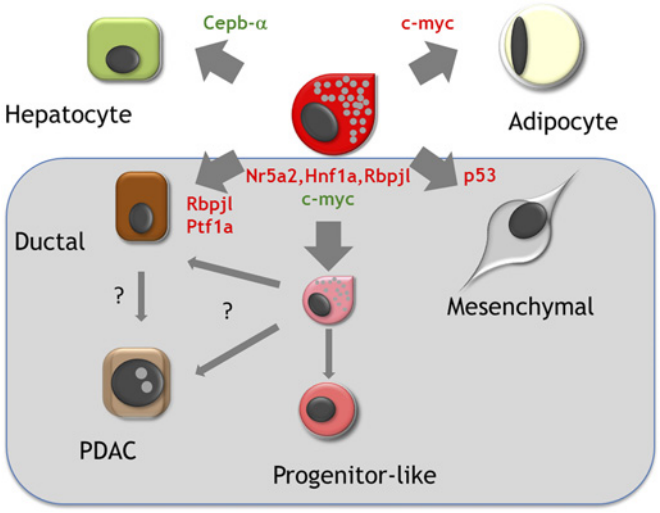

Figure 2 Key transcription factors and genes that govern acinar cell differentiation and plasticity are being unraveled ('what an acinar cell can do'), and this knowledge will advance our understanding of how this cell type may contribute to pancreatic ductal adenocarcinoma (PDAC) ('what it does do'). Transcription factors in green drive altered acinar differentiation: Cebp-α promotes hepatocytic differentiation, and c-myc can drive dedifferentiation. Lack of transcription factors depicted in red also affects the acinar differentiation: lack of Rbpjl, (Nr5a2) or hepatic nuclear factor 1a (Hnf1a) turns on a dedifferentiated, even progenitor-like phenotype. If both Rbpjl and Ptf1a are absent, a ductal differentiation is acquired. Acinar cells in the absence of c-myc install an adipocyte-like differentiation, whereas cells from *Trp53* null mice undergo epithelial—mesenchymal transition.

and adult ducts [22 43 58]; and Sry-box containing gene 9 (Sox9)[59] is not restricted to ducts.[44] Therefore an improved molecular cartography of ductal cells is needed.

Mouse acinar cells, identified using *Ela1*—Cre^{ERT2} lineage tracing, 'dedifferentiate' when cultured in suspension and acquire an embryonic-like phenotype.[44 54] These cells express low levels of Ptf1a, Pdx1 and selected digestive enzyme transcripts, features unique to multipotent pancreatic progenitors.[16] They also have the embryonic PTF1 complex sitting on its bona fide target promoters. These features discriminate pancreatic progenitors (dedifferentiation) from ductal cells (transdifferentiation). Transcriptomic analysis further distinguishes these cells from normal adult ductal cells.[44] Recent lineage tracing experiments indicate that also human acinar cells under specific culture conditions can acquire a ductal differentiation state.[60]

In vivo, the conversion of acinar cells into cells with ductal characteristics has been demonstrated using lineage tracing upon induction of pancreatitis with caerulein,[61] pancreatic duct ligation (PDL),[62] and in *Metallothionein*-TGFα (*MT*—TGFα) transgenic mice.[39] Acinar cells undergo transient dedifferentiation, in the 2-day caerulein acute pancreatitis model, with re-activation of pancreatic embryonic characteristics[63] and almost complete regeneration of the pancreas within 1 week. Upon prolonged caerulein exposure, chronic damage is induced and acinar dedifferentiation is not followed by acinar cell regeneration.[44 61] In other models of damage, such as PDL, acinar cells undergo apoptosis as well as dedifferentiation, similar to suspension cultures.[44]

Differentiation in PanIN and PDAC

Prominent markers displayed by dedifferentiated non-neoplastic acini are shared by PanIN/PDAC lesions. Besides the ductal keratins,[64 65] Pdx1 is expressed in dedifferentiated cells, PanIN lesions and PDAC.[66] Dedifferentiated acinar cells display low Ptf1a expression,which can be cytoplasmic.[67] In human samples, Ptf1a has been reported in some PanIN-A lesions, but it is undetectable in PDAC.[67 68] Bmi1, a member of the PRC1 complex, becomes induced on acinar cell dedifferentiation in vitro, in PDL and in caerulein pancreatitis, and during PDAC development in mice.[45]

Both in vitro and in vivo models thus support the dedifferentiation of adult acinar cells. We acknowledge that there is still some controversy about the contribution of ADM to PDAC. Telomere shortening and K-*Ras* mutations occur mainly in PanIN-associated ADM and rarely in isolated ADM in human samples.[8] This suggests that the latter lesions are an earlier, and potentially end-stage, lesion and that PanIN-associated metaplasia represent a more advanced stage. So far, little evidence supports their being retrograde extensions of PanINs, as has been hypothesised.[8] Mouse models should provide further insights.

The main pathways involved in acinar differentiation/dedifferentiation are discussed below (figure 3). Other reviews deal with their implications in other aspects of pancreas biology.

Differentiation: signalling cues and pathways

Ras and downstream from Ras

Ras proteins integrate signalling by membrane receptors and activate the extracellular signal-related kinase (ERK)/mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) and Ral. The effects of epidermal growth factor receptor (EGFR), Ras and MAPK signalling in adult pancreas are well documented, but less is known of their role in embryonic pancreas development. In *EGFR* knockouts, islet differentiation and ductal branching are affected, without acinar changes reported.[69] Mice overexpressing TGFα or amphiregulin develop normally, but show altered acinar differentiation and duct cell proliferation.[26 70]

Upon acinar cell injury, Ras and its downstream pathways are activated.[33 44] *Ela1*—TGFα transgenic mice develop ADM and carcinomas. In agreement, blocking of EGFR and MAPK signalling inhibits duct formation from cultured acinar cells.[26] It is not clear whether EGFR plays a unique role among receptor tyrosine kinases or it stands out because it has been more extensively studied. It has been proposed that Ras activity levels, regardless of whether it emanates from wild-type or mutant protein, determines acinar cell fate.[33] In human tissues, ~30% of CP samples harbour *K-RAS* mutations: they are generally absent from ADM lesions, but are more common in metaplastic lesions associated with PanINs.[71] Patients with PDAC who have wild-type K-*RAS* tumours are more likely to have a history of CP.[72]

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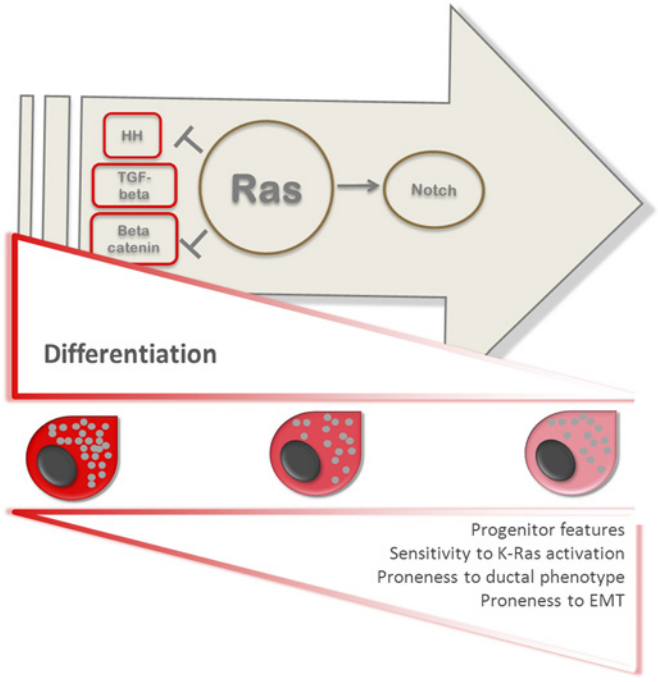

Figure 3 Ras and Notch pathway activation can negatively impinge on the differentiation programme of acinar cells. In contrast, Hedgehog (HH), Wnt and transforming growth factor (TGF)β signalling positively impact on maintenance/regeneration of the acinar component. Ras signalling plays a crucial role in this balance, and its overactivation promotes pancreatic ductal adenocarcinoma development, directly but also probably via repression of HH, by interfering with β-catenin signalling, and by activation of Notch signalling. Loss of differentiation results in acquired progenitor features, increased susceptibility to K-Ras, and proneness to ductal and epithelial–mesenchymal transition (EMT) features.

Acinar cells overexpressing the human cholecystokinin 2 (CCK2) receptor (*Elas*CCK2 transgenic mice) undergo dedifferentiation and malignant transformation.[73] Src/ERK activation contributes to this effect.[74] In other contexts, CCK receptors have been shown to activate Ras proteins.[75]

Regarding the PI3K/AKT pathway, overexpression of active Akt in acinar cells by activated Cre under the elastase promoter leads to acinar-derived metaplastic ducts displaying embryonic characteristics.[76] IPMN-like tumours—but no PanINs—develop, in line with the occurrence of *PIK3CA* mutations in 11% of IPMNs.[77] Constitutive Akt expression driven by *Pdx1*–Cre leads to the development of PDAC in old animals.[76] Knockout of *Pten*, a negative regulator of PI3K signalling, in acinar cells results in highly proliferative mucinous ducts expressing pancreatic progenitor markers. Some of these mice also develop tumours,[37] supporting a role for PI3K in loss of acinar cell differentiation. However, there is no direct evidence that PI3K inhibition can block acinar cell dedifferentiation.[78]

In conclusion, activation of Ras and downstream signalling is linked to acinar cell dedifferentiation and—eventually—tumour generation; whether this association is causal remains to be established.

β-Catenin signalling

Wnt-secreted ligands bind membrane receptors, inactivate the axin–glycogen synthase kinase-3–adenomatous polyposis coli (APC) complex, which promotes the proteolytic degradation of cytosolic β-catenin (canonical Wnt signalling), leading to β-catenin accumulation and nuclear translocation and activation of target genes, including c-myc.

β-Catenin hyperactivation at the time of pancreas specification causes pancreatic agenesis or hypoplasia. At later stages, Wnt signalling blockade reduces proliferation of endocrine and exocrine cells. Conditional β-catenin deletion leads to impaired acinar cell proliferation, with c-myc being a major effector of the β-catenin-induced expansion of the exocrine compartment. The effects on acinar differentiation vary depending on the experimental conditions.[79 80]

Cytoplasmic accumulation of β-catenin is often associated with acinar dedifferentiation.[63 78 81] β-Catenin signalling determines whether acinar regeneration or persistent dedifferentiation occur upon pancreatic damage.[81] Strong and persistent Ras activation interferes with canonical β-catenin signalling, blocks acinar regeneration, and favours ductal metaplasia.[81] In vitro studies of isolated acini are consistent with these observations.[44] β-Catenin cooperates with activated Ras in the generation of PanIN and PDAC.[82] However, activating mutations in *β-catenin* or other Wnt-related genes are rare in PDAC.[82]

Notch signalling

Notch–ligand interactions lead to proteolytic cleavage of Notch intracellular domain (NICD), which then binds to Rbpj and activates transcription. Mice lacking Notch1/2 develop a quite normal pancreas, but *Rbpj* inactivation results in loss of pancreatic mass and precocious endocrine differentiation.[83–85] Notch overexpression inhibits acinar differentiation.[83–85] Inactivation of the Notch target Hes1 results in pancreatic hypoplasia and ectopic pancreas in the gut and the stomach, as well as islet-like clusters and acini in the bile duct.[84 85] In the early embryonic pancreas, Hes1-expressing cells are multipotent progenitors in which Notch signalling installs a ductal phenotype. Later in embryogenesis, Hes1 is expressed in exocrine-restricted progenitors, where Notch activation promotes ductal differentiation at the expense of acinar cell fates. In the adult, Hes1 is restricted to a subpopulation of CACs and ducts and marks mainly cells in which the ductal programme is activated.[34 36] However, Hes1 is a readout not only of Notch, but of other pathways as well (ie, non-canonical NFκB,[86] Jnk pathway[87] and Hedgehog[88]).

During pancreatitis, Notch signalling is re-activated in both humans and mice,[63 89] and it is required for acinar regeneration in cooperation with Wnt signalling.[90] Notch activation blocks acinar differentiation, and its inhibition partially blocks acinar dedifferentiation.[34] NICD overexpression in mature acini does not result in ductal differentiation; yet, in cooperation with activated Ras, it leads to ADM in mice.[91] Therefore Notch contributes to acinar dedifferentiation and ductal metaplasia.

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Matrix metalloproteinase 7 (MMP7) is probably a common effector of Ras, Wnt and Notch.[92–94] In acinar cells, MMP7 cleaves Notch and initiates signalling, and NICD inhibits β-catenin-mediated transcriptional activity.[90] MMP7 appears to be required for ADM in vitro and in vivo.[93 95]

Notch2, rather than Notch1, appears to be the crucial player in pancreatic carcinogenesis.[96] Furthermore, Notch1 is tumour suppressive in a K-Ras knock-in tumour model (driven from embryonic stages on).[97] A more detailed assessment of the function of each of the Notch proteins is required. Altogether, these studies support the idea that Ras is a crucial determinant of the biological effects of Notch.

Hedgehog (HH) signalling

The three hedgehog ligands (Sonic hedgehog (Shh), Desert hedgehog (Dhh) and Indian hedgehog (Ihh)) bind to Patched (Ptch) receptors and release their inhibition of smoothened (Smo). The main HH effectors are Gli transcription factors. Shh down-regulation is key for endodermal specification into pancreas. Early work suggested that altered HH signalling in pancreas development affected the mesenchyme, but Gli2 epithelial overexpression is associated with acinar cell loss.[98 99]

HH signalling is required for damage-induced acinar regeneration as shown by its pharmacological or genetic inactivation in an acute pancreatitis model; in contrast, ductal metaplasia and proliferation are unaffected.[100 101] This effect may be mediated by the hyperactivation of Ras during CP and the resulting inhibition of it on HH signalling.[102]

Several HH components, including the receptors Ptch and Smo, are upregulated in PanINs and PDAC.[103 104] It has been shown that HH signalling acts in a paracrine manner: epithelial cells produce the ligand leading to pathway activation in the adjacent stroma and fibroblast proliferation/activation.[105] The limited autocrine activation of HH in PDAC is thought to result from: (1) Ras inhibition of autocrine HH signalling via DYRK1B kinase[102]; (2) a HH ligand deregulation of Gli in tumour cells[102]; (3) primary cilia-mediated attenuation of HH signalling downstream of Smo.[99] This mechanism may not operate since the primary cilium is typically lost in PDAC.[106]

TGFβ signalling pathway

The TGFβ pathway plays an important role in normal tissue homoeostasis, and it cross-talks with the MAPK and Wnt pathways. TGFβ restricts endodermal specification to pancreas,[107] suppresses proliferation and differentiation towards the endocrine lineage,[108] and can also affect embryonic acinar growth and survival.[109 110] Transgenic mice expressing a dominant-negative mutant type II TGFβ receptor under control of the *metallothionein* 1 promoter, which is active in acini but not in ducts, display increased acinar cell proliferation and ADM, suggesting acinar cell dedifferentiation because intermediate cell types were found.[111] These mice were less sensitive to caerulein-induced acute pancreatitis.[112]

Acinar cells: beyond the epithelium

Dedifferentiation of epithelial cells has also been linked to epithelial–mesenchymal transition (EMT), a process characterised by the down-regulation of epithelial markers and acquisition of mesenchymal features and migratory properties, E-cadherin loss being a hallmark of this process.[113] It has recently been suggested that human exocrine pancreatic cells can undergo EMT.[114] Dedifferentiation and EMT have been demonstrated by lineage tracing in human endocrine β-cells in culture.[115] Murine acinar cells can undergo massive EMT, which is favoured by loss of *Trp53*.[116] These effects may be pertinent to the acquisition of migratory and invasive properties in PDAC.

Overall, there is extensive evidence that acinar cells display plasticity with the potential to dedifferentiate, transdifferentiate to ductal cells in selected model systems, and acquire mesenchymal properties (figure 2). These processes can thus occur in non-neoplastic cells, and are similar to those occurring in tumours (figure 1).

TUMOUR-SUPPRESSIVE MECHANISMS

Other biological processes affecting acinar cells may also contribute to their fate when undergoing dedifferentiation. Senescence and autophagy are briefly discussed here.

Senescence is an early tumour-suppressive mechanism that results from several types of stress, including telomere erosion, oxidative stress, oncogenic stress and DNA damage. The senescence programme is mainly dependent on the integrity of the Rb and p53 pathways, both of which are altered in PDAC. Consistently, senescence has been reported in PanINs, but not in PDAC.[117] There is evidence of increased proliferation in ductal complexes in areas of CP, but also of an activated senescence programme in dedifferentiated acini in experimental CP and in in vitro models.[44 51] A role for Twist1 in overcoming mutant K-Ras-induced senescence has been proposed.[118] It seems likely that, in pancreatitis, subpopulations of acinar cells distinctly respond to signalling stress, leading to different cellular outputs whereby some acinar cells can overcome this tumour barrier.

Autophagy aims at preserving cellular integrity in response to stressful conditions (survival) and can also be a tumour-suppressive mechanism. In general, tumour suppressors activate autophagy, whereas oncogenic pathways suppress it.[119] It has been suggested that early stages of pancreas tumorigenesis are associated with a down-regulation of autophagy-related genes.[120] Autophagy is triggered during acute pancreatitis, and *autophagy protein 5 (Atg5)*-deficient acinar cells are partially protected from the damage induced by caerulein.[121 122] Therefore it will be important to further examine the role of autophagy in the context of acinar cell dedifferentiation.

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INFLAMMATION AND THE STROMA: THE ANSWER IS AROUND?

An essential question regarding a putative role of acinar cells in PDAC is: What turns off the acinar differentiation programme? We consider here the role of non-cell autonomous mechanisms.

Cell and tissue architecture: extreme makeover

Abnormal enzyme secretion can cause oedema and cellular stress and affect epithelial cell–cell contacts with a protumoural effect. These changes are reflected in altered differentiation, underscored by observations using dispersed acinar cells in culture,[44 54] including decisive roles for cadherin-mediated cell–cell adhesion, β-catenin and the PI3K pathway.[78] Organotypic slice cultures could help to distinguish effects of culture-induced stress and intercellular contact disruption on acinar cell differentiation.

Inflammation

Chronic inflammation leads to free radical formation, activation of cyclo-oxygenase-2, NFκB and inducible nitric oxide synthase, and inflammatory cytokines.[123] Oxidative stress also causes DNA damage.[117]

Interleukin 1β overexpression under the control of the *elastase* promoter is associated with ADM and inflammation, but no PanINs or tumours develop, even in the setting of mutant *Trp53*.[124] Inflammation can overcome the senescence barrier associated with PanINs, thus favouring PDAC development, in a K-RAS-induced mouse model of PDAC, and similar observations have been made in patients with CP treated with anti-inflammatory drugs.[125] Stat3, signalling downstream of interleukin 6, promotes ADM, induces proliferation and blocks apoptosis, through upregulation of chemokines and recruitment of activated macrophages. Ablation of *Stat3* in pancreatic epithelial cells in the K-RAS-induced mouse models attenuated PDAC at different levels, with fewer PanINs, lower tumour grade, and reduced metastases.[126 127]

The stroma

Altered activity of mesenchymal cells occurs during acute and chronic pancreatitis and may contribute to acinar dedifferentiation through qualitative or quantitative changes in cellular or matrix composition.[128 129] The extensive desmoplastic reaction associated with CP and PDAC may result from positive feedback loops that favour matrix deposition and pancreatic stellate cell activation and further help to block acinar cell regeneration via epigenetic mechanisms.[128] Dedifferentiated acini or tumour cells may impinge on the composition of the stroma.[128] Hypoxia may also contribute to cell differentiation at the onset of CP and PDAC.[130] In relation to the dense stroma of PDAC and the associated hypovascularisation, Shh inhibition causes stromal collapse, increased delivery of chemotherapeutic drugs, and increased antitumour effects.[131] Therefore the use of drug combinations that affect stroma and tumour cells may be effective in PDAC.[131]

The nerves, last but not least

The exocrine pancreas is mainly innervated by parasympathetic ganglia and preganglial fibres. Neural signals cooperate with humoral mechanisms (ie, cholecystokinin) to trigger excitation of sensory afferents of the enteropancreatic reflexes.[132] Tissue damage is possibly associated with loss of all these homoeostatic mechanisms; nerve fibre activation occurs in CP and PDAC, as has been studied in relationship to acinar cell innervation, pain and tumour cell invasion.[133 134]

WHERE TO LOOK AHEAD?

The aetiological heterogeneity of PDAC suggests that various routes can lead to PDAC, and a molecular taxonomy of PDAC is necessary. The existence of three PDAC subtypes with major therapeutic implications has been proposed.[135]

Our knowledge on acinar cell biology should be transferred to the clinical setting given the evidence that ADM could be a preneoplastic lesion. This is not generally accepted to be the case for PDAC. Yet, metaplasia is well established as a risk for cancer at other sites including the stomach and the oesophagus.

The molecular analysis of metaplastic lesions may provide insight into new biomarkers of risk and chemopreventive strategies, including epigenetic drugs. If ADM is a risk factor for PDAC, more emphasis should be placed on its early detection, possibly using non-invasive strategies—that is, serum markers.

The study of PDAC will identify new genes, proteins and pathways that may play roles at different stages of tumour development, including ADM. The International Cancer Genome Consortium (http://www.icgc.org) is an initiative to obtain comprehensive genomic, transcriptomic and epigenomic data on the main human tumour types, including pancreatic. The potential of such data to provide insight into tumour pathogenesis, including ADM and early neoplasia, is revealed by analyses of intratumoural heterogeneity as well as metastatic seeding.[9 10 135]

The biology of the pancreatic epithelium is and will be in the next few years a topic of intense research. Understanding cell differentiation may contribute to improved patient care, although—admittedly—this is unlikely to happen 'tomorrow'.

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Ilse Rooman and Francisco X Real

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