

## Review Article

# Role of Notch signaling pathway in pancreatic cancer

Jiankun Gao<sup>1</sup>, Bo Long<sup>2</sup>, Zhiwei Wang<sup>3,4</sup>

<sup>1</sup>Sichuan College of Traditional Chinese Medicine, Mianyang, Sichuan, China; <sup>2</sup>Department of Infectious Diseases, Mianyang 404 Hospital, Mianyang, Sichuan, China; <sup>3</sup>The Cyrus Tang Hematology Center, Jiangsu Institute of Hematology, The First Affiliated Hospital, Soochow University, Suzhou 215123, China; <sup>4</sup>Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, MA 02215, USA

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**Abstract:** Pancreatic cancer (PC) is one of the highly aggressive malignancies in the United States. It has been shown that multiple signaling pathways are involved in the pathogenesis of PC, such as JNK, PI3K/AKT, Rho GTPase, Hedgehog (Hh) and Skp2. In recent years, accumulated evidence has demonstrated that Notch signaling pathway plays critical roles in the development and progression of PC. Therefore, in this review we discuss the recent literature regarding the function and regulation of Notch in the pathogenesis of PC. Moreover, we describe that Notch signaling pathway could be down-regulated by its inhibitors or natural compounds, which could be a novel approach for the treatment of PC patients.

**Keywords:** Pancreatic cancer, notch, cellular signaling, therapy, target

## Introduction

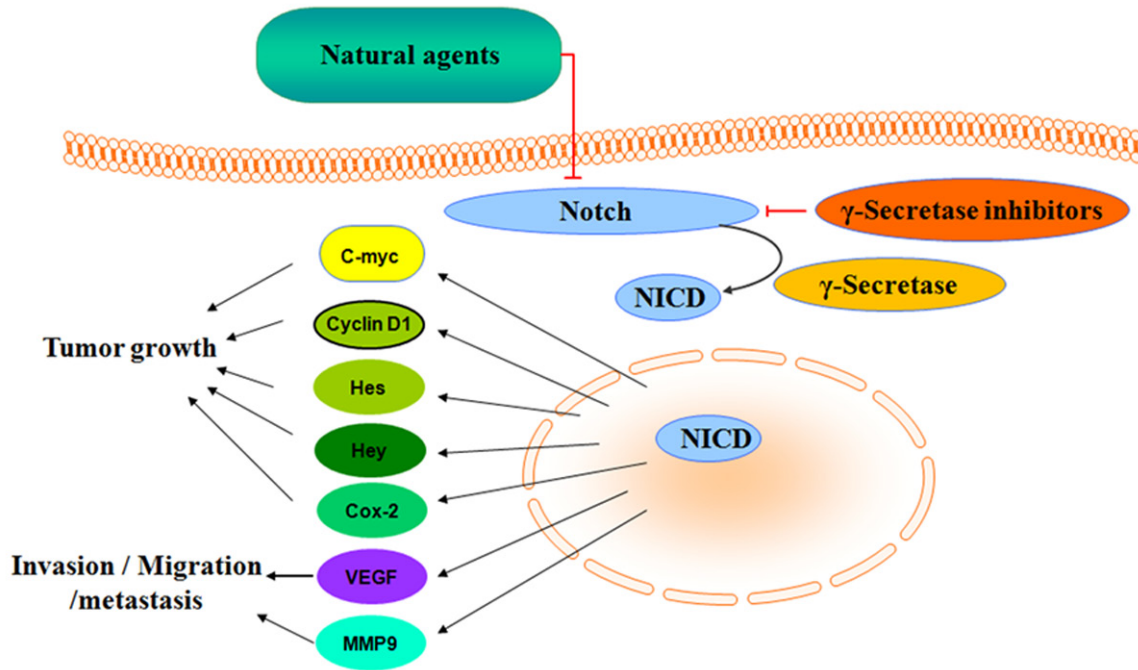
Pancreatic cancer (PC) is a highly aggressive malignancy and ranks the fourth leading cause of cancer related death in the United States [1]. In China, PC belongs to one of the ten most common cancers for men, and the incidence and the mortality of PC are increased in recent years [2]. This high mortality is partly due to the absence of specific symptoms and signs, and the lack of early detection tests for PC, as well as the lack of effective chemotherapies [3]. Although the molecular mechanisms of PC development remain largely unclear, many factors have been reported to be associated with increased incidence of PC [4]. For example, a history of diabetes or chronic pancreatitis, chronic cirrhosis, a family history of PC, a high-fat and high-cholesterol diet, tobacco smoking, alcohol and coffee intake, use of aspirin and specific blood type have been found to contribute to PC development [5]. In recent years, studies have shown that multiple cellular signaling pathways such as JNK [6], PI3K/AKT [7], nuclear factor kappaB (NF- $\kappa$ B) [8], Hedgehog [9], and Skp2 [10] are believed to play critical roles in the aggressive pathological progression of PC. It is important to note that the exact

mechanisms by which PC develops and progresses still remain poorly understood. However, robust evidence has been accumulated to suggest that Notch plays an important role in the development of PC [11-14]. Therefore, in this review article, we will focus our discussion on the role of Notch in the development and progression of PC, and further summarize potential approaches by which Notch could be inhibited.

## Notch signaling pathway

It has been well documented that the Notch signaling pathway is critical for cell proliferation, differentiation, development and homeostasis [15]. It is known that mammals express four transmembrane Notch receptors (Notch-1, Notch-2, Notch-3 and Notch-4) and five canonical transmembrane ligands (Delta-like 1, Delta-like 3 and Delta-like 4, Jagged-1 and Jagged-2) [16]. Notch signaling pathway will be activated after Notch-ligand binding and three consecutive proteolytic cleavages by multiple enzyme complexes including  $\gamma$ -secretase complex [17]. This produces an active fragment, NICD (Notch intracellular domain), which enters the nucleus and binds to CSL, and displaces co-repressors

## Notch in pancreatic cancer



**Figure 1.** Diagram of Notch signaling. Notch signaling pathway plays a pivotal role in the development and progression of PC via regulating its target genes.  $\gamma$ -secretase inhibitors and natural agents could be inhibitors of Notch pathway in PC cells.

from CSL, and subsequently recruits a co-activator complex containing mastermind, p300, and other co-activators, leading to the activation of Notch target genes [18]. So far, many Notch target genes have been identified such as Hes (hairy enhance of split) family, Hey family, Akt, cyclin D1, c-myc, COX-2 (cyclooxygenase-2), ERK (extracellular signal-regulated kinase), MMP-9 (matrix metalloproteinase-9), mTOR (mammalian target of rapamycin), NF- $\kappa$ B, p21, p27, p53 and VEGF (vascular endothelial growth factor) [15]. Since these target genes are critically involved in tumorigenesis [19], Notch signaling pathway plays a pivotal role in the development and progression of human cancers via regulating its target genes (**Figure 1**).

### The role of notch in the development and progression of pancreatic cancer

It is noteworthy that the Notch signaling pathway exerts both oncogenic and tumor suppressive functions, depending on the cellular context [20]. For example, one study has shown that Notch-1 has an oncosuppressive function in skin cancer [21]. Another study has demonstrated that Notch1 suppresses PanIN (Pan-

creatic Intraepithelial Neoplasias), the proposed precursor lesions of PC formation in a mouse model of PC [22]. More evidence suggests that Notch plays important oncogenic roles in pancreatic tumorigenesis. For example, it has been reported that Notch can promote PanINs [23]. Many literatures also strongly suggested that increased expression of Notch is detected in PC cells and tissues. Moreover, reactivation of Notch signaling is observed in early PC pathogenesis and persists throughout the progression of the disease [24-28], suggesting that Notch could be useful as a prognostic biomarker. However, the molecular mechanism(s) by which Notch induces PC growth has not been fully elucidated. However, multiple signaling pathways, such as MEK/ERK [24], plasma growth factor receptor-c-Src [29], Hedgehog [30], TGF- $\beta$  (transforming growth factor-beta) [31], and Wnt [32] signaling have been reported to crosstalk with Notch in PC, and so it is believed that the crosstalk between Notch and these signaling pathways may play critical roles in pancreatic tumorigenesis. In the following sections, we will discuss the recent advances in our understanding of the role of Notch in PC progression. We will also summarize the results of emerging studies on Notch,

including the upstream regulators and downstream effectors of this protein, as well as its implication in human PC.

### *Upstream regulators of notch in PC*

Little is known regarding the upstream regulators of Notch in PC. Several groups have found that some genes can regulate Notch expression. For instance, EGFR (epidermal growth factor receptor) can regulate Notch-1 expression and EGFR-mediated Notch-1 activation leads to the up-regulating MMP-9 and VEGF expression, and stimulating cell invasion and metastasis in PC cells [29]. Notch was also proved as a target for CCN1 regulation that provides signals that support tumorigenic activities [33]. Additionally, Tremblay et al. reported that activation of the MEK/ERK pathway promotes Notch signaling [24]. More recently, it has been found that over-expression of ASPH (Aspartate  $\beta$ -hydroxylase) activates Notch by promoting cleavage of Notch1 ICN to liberate the C-terminal ICN and subsequently upregulates a number of downstream Notch responsive genes in PC cells [34]. DNMA1L (Dominant-Negative form of Mastermind-like1) expression successfully inhibited Notch signaling in the pancreas in vivo [23], leading to degradation of nuclear Notch1 thereby inducing PC cell death [35]. The mechanisms by which these upstream genes regulate Notch are discussed in the following paragraphs.

### *EGFR regulates notch in PC*

EGFR is a member of the ErbB family of receptors and has tyrosine kinase activity and it is overexpressed in PC [36]. EGFR could dimerize with other members of the EGFR family as a homodimer or heterodimer, after ligand binding. Then, EGFR is activated by auto-phosphorylated or trans-phosphorylated at specific tyrosine residues, then multiple downstream signaling pathways, including phosphatidylinositol 3'-kinase and AKT, ERK, and the Notch pathway are activated, finally, leading to increased cellular proliferation and prevention of apoptosis [37, 38]. EGFR over-expression is supposed to confer a poor survival, relating to a more advanced stage and the presence of metastases in PC. Consequently, inhibition of the EGFR signaling pathway is a tempting therapeutic target. The relationship between Notch and EGFR is mostly antagonistic, which may in part be

based on the phosphorylation of the Notch signal transducer Suppressor of Hairless, a transcription factor that together with several cofactors regulates the expression of Notch target genes by MAPK (Mitogen-activated protein kinase) [39].

We have found that ERRP (EGFR-related peptide) inhibited cell growth of PC cells by attenuating EGFR activation in vitro and in vivo [40, 41], then, we strongly proved that ERRP down-regulate Notch-1 and its downstream target genes which are mechanistically linked to apoptotic processes in PC [38]. That is ERRP inhibited the activation of EGFR and also reduced the activity of Notch-1 signaling.

### *CCN1 regulates notch in PC*

CCN1, formerly known as *cyr61*, belongs to the Cyr61-CTGF-Nov (CCN) family and is a secreted protein that functions in a paracrine and/or autocrine manner [42, 43]. CCN1's functions include but not limited to angiogenesis, cell adhesion, migration and cytoprotection [43-46]. It is abnormal expression in a variety of cancers including PC [33]. Haque et al. showed that CCN1 mRNA and protein expression were elevated in most of PC specimens and pancreatic cancer cell lines, and silencing CCN1 inhibited cell migration, EMT and tumor growth in nude mice [47].

CCN1 is a potent regulator of Shh (sonic hedgehog) pathway via Notch-1. CCN1 activity was mediated in part through altering proteasome activity [33]. It has been shown that CCN1 impacts both the Shh and Notch pathways [48]. CCN1 acts, at least in part, by altering proteasome activity. Neutralizing anti-integrin  $\alpha$ v or anti-integrin  $\beta$ 3 antibodies markedly blocked CCN1-induced activation of Notch-1 and Shh in PC cells, emphasizing the importance of these integrins in CCN1-mediated activity. These results suggest that CCN1 may be an ideal target for treating PC.

### *MEK/ERK regulates notch in PC*

It is well documented that MEK/ERK signaling is directly involved in the prevention of apoptosis [49]. The MEK/ERK pathway has been shown to play a pivotal role in controlling cell growth, radioresistance and differentiative signals [50]. MEK/ERK pathway plays a prominent role in maintaining the stem-like phenotype of

rhabdomyosarcoma cells [51]. Abnormal activation of the MEK/ERK signaling pathway is tightly associated with tumorigenesis including PC [52].

Tremblay et al. founded that MEK/ERK signaling pathway is efficient to, immediately after Notch1 cleavage, directly influence NIC1 (Notch1 intracellular domain) transcriptional function [24]. They suggested that the MEK/ERK pathway promoted expression of Notch target genes, therefore influencing Notch signal strength by promoting the assembly of a functional NIC1 transcriptional unit with CSL and MAML1 (MASTERMIND-LIKE 1) in PC. However, further studies are required to delineate the precise mechanisms by which the MEK/ERK pathway promotes Notch signaling.

### *ASPH regulates notch in PC*

Aspartate  $\beta$ -hydroxylase (ASPH) is an 86 KD Type II transmembrane protein. It is also a member of the  $\alpha$ -ketoglutarate-dependent dioxygenase family [34, 53]. ASPH catalyzes the  $\beta$ -hydroxylation of aspartyl and asparaginyl residues located in the EGF-like repeats of various proteins including Notch, Jagged and Delta-like [54]. ASPH is expressed in many organs during embryogenesis, but has very low or negligible expression in adult tissues [55]. Then, it re-emerges in tumors of pancreas and lung [34, 56], suggesting it may be an oncogene involved in the transformation of normal cells to a malignant phenotype [57]. ASPH could promote tumor growth and cell migration and invasion [58, 59]. ASPH may play an important role in PC pathogenesis [34].

Notch receptors contain 36 EGF-like repeats in the extracellular domain, which are the substrates of ASPH  $\beta$ -hydroxylase. Notch signaling pathway can be activated by ASPH upregulation to promote tumor cell migration, invasion and metastases [58, 60]. Dong et al. reported that ASPH may promote the interactions of the Notch receptors with their ligands (such as Jagged and Delta-like) [34]. It is suggested that enhanced Notch receptor-ligand interaction leads to the generation of activated Notch1 ICN followed by upregulation of downstream target genes.

### *DNMAML regulates notch in PC*

DNMAML contains amino acids 13-74 of MAML1, which binds the Notch-CSL/RBP-J

complex, but lacks the MAML1 sequences needed to recruit transcriptional coactivators [61]. Tu et al. supposed that DNMAML is a pan-Notch inhibitor, blocking signaling from all four Notch receptors [62]. DNMAML expression was previously shown to lead to both cell proliferation and invasion [63]. Thomas et al. found that DNMAML expression efficiently inhibits epithelial Notch signaling and delays PanIN formation [23].

### *FBW7 regulates notch in PC*

FBW7 (F-box and WD repeat domain-containing 7), also known as Fbxw7, is the F-box protein subunit of a Skp1-Cul1-F-box protein (SCF)-type ubiquitin ligase complex. FBW7 contains 3 isoforms (FBW7 $\alpha$ , FBW7 $\beta$ , and FBW7 $\gamma$ ), and they are differently regulated in subtract recognition [64]. Besides, FBW7 activity is controlled at different levels, resulting in regulation of the abundance and activity of its substrates in a variety of human solid tumors, including PC [65]. FBW7 has been found to be related to numerous cellular processes such as cell proliferation, apoptosis, cell cycle and differentiation [66-68]. Furthermore, FBW7 is considered as a tumor suppressor protein for that it targets multiple well-known oncoproteins including Notch-1 by ubiquitination-mediated destruction [35, 69]. It is well documented that FBW7 binds to phosphorylated Notch 1C and mediates its ubiquitination and then rapid degradation [70]. Furthermore, FBW7 regulates Notch1 downstream signaling pathways through ubiquitin ligase-mediated degradation [71].

We observed that accumulation of FBW7 can lead to down-regulation of Notch1 and related pathways (Hes-1, C-Myc and VEGF) in PC cells which was correlated with growth inhibition, cell cycle arrest and apoptosis of PC in vitro and in vivo [35]. More importantly, we found that miR-223 governs gemcitabine-resistant (GR)-induced epithelial-to-mesenchymal transition (EMT) in part due to down-regulation of its target FBW7 and subsequent upregulation of Notch-1 in PC [72].

### **Downstream effectors of notch in pancreatic cancer**

Studies have demonstrated that Notch regulates a variety of cellular processes including cell cycle progression, cell proliferation, apop-

tosis, differentiation, migration, invasion, and survival, all of which are related to cancer development and progression. This is mainly achieved through directly promoting the degradation of Notch downstream targets. Here, we mainly focus on discussing the recent advances in the understanding of the role of Notch in PC progression.

### *Notch regulates Akt in PC*

Serine/threonine protein kinase Akt named protein kinase B (PKB), the virus oncogenes V-akt homologue. The function of the Akt involves nutrient metabolism, cell survival and cell growth, cell apoptosis, and cell cycle regulation [73-76]. Akt has three isoforms Akt1, Akt2 and Akt3 (or PKB $\alpha$ / $\beta$ / $\gamma$  respectively) [77]. Aberrant expression of Akt leads to many diseases such as cancer, diabetes, cardiovascular and neurological diseases [78]. Akt is found to be activated in 59% of tumors [13]. It is well known that Akt could be a central node in signaling pathways consisting of many downstream components, such as mTOR [79].

Li et al. found that Notch-1 activates Akt in breast cancer [80]. Vo et al. discovered that Notch modulates the Akt pathway through regulation of PTEN (phosphatase and tensin homologue) phosphorylation [13]. Moreover, the regulation is dependent on RhoA, a member of the Rho family of small GTPases which is required for Rock1 activation [81]. The Notch-dependent increase in PTEN phosphorylation is inhibited by Rock1 inhibitor, suggesting that Notch regulates PTEN through the RhoA/Rock1 pathway in PC. Therefore, targeting both pathways will lead to a greater efficacy in the treatment of patients with PC.

### *Notch regulates PKD1 in PC*

Protein Kinase D family members are serine/threonine kinases that consists of three isoforms: PKD1/PKC $\mu$ , PKD2 and PKD3/PKC $\nu$  [82]. PKDs effect on diverse biological processes such as protein transport, cell migration, proliferation and apoptosis, which are characteristics in the stepwise pathogenesis of neoplasia. PKDs have distinct impact on these functions. PKD1 was the first isoform identified and is the most widely studied [83]. PKD1 blocks EMT and cell migration [84], and it was overexpressed in PC patient samples [85]. In

PC cell lines PKD1 contributes to cell proliferation and cell survival [86, 87]. Furthermore, it was shown that inhibition of PKD1 decreases orthotopic growth of PC cell lines in mice [86].

Liou et al. show that PKD1 signaling can contribute to very early events that alter pancreas cellular plasticity. Their results show that PKD1 is necessary to mediate TGF $\alpha$ - and active Kras-induced reprogramming of pancreatic acinar cells to a duct-like phenotype that can give rise to PanIN lesions [88]. They identified that Notch signaling pathway activates the TGF $\alpha$ -Kras-PKD1 [88].

### *Notch cross-talks with other major signaling pathways in PC*

Notch has been reported to cross-talk with other pathways, such as Bcl-2, and NF- $\kappa$ B [89, 90]. Thus, cross-talks between Notch and other pathways could play pivotal roles in pancreatic tumorigenesis.

#### *The cross-talk between notch and Bcl-2 in PC*

B-cell leukemia/lymphoma-2 (Bcl-2) and its family members were overexpressed in PC [91, 92]. Bcl-2 is the strongest apoptosis inhibitor among all known cell proteins [93], and it includes three subgroups of proteins. Defects in apoptosis are now considered to be a hallmark of most cancers [94]. Bcl-2 family proteins decide mitochondrial involvement in cell death cascades, modulating commitment to apoptosis to diverse stimuli [95]. It has been well documented that Bcl-2 functions through heterodimerization with proapoptotic members of the Bcl-2 family to prevent mitochondrial pore formation and prevent cytochrome c release and initiation of apoptosis [89, 96]. A number of studies have shown that activated Notch can regulate expression of Bcl-2 [97, 98], suggesting that Bcl-2 is downstream of Notch inhibition. Additionally, overexpression of Bcl2 can increase Notch-1. However, down-regulation of Bcl-2 can inhibit the Notch-1 expression in PC cells [89]. We found that the inactivation of Bcl-2 down-regulated the Notch-1 activity, resulting in the inhibition of the growth of PC cells in vitro and in vivo [89].

#### *The cross-talk between notch and NF- $\kappa$ B in PC*

The NF- $\kappa$ B family includes homo- or heterodimers formed by p50, p65 (RelA), c-Rel, p52, and RelB [99, 100]. NF- $\kappa$ B plays an important

role in the control of cell growth, differentiation, apoptosis and inflammation by mediating survival signals [101, 102]. NF- $\kappa$ B and Notch pathways are activated in many types of cancers including PC [103, 104]. Numerous reports have described regulation of NF- $\kappa$ B by Notch and vice versa through different, context-dependent mechanisms [105]. It is well documented that transcriptional regulation of NF- $\kappa$ B pathway members by Notch and transcriptional regulation of Notch pathway members by NF- $\kappa$ B are physical interaction between Notch and NF- $\kappa$ B [105]. Maniati et al. supposed that the interaction between NF- $\kappa$ B and Notch signaling and a coordinated downregulation of PPAR- $\gamma$  acted as a forward feedback loop that sustains expression of inflammatory cytokines and chemokines by the transformed cells in PC, which highlight the requirement for inflammatory signaling pathways in the development of PC [90].

### *The cross-talk between notch and c-Src in PC*

C-Src is a non-receptor tyrosine kinase product of the proto-oncogene c-Src [106], which has been shown to exert as a target of G protein-coupled receptors (GPCRs) in transactivation of growth factor receptors such as EGFR [107]. c-Src also takes part in cytokine-activated transactivation of growth factor receptors such as EGFR in several cell types [108]. More and more evidence suggests Src as an important determinant of tumorigenesis, invasion, and metastasis [109]. c-Src is overexpressed in over 70% of PC cell lines, and Src kinase activity is often elevated [106].

Some studies have demonstrated colocalization of Notch and c-Src proteins in PC cells, where Src is required for proteolytic activation of Notch [29], and of Notch and the T-cell-specific Src family member Lck in T cells [110]. Ho et al. revealed a functional relationship between the two genes that is Notch-Src accesses JNK in a significantly different fashion than Notch-Mef2 (Myocyte enhancer factor 2) [111]. Ma et al. indicated that Notch-1 and c-Src proteins are physically associated and that this kind of association mediates Notch-1 processing and activation [29].

### *The cross-talk between notch and hedgehog in PC*

The hedgehog (Hh) signaling pathway is a critical embryological signaling pathway, both dur-

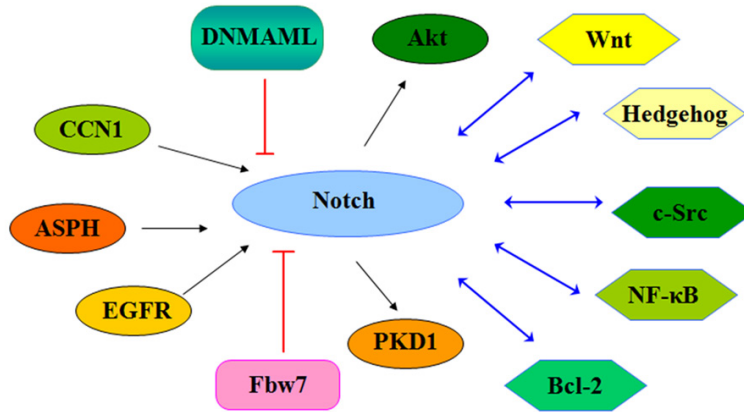
ing development and in adult tissues. The pathway is regulated by two twelve-pass transmembrane receptors, Patched1 (Ptch1) and Patched2 (Ptch2), which are localized to the primary cilium [112]. There are three homologous Hh ligands: Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert Hedgehog (Dhh) exist in most vertebrate species. Each homologous has different expression patterns and functions, which probably helped promote the increasing complexity of vertebrates and their successful diversification [113]. It has been discovered that many cancers contain abnormal Hedgehog signaling activation which is associate with 1/3 of cancer-related deaths. Hedgehog signaling pathway is an ideal target for chemotherapeutic development [114]. Gli1, a downstream transcription factor of the Hedgehog signaling pathway, is overexpressed in many cancers and is supposed to play a role in the development and progression of metastatic diseases [115]. Aberrant Hedgehog/Gli1 signaling pathway activity has been related to growth and progression in many types of cancers including PC [116, 117].

Notch and Hedgehog signaling have been implicated in the survival of cancer stem cells (CSC), suggesting that both pathways will need to be targeted simultaneously when we aim to eradicate some kinds of cancer in patients [118]. Schreck et al. found that direct upregulation of the Hedgehog pathway through a novel cross talk mechanism, which is Notch like directly suppress Hedgehog via Hes1 mediated inhibition of Gli1 transcription [119]. Vice versa, Hedgehog pathway components Gli1 and Gli2 are able to positively regulate Hes1 independently of Notch [120, 121]. Targeting both pathways simultaneously may be more effective at eliminating cancer cells. Downregulation of Notch leads to the cell growth inhibition and apoptosis of PC cells, whereas Hedgehog inhibition will contribute to enhanced delivery of drugs to the tumors. Both pathway inhibitors seem to have synergistic effects for therapeutics for PC [122].

### *The cross-talk between notch and wnt in PC*

The Wnt signal pathway is an important embryonic signaling pathway that is required for proliferation, morphogenesis and differentiation of several organs, including the pancreas [123]. Wnt ligands bind to receptors of the Frizzled

## Notch in pancreatic cancer



**Figure 2.** Diagram of signaling pathways that control Notch expression in PC.

(FZD)/low-density lipoprotein receptor related protein then inactivate a complex of cytoplasmic proteins that promote the proteasomal degradation of  $\beta$ -catenin, resulting in its cytoplasmic accumulation and nuclear localization and subsequent transcriptional regulation of target gene [124-126]. Wnt signaling regulates many aspects of pancreatic biology, and its activity is gradually increased during pancreatic carcinogenesis. The accumulation of  $\beta$ -catenin and activation of Wnt target genes have been observed in PanINs and PC [127, 128].

Notch and Wnt signaling are both pivotal pathways that control the proliferation and differentiation of stem/progenitor cells [129]. In the early pancreatic lineage commitment, there is a crosstalk between Notch and Wnt signaling. Notch pathway promotes the lineage commitment and differentiation of pancreatic progenitors, whereas Wnt signaling maintains the stem cell state [130-133]. Wnt pathway regulates Notch signaling by the negative effect of Wnt on the Dishevelled 2 (Dvl2)-mediated GSK3 $\beta$  activity. GSK3 $\beta$  stabilizes the Notch-IC by binding and phosphorylating Notch-IC in the embryonic fibroblasts and N2a cells [134], and GSK-3 $\beta$  inhibition leads to the degradation of Notch-IC mediated by the proteasome [133]. Furthermore, Wnt signaling inhibits Notch activity through Pygopus2 to promote self-renewal and to prevent the premature differentiation of mammary stem cells [135]. On the other hand, Notch has been demonstrated to directly bind to  $\beta$ -catenin to inhibit its function by promoting  $\beta$ -catenin lysosomal sequestration and degradation in embryonic stem cells and colon cancer cells [136]. Notch and Wnt pathways appear

to be interlinked in that Notch signaling functions as a negative regulator  $\beta$ -catenin dependent signaling both in pancreas organogenesis and oncogenesis [32].

### Notch inhibition is a novel strategy for PC treatment

Since Notch signaling pathway is involved in tumor cell cycle regulation, cell growth, apoptosis, migration, and metastasis and its cross-talk with many signaling pathways in human cancers including PC,

Notch has emerged as an attractive pharmacological target for the development of novel cancer therapy. Due to that Notch signaling is activated via the activity of  $\gamma$ -secretase,  $\gamma$ -secretase inhibitors (GSIs) could be useful for cancer therapy [16, 137]. Indeed, emerging evidence has suggested that several forms of GSIs inhibited tumor cell growth, migration and invasion in various human cancers including PC [138]. For example, the inhibition of Notch activity by GSIs retarded tumor development in a murine model of PC [138]. Notably, GSI can block EMT, migration and invasion in PC cells, and suppress pancreatic CSCs in a xenograft mouse model [139]. Although GSI has shown the anti-tumor activity in human cancer, GSIs exhibits multiple side-effects. For instance, GSIs could block the cleavage of all four Notch receptors and multiple other  $\gamma$ -secretase substrates, which could be important for normal cell survival [138]. Additionally, GSIs has unwanted cytotoxicity in the gastrointestinal tract [138].

To overcome the limitations of GSIs, several studies have used less toxic alternative therapies such as Quinomycin [140]. or other natural compounds, which are typically non-toxic to human cells, to inhibit Notch signaling pathway in human malignancies. For example, nature agents such as genistein, curcumin, sulforaphane have been reported to inhibit Notch expression. We revealed that genistein inhibited cell growth, migration, invasion, EMT phenotype, formation of pancreatospheres via suppressing Notch-1 expression in PC cells [141]. Consistently, we observed that genistein inhibited Notch-1 expression through up-regulation of miR-34a in PC cells [142]. Sulforaphane, a

natural compound derived from cruciferous vegetables, was shown to target the pancreatic CSCs [143-145]. Moreover, the synergistic activity of sulforaphane and sorafenib was found to eliminate CSCs from PC cells [146]. Furthermore, sulforaphane increased the sensitivity of cells to chemotherapeutic agents such as cisplatin, gemcitabine, doxorubicin and 5-fluorouracil through targeting CSCs and inactivation of Notch-1 in PC. [143] More recently, Xu et al. found that Qingyihuaji formula (QYHJ), composed of traditional Chinese herbs, down regulated Notch-targeted genes Hes-1 and Hey-1 expression in both mRNA level and protein level, which was more effective than gemcitabine treatment in PC [147]. Taken together, these findings suggest that natural compounds could be non-toxic inhibitors of Notch pathway in PC cells.

### Conclusions

In conclusion, Notch signaling pathway plays an important role in the development and progression of human cancers including PC. Therefore, development of inhibitors that target Notch could be a novel strategy for the treatment of PC. One alternative strategy may be to target several signaling pathways that control Notch expression (**Figure 2**). Since natural compounds have less toxic to human, these natural agents could be useful for prevention of tumor progression and successful treatment of PC through inactivation of Notch signaling pathway.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Zhiwei Wang, Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave., CLS 628B, Boston, MA 02215, USA. Tel: 617-682-2680; Fax: 617-735-2480; E-mail: zwang6@bidmc.harvard.edu

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## Notch in pancreatic cancer

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