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REVIEW

Therapeutic potential of targeting acinar cell reprogramming in pancreatic cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a common pancreatic cancer and the fourth leading cause of cancer death in the United States. Treating this life-threatening disease remains challenging due to the lack of effective prognosis, diagnosis and therapy. Apart from pancreatic duct cells, acinar cells may also be the origin of PDAC. During pancreatitis or combined with activating $KRas^{G12D}$ mutation, acinar cells lose their cellular identity and undergo a transdifferentiation process called acinar-to-ductal-metaplasia (ADM), forming duct cells which may then transform into pancreatic intraepithelial neoplasia (PanIN) and eventually PDAC. During ADM, the activation of mitogen-activated protein kinases, Wnt, Notch and phosphatidylinositide 3-kinases/Akt signaling inhibits the transcription of acinar-specific genes, including Mist and amylase, but promotes the expression of ductal genes, such as cytokeratin-19. Inhibition of this transdifferentiation process hinders the development of PanIN and PDAC. In addition, the transdifferentiated cells regain acinar identity, indicating ADM may be a reversible process. This provides a new therapeutic direction in treating PDAC through cancer reprogramming. Many studies have already demonstrated the success of switching PanIN/PDAC back to normal cells through the use of PD325901, the expression of E47, and the knockdown of Dickkopf-3. In this review, we discuss the signaling pathways involved in ADM and the therapeutic potential of targeting reprogramming in order to treat PDAC.

Key words: Acinar cells; Acinar-to-ductal metaplasia; Pancreatic ductal adenocarcinoma; Signal transduction; Reprogramming

Core tip: Treating pancreatic ductal adenocarcinoma

(PDAC) remains challenging due to the lack of effective therapeutics. Apart from pancreatic duct cells, acinar cells may also be the origin of PDAC. During pancreatitis or combined with activating $KRas^{G12D}$ mutation, acinar cells undergo a transdifferentiation process called acinar-to-ductal-metaplasia (ADM), forming duct cells which may then be transformed into PanIN and eventually PDAC. This process involves MAPK, Wnt, Notch and PI3K/Akt signaling. Since ADM may be a reversible process, switching PDAC back to normal cells may also be achieved and developed as a novel therapy.

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INTRODUCTION

Pancreatic cancer is still the fourth leading cause of cancer death in the United States and Europe, although the incidence rate and death rate in the United States have been stable since the 1970s (but continue to increase in European countries)[1,2]. Most (96%) of the pancreatic cancer is from exocrine pancreas, including pancreatic ductal adenocarcinoma (PDAC)[1]. Due to the lack of reliable diagnostics, over half of patients are usually diagnosed with pancreatic cancer at an advanced stage. This also contributes to the low 5-year survival rate (7%), with median survival around 4 mo[1,3]. Surgery, chemotherapy and radiotherapy are commonly used to treat pancreatic cancer, although they can only extend survival for several months or can be used as an alleviative instead of for curing the patient[3,4]. Also, since many patients already have cancer metastasis when they are diagnosed, only a few patients can receive surgery. The chemotherapy drug gemcitabine is usually used together with surgery or the targeted anti-cancer drug erlotinib. In addition, combining gemcitabine with other chemotherapy drugs, such as nab-paclitaxel or the human monoclonal antibody AGS-1C4D4, can only provide a limited extension of survival by months in exchange for developing adverse effects, including abdominal pain, peripheral neuropathy and myelosuppression[5,6]. Therefore, a novel therapy is urgently needed. Here, we briefly introduce transdifferentiation of acinar cells to ductal cells with tumorigenic potential under the activation of cellular signaling pathways. Also, we discuss the therapeutic potential of reprogramming PDAC back to normal pancreatic cells, such as acinar cells, in order to treat cancer.

ROLE OF ACINAR-TO-DUCTAL METAPLASIA IN THE INITIATION AND DEVELOPMENT OF PANCREATIC DUCTAL ADENOCARCINOMA

Pancreas is composed of the endocrine and exocrine compartments, which are responsible for maintaining body homeostasis and digesting food respectively[7]. The endocrine pancreas is formed by islets cells, which secrete hormones such as insulin and glucagon to regulate the glucose level in the body. The exocrine pancreas consists of acinar cells, which synthesize digestive enzymes such as amylase and duct cells and are responsible for transportation of digestive enzymes to the duodenum for digestion. Studies show that PDAC may originate from pancreatic duct cells through the development of pancreatic intraepithelial neoplasia (PanIN) after the first activating gene mutation in KRas[8]. However, the $KRas^{G12D}$ mutation alone is insufficient to drive the development of PDAC[8]. Further genetic changes, such as in p16, p53 and SMAD4, and overexpression of epidermal growth factor receptor (EGFR), EGF and transforming growth factor alpha (TGFα) are required for development to a higher-grade PanIN and eventually PDAC[9-12].

Apart from pancreatic duct cells, pancreatic acinar cells, which are the source of acinar cell carcinoma, may also contribute to the development of PDAC through acinar-to-ductal metaplasia (ADM)[13]. ADM is a type of transdifferentiation by which a mature and differentiated cell type changes its identity to another differentiated cell type[14]. During ADM, acinar cells leave the quiescent state and transdifferentiate to duct cells, losing their grape-like morphology and changing the transcriptome from acinar-like, such as with expression of Mist, amylase, carboxypeptidase and elastase, to duct-like, such as with expression of cytokeratin-19 (CK-19), cytokeratin-20 (CK-20) and carbonic anhydrase II[13,15]. This transdifferentiation process involves a nestin-positive intermediate[15]. Also, studies using an *in vivo* model showed that pancreatitis, both acute and chronic, can result in ADM[16,17]. These observations are consistent with the suggestion that chronic pancreatitis may be one of the causes of pancreatic cancer[18].

MAPK signaling is involved in ADM

It has been reported that more than 90% of PDAC harbor a KRas activating mutation[10]. In addition, compared to normal acinar cells, acinar cells with KRas mutation fail to regenerate during pancreatitis and undergo ADM-forming PanIN. These findings indicate the importance of an activating mutation of KRas in acinar reprogramming and PDAC development. KRas is involved in many signaling pathways, such as the mitogen-activated protein kinases (MAPK)

signaling pathway; therefore, the importance of the MAPK signaling pathway has been studied using three-dimensional culture of mouse acinar cells with activating mutation of $KRas^{G12D}$ after pancreatitis induction[16]. Inhibiting the MAPK signaling pathway after pancreatitis maintains cells in the grape-like acinar morphology and expressing amylase without undergoing ADM. In contrast, ADM is observed for acinar cells without inhibition of the MAPK signaling pathway after pancreatitis.

Upon the activation of EGFR by the EGF family members, such as EGF and TGFα, the growth factor receptor-bound protein 2 (GRB2) binds to the tyrosine kinase on the cytoplasmic side of EGFR through its SH2 domain[19]. This binding recruits the guanine nucleotide exchange factor son of sevenless (SOS) to interact with the SH3 domain of GRB2, resulting in SOS activation. The activated SOS exchanges the GDP in resting Ras with GTP. Therefore, Ras is activated, which in turn activates the protein kinase activity of the serine/threonine kinase Raf. Raf phosphorylates and activates mitogen-activated protein kinase kinase1/2 (MEK1/2). MEK1/2 phosphorylates and activates MAPK.

The nuclear factor of activated T-cells (NFAT) transcription factor family actively participates in the immune system, which includes T-cells, B-cells and dendritic cells[20]. Apart from the immune system, NFAT is also involved in proliferation, invasion, angiogenesis and drug-resistance in various cancer cells, such as those of prostate, breast and lung[20-24]. In addition, it has been discovered that two family members, NFATc1 and NFATc4 (NFATc1/4), may be the downstream targets of the MAPK signaling pathway and may correlate with ADM and PanIN development[25,26]. NFATc1 is expressed, activated and accumulated in the nucleus of pancreatic cancer cells[27]. In addition, after the activation of MAPK signaling by TGFα, both NFATc1/4 are up-regulated, in contrast to the other NFAT family members such as NFATc2 and NFATc3[25]. Furthermore, acinar to ductal morphological transdifferentiation is observed in NFATc1/4-expressing acinar cells. It has been demonstrated that during ADM, NFATc1 interacts with c-Jun which is activated by the MAPK family member c-Jun N-terminal kinases (JNK) after activation of MAPK signaling, as indicated by co-immunoprecipitation assay[26]. The NFATc1-c-Jun complex is also observed in T-cells, mediating immune response[28,29]. It binds to the promoter of sex determining region Y-box 9 (*Sox9*), resulting in activation through histone 3 lysine 4 trimethylation[26]. Hessmann *et al*[25] also reported similar c-Jun complex formation and *Sox9* activation involving NFATc4. Disrupting the NFATc1/4-c-Jun complex results in failure in inducing *Sox9*. These observations consistently indicate the involvement of Sox9 in ADM.

Although the Sox9 transcription factor is involved in ductal, acinar and endocrine development, it is not expressed in acinar cells[30]. However, it is induced during chronic pancreatitis and ADM[25,26,31]. In addition, it is expressed in ductal cells, PanINs and PDAC, indicating its association in acinar reprogramming, cancer initiation and development[25,26]. On the other hand, acinar cells with *Sox9* deletion can still undergo ADM upon pancreatic injury. Kopp *et al*[31] suggested that another pancreatic development-related transcription factor, hepatocyte nuclear factor 6 (Hnf6), can replace Sox9 with lower ADM-promoting efficiency. Like Sox9, hepatocyte nuclear factor 1 beta (Hnf1β) and Hnf6 are expressed in normal ductal cells but not in acinar cells[32]. However, in contrast to the suggestion by Roy and colleagues[33] that Hnf1β is up-regulated during acinar dedifferentiation, the expression of Hnf1β is not observed during ADM in either human or mouse tissue samples[32].

On the other hand, overexpression of Hnf6 triggers ADM, as indicated by the increase in CK-19 level and decrease in amylase and the acinar transcription factor Mist levels. Although, how Hnf6 is regulated still needs further study. Prévot *et al*[32] demonstrated that Hnf6 can induce the expression of Sox9, while knockdown of *HNF6* alleles cannot fully deplete Sox9. This indicates that Hnf6 is not the sole regulator of Sox9. In addition, the Prrx1b isoform of another transcription factor, Prrx1, is also up-regulated during acinar transdifferentiation and positively regulates the expression of *Sox9* through binding to its promoter and induces ADM[34]. Therefore, this may suggest that Sox9 is regulated by at least NFATc1/4-c-Jun, Hnf6 and Prrx1. Although depleting *Sox9* can still drive the reprogramming of acinar cells to ductal cells, no progression to PanIN is observed[31]. In addition, *Sox9* is expressed in PanIN and PDAC, but not for Hnf6[32]. Although, this may suggest that Sox can be replaced partially by Hnf6 during ADM, but it may still be required for PanIN progression.

Current evidence suggests that there may be a functional correlation between Sox9 and protein kinase D1 (PKD1)[35]. Similar to Sox9, PKD1, which is up-regulated under the activation of MAPK signaling by either EGFR or $KRas^{G12D}$, promotes acinar cell reprogramming. Also, knockdown of *PKD1* or inhibiting PKD1 significantly suppress ADM. PKD1 bridges the MAPK and Notch signaling pathways and promotes PanIN and adenocarcinoma formation[35]. When PKD1 is actively expressed in acinar cells, matrix metalloproteinase 7 (MMP7), a disintegrin and metalloproteinase 7 (Adam7) and Adam10, which are regulators of Notch, are significantly up-regulated, while Notch-suppressor suppressor enhancer lin12/Notch 1-like and casitas B-lineage lymphoma are down-regulated[35,36]. Sawey *et al*[36] also reported similar findings that MMP7 is involved in ADM *via* MAPK signaling. In addition, targets of the Notch signaling pathway, such as hairy and enhancer of split-1 (Hes-1), Hairy/enhancer-of-split related with YRPW motif protein-1 (Hey-1) and p21, are significantly up-regulated when PKD1 is activated[32,33]. These results indicate that Notch signaling is involved in ADM.

Wnt signaling is involved in ADM

Wnt signaling is directly and indirectly involved in development and repair of various organs, including pancreas, bone, kidney and heart[37-40]. The Wnt signaling pathway is complex, and the three well-studied pathways are the canonical pathway (or Wnt/β-catenin pathway), the non-canonical planar cell polarity pathway and the non-canonical Wnt/calcium pathway[41]. The canonical pathway is different from the other two in the fact that involvement of β-catenin is required for ADM. When there is no Wnt signaling activation, β-catenin is originally degraded by phosphorylation and then ubiquitination involving the β-catenin destruction complex (formed by protein phosphatase 2A, glycogen synthase kinase3, casein kinase 1α, adenomatosis polyposis coli, and Axin). Wnt signaling can be activated by the binding of Wnt protein to the Frizzled family receptor complex Fz/LRP5/6. This results in membrane translocation and binding of Axin to LRP5/6 through microtubule actin-linking factor 1. In addition, Dsh is activated and binds to the receptor complex, inhibiting glycogen synthase kinase 3. As a result, the β-catenin destruction complex is disrupted and β-catenin accumulates. β-catenin then translocates to the nucleus, activating its target genes.

A growing number of studies suggest the role of the canonical pathway in ADM. However, its role-whether as a promoter or inhibitor-depends on the progression of the reprogramming. Since the canonical pathway is involved in the regeneration process of many organs, it may also actively participate in pancreatic regeneration. During pancreatitis, the canonical pathway is activated within 1 wk, as indicated by the accumulation of β-catenin and Axin[42,43]. Recovered pancreas, with normal grape-like morphology and amylase expression, is observed after 1 wk. In addition, activation of the canonical pathway blocks $KRas^{G12D}$-driven ADM during pancreatitis, resulting in a normal recovery process. Similarly, overexpressing β-catenin does not result in PDAC but instead in an uncommon intraductal tubular tumor[44,45].

On the other hand, gradual accumulation of β-catenin is observed in acinar cells with $KRas^{G12D}$ after 1 mo of pancreatitis[42]. However, normal acinar morphology and acinar maker expression are not observed. Instead, mice with functional β-catenin and active mutation of $KRas^{G12D}$ express CK-19 and mucin, indicating progression of ADM and PanIN development respectively[44]. Also, inhibition of Frizzled receptor, which is the starting point of the Wnt signaling pathway, by the monoclonal antibody OMP-18R5 hinders progression of ADM. The decrease in Wnt target genes Axin2, lymphoid enhancer-binding factor-1 and MMP7 in mice after treatment with OMP-18R5 confirms the inhibition of Wnt signaling. Therefore, canonical Wnt signaling functions as an inhibitor during the initiation of ADM but promotes the formation of PanIN in the late process.

Sirtuin-1 (Sirt-1) functions as a protein deacetylase, controlling the development of heart, brain and spinal cord[46]. It is also found in both exocrine and endocrine pancreas. In endocrine pancreas, especially in the β-cells, Sirt-1 regulates the secretion of insulin[47]. While in injured exocrine pancreas, it promotes acinar transdifferentiation. During pancreatitis, Sirt-1 is transiently translocated from nucleus to cytoplasm, which is important for ADM[48]. Blocking Sirt-1 translocation significantly interferes with the expression of ductal genes. In addition, inhibiting Sirt-1 expression in nucleus by non-functional gene mutation significantly accelerates and prolongs the progression of acinar reprogramming. These results indicate that Sirt-1 functions as an ADM suppressor in nucleus. On the other hand, similar to suppressing MAPK signaling, the use of nicotinamide, which is an end-product inhibitor of Sirt-1 in protein acetylation, inhibits ADM after cytoplasmic shift of Sirt-1[48,49]. The cytoplasmic Sirt-1 deacetylates β-catenin and acinar-specific transcription factors, thereby inhibiting the acinar regeneration.

Notch signaling is involved in ADM

Notch signaling is required for regulating the development of various vital organs, including pancreas, bones and blood[50-53]. Notch protein is composed of the Notch extracellular domain (NECD), Notch transmembrane fragment (NTF) and Notch intracellular domain (NICD). Maturation of the Notch receptor involves cleavage of the NECD from the non-NECD and integration of the NECD heterodimer to the cell membrane. During organ development, especially of the pancreas, the Notch receptor is activated by type Ⅰ transmembrane ligands including jagged-1, resulting in activation of a membrane-bound protease which cleaves the non-NECD into NTF and NICD. The released NICD translocates to the nucleus, resulting in expression of target genes such as Hes-1 and nuclear factor-κB, involving Rbp-J, Mastermind-like, and DEAD-box helicase.

Notch signaling is activated in PDAC and its precursor. Compared to normal pancreatic tissues, the Notch ligand jagged-1, the Notch receptors Notch2 and Notch3, and its target genes Hes-1 and Hey-1 are up-regulated during pancreatitis, ADM and cancer development[13,43,54]. In addition, PDK1, which is up-regulated during ADM, activates the Notch signaling pathway through at least MMP7[35]. Also, MMP7 is the downstream target in Wnt signaling during ADM, since inhibition of Wnt signaling decreases the level of MMP7. MMP7 is one of main metalloproteinases responsible for activating cleavage of the Notch receptor[55]. Therefore, the increase in MMP7 level indicates involvement of Notch signaling during acinar cell reprogramming[36,56].

The downstream effector Hes-1 in the Notch signaling pathway negatively regulates the basic helix-loop-helix (bHLH) transcription factor family, which is involved in pancreatic development. One of

the members, neurgenin3, is involved in endocrine differentiation, whereas acinar cell development requires Mist[57]. During development of the exocrine pancreas in the embryo, Notch signaling is activated in the exocrine precursor but it is repressed in normal differentiated acinar cells. Since the downstream target Hes-1 in Notch signaling inhibits the transcription of acinar transcription factors, inactivation of Notch signaling promotes the development of pancreatic acinar cells[58]. During acinar differentiation, Mist activates the genes involved in producing digestive enzymes, such as amylase and carboxypeptidase, in stimulating exocytosis and in maintaining acinar cell homeostasis[57,59]. Inhibition of Mist in pancreatic acinar cells results in ADM, as indicated by the loss of cell organization, the decrease in acinar gene expression and the expression of ductal marker CK-19[60].

Phosphatidylinositide 3-kinase/Akt signaling is involved in ADM

Although many studies have focused on the loss of digestive enzymes in acinar cells, little emphasis has been put on the morphological changes during ADM. It has been suggested that activation of Akt signaling is responsible for the morphogenesis of mammary acinar cells during malignant transformation[61]. Similarly, Phosphatidylinositide 3-kinase (PI3K)/Akt signaling is also involved in structural changes, from acinar to ductal, through actin remodeling[62,63]. Under KRas mutation, PI3Kα, but not the other isoforms (β, γ or δ), induces the morphological changes in acinar cells[63]. The activation of PI3Kα phosphorylates phosphatidylinositol 4, 5-bisphosphate (PIP_2), forming phosphatidylinositol-3, 4, 5-trisphosphate (PIP_3), which activates phosphoinositide-dependent kinase 1 (PDK1) through binding to the pleckstrin homology (PH) domain and eventually activates Akt[64]. In addition, Rho GTPase, especially the RAC subfamily, can be activated by PIP3 *via* RAC-guanine nucleotide exchange factors (GEFs), such as Vav-1 and Tiam-1, with a PH domain[65]. The downstream effectors RAC and Akt are important for the dynamic changes in actin structure[61,63-66]. Arrangement of actin *via* polymerization results in morphological changes during ADM[63]. Inhibition or knockdown of PI3K, RAC or PDK1 not only maintains acinar morphology but also inhibits the development of PanIN and PDAC[62-64].

Mutation in p53, which is also a downstream effector in PI3K/Akt signaling, can be found in > 75% of PDAC patients[10]. The importance of p53 inactivation in cancer metastasis has been demonstrated[67,68]. However, whether p53 is involved in the early stage of PDAC development is still poorly understood. It may not participate in ADM but is involved in the development of higher grades of PanINs and PDAC[63].

Inactivation of acinar transcription factors in ADM

In addition to Mist, down-regulation of other acinar-specific transcription factors, including Gata6, pancreas transcription factor 1 complex (PTF1), nuclear receptor subfamily 5 group A member 2 (Nr5a2) and Hnf1α are also observed during ADM. During pancreas development, Gata4 and Gata6 are expressed in developing pancreas, as opposed to the other Gata factors, *i.e.* Gata1-3, 5[69]. In the later stage of development, there is a linage preference for Gata4 and Gata6 expression. Gata4 mRNA and protein can be found only in exocrine pancreas, together with amylase but not glycogen, whereas Gata6 is in the endocrine pancreas[69,70]. However, in the adult pancreas, Gata4 is also expressed in islet cells such as the β-cell[69]. Gata6 expression is also observed in both acinar and islet cells in the adult pancreas[71]. Gata6 regulates the expression of other acinar transcription factors, such as Mist and PTF1, through binding to their promoters[71,72]. The knockdown of *Gata6* hinders the expression of Mist, pancreas specific transcription factor1a (*Ptf1a*) and recombination signal binding protein for immunoglobulin kappa J region1 (*Rbpj1*), and also their downstream acinar genes, such as amylase, while expression of CK-19 is promoted. In addition, further activation of MAPK signaling upon Gata6 silencing emphasizes the role of Gata6 down-regulation in promoting ADM[72].

PTF1, which is composed of Ptf1α and Rbpj1, is involved in exocrine pancreas development and maintains correct spatial orientation under the regulation of Notch signaling[73-75]. Nr5a2, under the regulation of Gata6 and PTF1, is important in acinar development, maintaining acinar identity and acinar regeneration during pancreatitis[76,77]. During $KRas^{G12D}$-driven ADM, PTF1 and Nr5a2 are down-regulated and their low expressions are maintained in PanIN and PDAC[76,78]. The loss of these acinar transcription factors sensitizes the cells to KRas mutation, promoting ADM and PDAC development.

Targeting PDAC reprogramming

Under the stimulation of $KRas^{G12D}$ mutation or combined with pancreatitis, normal acinar cells can be transdifferentiated into ductal cells, followed by the formation of PanIN and PDAC. This process involves the activation of MAPK, Wnt, Notch and PI3K/Akt signaling (Figure 1). Inhibition of these pathways keeps cells in their acinar state. Importantly, treating the transdifferentiated duct cells with nicotinamide reprograms them back to acinar cells, with re-expression of amylase and repression of CK-20, and indicating that ADM may be a reversible process[49]. Therefore, PDAC development from its progenitor acinar cells may also be reversed upon the inhibition of cancer-related signaling (Figure 2).

A growing number of studies have proved the concept of reprogramming PanIN or PDAC to normal pancreatic cell types such as acinar cells and endocrine cells (Table 1). Since the MAPK signaling pathway

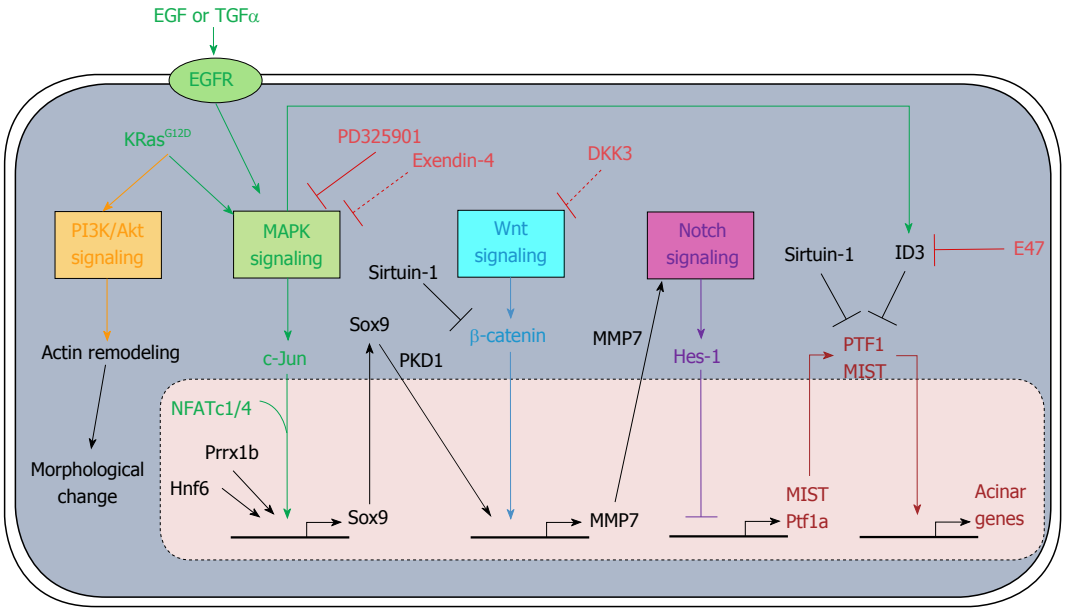

Figure 1 MAPK, Wnt, Notch and PI3K/Akt signaling is involved in acinar-to-ductal metaplasia. The potential therapies are shown in red (retinoic acid is not shown). EGF: Epidermal growth factor; TGFα: Transforming growth factor alpha; MMP7: Matrix metalloproteinase 7.

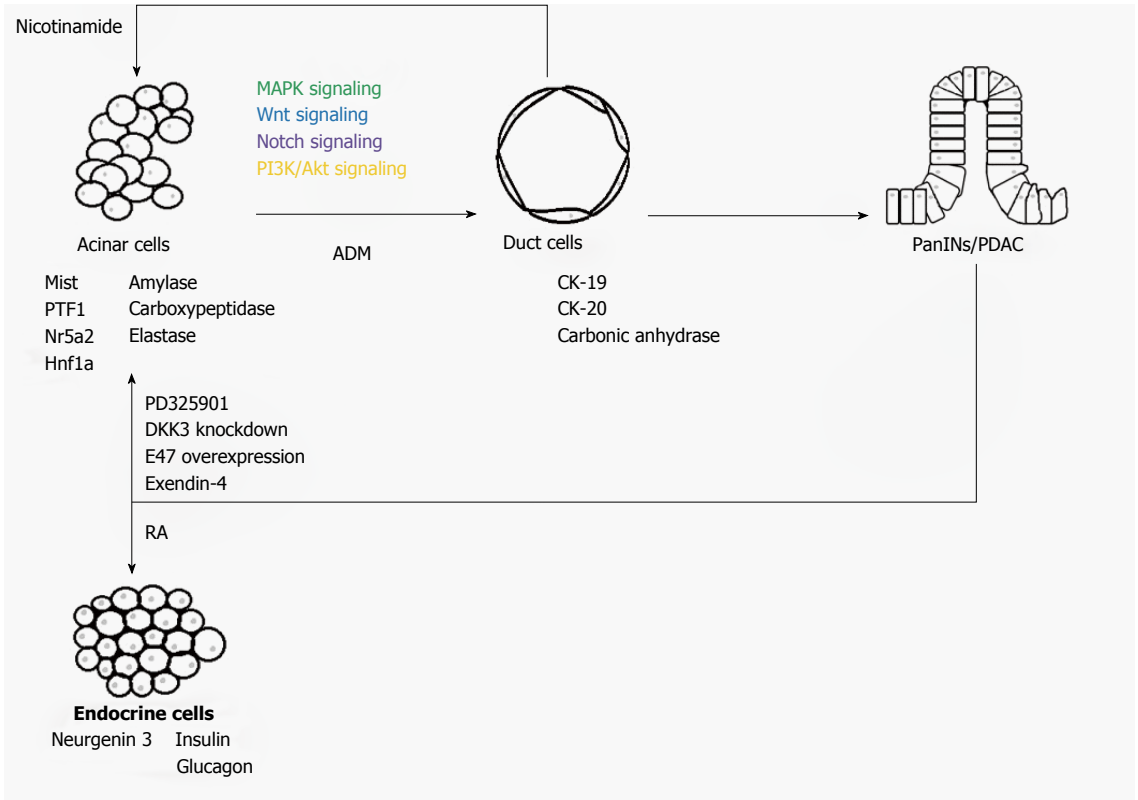

Figure 2 Development of pancreatic intraepithelial neoplasia pancreatic ductal adenocarcinoma from acinar cells through acinar-to-ductal metaplasia. During ADM, cells in each state have their own morphology and protein expression pattern. ADM: Acinar-to-ductal metaplasia.

is up-regulated and is required during ADM and carcinogenesis, attenuation of this pathway may promote cancer transdifferentiation back to quiescent acinar cells. The MEK1/2 inhibitor PD325901 is effective in treating colon cancer, breast cancer and melanoma and has entered phase II clinical trial[79]. In addition, PD325901 can enhance the anti-cancer ability of the PI3K/mTOR inhibitor PF-04691502 in lung cancer and gemcitabine in pancreatic cancer[80]. A recent report has suggested that through inhibiting the MAPK signaling pathway, PD325901 may re-differentiate PanIN back to acinar state in addition to the reduction of the proliferation rate of cancer cells both *in vitro* and *in vivo*[16]. However, whether

Table 1 Therapeutic approach in reprogramming pancreatic ductal adenocarcinoma

Therapeutic approach	Product	Mechanism	Model studied	Ref.
Chemical	PD325901	Inhibits MEK1/2 in MAPK signaling	Block ADM in *in vitro* experiment Reprogram pancreatitis- induced PanIN back to acinar cells in *in vivo* experiment	[16]
	Exendin-4	May inhibit ERK1/2 in MAPK signaling	Reprogram PANC-1 cells back to acinar cells and endocrine cells	[82]
	RA	Detailed study is needed	Reprogram HPAF cells to endocrine cells	[107]
Genetic	DKK3 knockdown	Detailed study is needed	Reprogram PANC-1 cells back to acinar cells and endocrine cells	[82]
	E47 overexpression	Functions as a sponge of ID3 to remove the inhibition of acinar transcription factors	Reprogram PDAC cell lines back to acinar cells using both *in vitro* and *in vivo* model systems	[100]

PD325901 can also reprogram cancer cells to acinar cells is still questioned, since more genetic alternations are accumulated in PDAC. Apart from PD325901, exendin-4, for which its synthetic version exenatide is used in treating type II diabetes, may reprogram PDAC[81]. It has been reported that exendin-4 induces cell cycle arrest *via* p27 and pancreatic cancer reprogramming, as indicated by expression of such digestive enzymes as amylase and chymotrypsinogen B1[82]. However, the detailed mechanism underlying how exendin-4, which is a glucagon-like peptide-1 receptor agonist, initiates cancer reprogramming is not fully understood[83]. In prostate cancer, exendin-4 inhibits cancer proliferation through suppressing MAPK signaling, as indicated by a decrease in phosphorylated ERK1/2 level[84]. On the other hand, ERK1/2 phosphorylation is observed during the process of liver transdifferentiation to pancreas involving exendin-4[85].

Dickkopf-3 (DKK-3) belongs to the DKK family, which regulates the Wnt signaling pathway. Unlike the other DKK members DKK-1, DKK-2 and DKK-4 which bind to LRP5/6 and Kremem1/2 (Krm1/2) receptors on the cell surface to form a trimer that inhibits Wnt signaling through receptor internalization, DKK-3 interacts with Krm1/2 receptors but not LRP5/6 intracellularly, probably on the membranes of endoplasmic reticulum or Golgi apparatus, before transporting to the cell surface[86,87]. The expression of DKK-3 is regulated by Pax6, which is one of the paired box transcription factors involved in development of the pancreas. DKK-3 can be activated or repressed by Pax6, depending on cancer type; in pancreatic cancer, DKK-3 is positively regulated in pancreatic cells[88]. Pax6 is a downstream target of the MAPK signaling pathway through interaction with Erk1/2 and p38 MAPK[89]. Therefore, DKK-3 links the MAPK and Wnt signaling pathways, controlling development of the pancreas. Although it has been reported that DKK-1 functions as a Wnt signaling inhibitor, the role of DKK-3 may be dependent on cell type; for example, DKK-3 positively regulates Wnt signaling in the embryonic kidney HEK293 cell line but functions as an inhibitor in lung and bone cancer[90-92]. Since it is reported that the Wnt signaling pathway contributes to cancer development, DKK-3 may function as a tumor suppressor through inhibiting the Wnt signaling pathway. Consistently, DKK-3 is down-regulated in various cancer types, such as lung and papillary thyroid cancer, although it promotes cancer invasion in oral squamous cancer[90,93-95]. Also, its expression is significantly decreased or even lost in some pancreatic cancer cell lines and pancreatic cancer tissues, probably *via DKK-3* promoter hypermethylation[82,96,97]. However, overexpression of DKK-3 does not inhibit the proliferation of pancreatic cancer cells significantly, although Uchida and colleagues[98] reported inhibition is involved in cancer cell proliferation and induction of apoptosis[82,97]. Interestingly, knockdown of the tumor suppressor DKK-3 also inhibited cell growth, but without apoptosis[82]. In addition, cell cycle arrest, morphological changes to endocrine cell-like status, and expression of the endocrine proteins insulin and glucagon and the acinar proteins amylase, chymotrypsinogen B1 and elastase are observed when DKK-3 is knocked-down. However, these changes in expression of acinar proteins or endocrine proteins have only been studied at the mRNA level but not at the protein level. In addition, DKK-3 level is up-regulated during exendin-4-induced cancer reprogramming. Therefore, whether inhibiting DKK-3 can transdifferentiate pancreatic cancer cells to normal pancreatic endocrine cells or acinar cells is still questioned.

During differentiation of the exocrine pancreas, the bHLH transcription factors Mist and PTF1 are expressed resulting in the development of acinar identity[57]. Since Mist and PTF1 are involved in acinar development, inhibiting Mist and PTF1 at the gene level results in losing acinar identity and promoting ADM[60,78]. In addition, sustained Mist expression hinders the progression of ADM and the development of PanIN[99]. Therefore, targeting acinar transcription factors can be used in cancer reprogramming. One representative example suggested by Kim *et al*[100] is the use of another bHLH transcription factor, E47. Since the bHLH family usually forms dimers, either homodimers or heterodimers, which then bind to target genes, it has been reported that E47 interacts with Mist or

PTF1 to facilitate their entry into the nucleus, followed by activation of acinar genes[101]. This bHLH dimer formation can be disrupted by the inhibitor of DNA (ID) family member ID3, which is mediated by MAPK signaling. This disruption can result from interaction between ID3 and bHLH transcription factors, forming a non-functional ID3/bHLH dimer, or resulting in the decrease in E47 expression[101]. Apart from regulating acinar gene expression, E47 also inhibits the progression from G1 phase to S phase in cell cycle through binding to the p21 promoter[100]. In PDAC, the elevated protein level of ID3 results in decreased E47 activity and cell cycle entry. Because of its role in normal acinar cells and its decreased protein level in PDAC, it has been reported that overexpression of E47 induces growth arrest[100,101]. In addition, Kim *et al*[100] demonstrated that E47 may reprogram PDAC back to acinar cells, as indicated by the expression of Mist and its target genes such as trypsinogen, connexin32 and ZO.1 which are responsible for protein digestion, gap junctions and tight junctions respectively. The role of overexpressed E47 in suppressing tumor growth and cancer reprogramming has also been confirmed by *in vivo* study. Therefore, overexpression of bHLH transcription factors that directly target acinar genes may reprogram PDAC to acinar cells.

In addition to pancreatic acinar cells, normal endocrine cells, such as β-cells, may also be another goal in cancer reprogramming. During pancreatic development, retinoic acid (RA) stimulates the development of endocrine progenitor cells and the differentiation into β-cells[102]. Also, RA can be used in treating diabetes through regenerating functional endocrine cells or human embryonic stem cells differentiation to β-cells[103,104]. In addition, RA, which has already been approved for treating acute promyelocytic leukemia, may inhibit the growth or promote differentiation of pancreatic cancer stem cells[105,106]. Since pancreatic duct cells and endocrine cells originate from the same bipotent progenitor, the close developmental relationship favors the transdifferentiation between these two cell types[33]. El-Metwally *et al*[107] demonstrated the use of RA in reprogramming PDAC back to normal quiescent endocrine cells secreting insulin, glucagon and somatostatin; although, the detailed mechanism needs further study. In addition, induction of insulin production by glucose indicates expression of the insulin receptor gene. This further confirms the formation of functional endocrine cells. Interestingly, apoptosis is observed after cancer transdifferentiation, which contrasts to other reprogramming methods that suggest the formation of quiescent acinar cells without undergoing apoptosis[107]. Increasing attention has been paid to the role played by the mitochondria during cancer reprogramming. Increased mitochondrial activity and mass are observed during cancer trans-differentiation, which is followed by apoptosis[108]. It has been suggested that apoptosis occurring after cancer transdifferentiation to normal endocrine cells may be due to an uncorrectable genetic alternation during cancer development.

CONCLUSION

It is generally believed that pancreatic duct cells are the origin of PDAC. However, during pancreatitis, acinar cells can undergo ADM, forming duct cells and subsequently PanIN and PDAC under the activation mutation of $KRas^{G12D}$. Therefore, acinar cells may also be the origin of PDAC through ADM. This acinar transdifferentiation process involves at least MAPK, Wnt, Notch and PI3K/Akt signaling. Inhibition of ADM in transdifferentiated cells can facilitate regaining the acinar identity, indicating ADM may be a reversible process and can be used as a chemopreventive. In addition, PanIN or even PDAC may be reprogrammed back to normal cells. The use of PD325901, exendin-4 and RA, overexpression of E47 and knockdown of DKK-3 have demonstrated the potential of cancer reprogramming. However, more evidence is needed to confirm the success of reprogramming. Future studies are needed to explore other therapeutic targets for regaining normal identity of PDAC.

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