Pancreatic cancer is the most aggressive malignant disease once it is diagnosed and it remains the fourth leading cause of cancer-related death in the U.S.A. Recent data indicates that the Notch signaling pathway plays an important role in the development and progression of pancreatic cancer. Emerging evidence also suggests that the activation of the Notch signaling pathway is mechanistically associated with molecular characteristics of cancer stem cells (CSCs) in pancreatic cancer. Moreover, CSCs are known to be highly drug-resistant, suggesting that targeted inactivation of Notch signaling would be useful for overcoming drug resistance and the elimination of CSCs. This review describes the roles of the Notch signaling pathway in pancreatic cancer with a special emphasis on its novel functions in the regulation of pancreatic CSC. Moreover, the review also proposes that targeting the Notch signaling pathway by natural agents may represent a novel strategy for overcoming drug resistance and the elimination of CSCs, which would be useful for the successful treatment of patients diagnosed with pancreatic cancer. 

Pancreatic cancer is a lethal disease. In 2010, pancreatic cancer was ranked as the fourth leading cause of cancer-related death, with over 43,140 newly diagnosed cases and approximately 36,800 deaths in the U.S.A. (1). Due to the absence of specific symptoms and signs and the lack of early detection tests, pancreatic cancer is usually diagnosed at an advanced stage, and thus it is incurable (1). Therefore, despite some advances in the surgical technique, radiation therapy and chemotherapy, the one-year survival rate is only 24% and only a dismal 6% of all patients with pancreatic cancer will survive five years (1). These figures have remained relatively unchanged over the past 25 years. One reason for this disappointing outcome is due to the fact that the majority of patients present with locally advanced cancer or metastases at the time of diagnosis. In addition, the lack of effective chemotherapies also contributes to high mortality of patients diagnosed with pancreatic cancer (2).

The molecular causes of pancreatic cancer are not yet fully understood (3). Several studies have shown an increased incidence of pancreatic cancer among patients with diabetes, chronic pancreatitis and family cancer syndromes (4). Several risk factors such as cigarette smoking, alcohol and coffee intake, use of aspirin, chronic cirrhosis, a high-fat and high-cholesterol diet and specific blood type have been found to be associated with an increased incidence of pancreatic cancer (5). In the past decades, many genes and signaling pathways have been investigated and believed to play critical roles in pancreatic cancer (6, 7). Recent evidences suggest that the Notch signaling pathway is one of the most important pathways whose activation contributes to pancreatic cancer (8). The molecular knowledge of the Notch signaling pathway with respect to pancreatic cancer is considered important for discovering new drugs and the design of novel therapeutic strategies for the treatment of pancreatic cancer with improved outcome.

Notch Signaling Pathway: An Overview

The Notch signaling pathway has been known to play critical mechanistic roles in the development of organs, tissue proliferation, differentiation and apoptosis (9). So far, four Notch transmembrane receptors (Notch1-4) and five ligands (Delta-like 1, 3, 4 and Jagged-1, -2) have been identified in mammals (10). Notch signaling is activated when a Notch ligand binds to an adjacent Notch receptor between a cell...
expressing a transmembrane-associated ligand and a cell expressing a transmembrane receptor (Figure 1). Upon activation, Notch is cleaved through a cascade of proteolytic cleavages by the metalloprotease, tumor necrosis factor-α-converting enzyme (TACE) and γ-secretase (11). The cleavage site by TACE is at an extracellular domain between Ala (1710) and Val (1711) residues, leading to the generation of Notch extracellular truncation (NEXT). NEXT is then cleaved by the γ-secretase complex, which consists of presenilin and nicastrin, releasing the active fragment Notch intracellular domain (NICD). In the absence of NICD, transcription of Notch target genes is maintained in an inactive state through a repressor complex mediated by the CBF1, suppressor of hairless and lag-1 (CSL). When NICD is in the nucleus, it binds to CSL, which displaces co-repressors such as SKIP, SHARP, histone deacetylases from CSL. The CSL-NICD complex recruits a co-activator complex containing mastermind, p300, and other co-activators, leading to the activation of Notch target genes (9, 11, 12) (Figure 1). The complexity of the various repressor and activator nuclear complexes in Notch signaling is not yet fully understood. However, Notch target genes are well characterized, namely hairy enhancer of split (Hes) and hes-related (Hey) family. In recent years, more Notch target genes have been identified such as Akt, cyclin D1, c-myc, cyclooxygenase-2 (COX-2), extracellular signal-regulated kinase (ERK), matrix metalloproteinase-9 (MMP-9), mammalian target of rapamycin (mTOR), nuclear factor-kappa B (NF-κB), p21<sup>cip1</sup>, p27<sup>kip1</sup>, p53 and vascular endothelial growth factor (VEGF) (9, 10, 12-14). These findings suggest that Notch signaling certainly plays a critical role in the development and progression of human cancer types due to the regulation of these target genes and their cross-talks.

Overall, Notch signaling plays an important role in tumor progression (9). It has been shown that the function of Notch signaling in tumorigenesis can be either oncogenic or oncosuppressive and the function is also context dependent (9). Notch signaling is oncosuppressive in several tumor types such as skin cancer, hepatocellular carcinoma, and small-cell lung cancer (13, 15). In contrast, most studies have shown Notch to have an oncogenic function in many human carcinomas including cervical, lung, colon, head and neck and renal carcinoma, prostate, acute myeloid, Hodgkin and large-cell lymphomas and pancreatic cancer (8, 14, 16-20). It is believed that Notch signaling plays a critical role in the early developmental stages by maintaining pancreatic epithelial cells in a progenitor state and, thus, delaying their differentiation until it becomes appropriate. In the adult pancreas, little-to-no expression of Notch signaling has been found (16). However, pancreatic cancer has been shown to overexpress the Notch signaling pathway-related molecules. High levels of expression of Notch receptors, Notch ligands and Notch target genes have been observed in pancreatic cancer (21-27). Moreover, Notch activity is required for TGF-α-induced acinar-to-ductal transition. Prevention of Notch activation by γ-secretase inhibitors (GSI) prevents acinar-to-ductal metaplasia in TGF-α-treated cells (22). Down-regulation of Notch-1 using specific siRNA or treatment with GSI has been found to be correlated with decreased proliferative rates, increased apoptosis, reduced cell migration and decreased invasive properties of pancreatic cancer cells (17, 18). Recently, it was shown that both Notch activation and activated K-Ras signaling act cooperatively to initiate pancreatic carcinogenesis (23, 24). Interestingly, Hanlon et al. reported that Notch-1 functions as a tumor suppressor gene in a model of K-ras-induced pancreatic ductal adenocarcinoma (28). However, the reason for this controversial finding is not known. Aberrant Notch signaling leads to pancreatic cancer although with different net effects (oncogenic vs. oncosuppressive), depending on tumor subtypes, the timing, intensity and interaction with other signaling pathways. The molecular mechanisms by which Notch contributes to pancreatic cancer are poorly understood. The basic features and the possible biological roles of Notch signaling in pancreatic cancer have been reviewed (8, 16, 29). Recently, Notch signaling was also found to be involved in pancreatic cancer stem cells (CSCs), which may be one of the mechanistic roles of Notch signaling for pancreatic cancer aggressiveness. Therefore, the novel function of Notch in pancreatic CSCs is now discussed.

**Notch Signaling in Pancreatic CSCs**

In recent years, the concept of CSCs has become more attractive due to advances in stem cell biology, leading to the identification of these cells from a wide variety of human cancer types. CSCs are characterized by properties of normal stem cells such as slow proliferation rates, indefinite self-replication capacity and resistance to toxic agents (drug resistance). CSCs have been identified and isolated, based on the expression of a specific molecule or combination of molecules such as CD24, CD34, CD44, CD133, epithelial-specific antigen (ESA) and aldehyde dehydrogenase (ALDH) (30, 31) and are able to self-renew, differentiate and regenerate to phenotypic cells of the original tumor when implanted into severe combined immunodeficient or nude mice. Furthermore, CSCs have been isolated from tumors of the hematopoietic system, breast, lung, prostate, colon, brain, head and neck and pancreas (30-38). The biology of pancreatic CSCs is further discussed in the following paragraphs.

**Pancreatic CSCs.** In 2007, scientists from two different groups claimed and published the identification and isolation of CSCs from human pancreatic cancer using two different sets of cell-surface markers (30, 31). Li et al. firstly described
that CD44⁺/CD24⁺/ESA⁺ pancreatic cancer cells show the stem cell properties of self-renewal, the ability to produce differentiated progeny and increased tumorigenic potential compared with marker-negative cancer cells (31). Hermann et al. reported that human pancreatic cancer tissue contain CSCs defined by CD133 and CXCR4 expression (30). They also found a distinct subpopulation of CSCs in the invasive front of pancreatic tumors and that the depletion of the CSC pool for these migrating CSCs virtually abrogated the metastatic phenotype of pancreatic tumors (30). Moreover, work from the same group pointed to prominin-1 as a marker for CSCs in primary pancreatic cancer and pancreatic cancer cell lines (30). The existence of pancreatic CSCs was further confirmed by Olempska et al. (39) who found that ABCG2 and CD133 may represent markers for pancreatic CSCs (39). In 2010, Rasheed et al. identified CSCs from pancreatic adenocarcinoma based on ALDH activity. They found that ALDH(+) and CD44⁺/CD24⁺ pancreatic CSCs are similarly tumorigenic, but ALDH(+) cells are relatively more invasive (40, 41). The following section will summarize what is known regarding the Notch signaling pathway in guiding the molecular events that occur in the processes of pancreatic CSCs, and will further discuss what is known regarding the design of novel therapeutic strategies for targeting Notch signaling in order to target pancreatic CSCs for the treatment of pancreatic cancer.

**Figure 1. Schematic of Notch signaling, described in detail in the section “Notch Signaling Pathway: An Overview”**.

**Figure 2. The connection between CSCs and the Notch signaling pathway.**

**Notch regulates pancreatic cancer stem cells.** The Notch signaling pathway is believed to play a critical role in CSCs. It has been reported that the fate of CSCs is controlled by the Notch pathway through induction of Jagged-1 in breast cancer (42). Farnie et al. also provided evidence for breast CSCs and their studies have consistently demonstrated that breast CSCs show up-regulated Notch genes (43). Hermann et al. have reported that human pancreatic CSCs are
exclusively tumorigenic and highly resistant to standard chemotherapy (30), suggesting that CSCs are drug-resistant cells which are responsible for tumor recurrence and metastasis. Another study has also shown that pancreatic CSCs are gemicitabine-resistant cells, suggesting that pancreatic CSCs contribute to drug resistance and metastasis (44). Wang et al. found that pancreatic CSCs show considerably higher levels of expression for Notch-1 than non-pancreatic CSCs (45). Ji et al. also reported that pancreatic CSCs contain high levels of Notch-1 and Notch-2 (46). These data suggest that the activation of Notch signaling may be involved in pancreatic CSCs self-renewal. However, the molecular mechanism how Notch signaling regulates pancreatic CSCs self-renewal remains undefined, the next section will summarize what is known to-date regarding the role of Notch in the molecular regulation of CSCs in pancreatic cancer.

MicroRNAs (miRNAs) regulate Notch signaling in pancreatic CSCs. Recently, it has been reported that miRNAs play a critical role in the Notch signaling pathway in pancreatic CSCs. In general, miRNAs are endogenous small RNA molecules that regulate gene expression and they are thought to have either oncogenic or oncosuppressive activity. Oncogenic miRNAs are up-regulated in cancer, whereas oncosuppressive miRNAs are down-regulated in cancer. Several miRNAs have been shown to crosstalk with the Notch pathway in human cancer. Recently, Ji et al. reported that Notch-1 and -2 are downstream genes of miR-34 in pancreatic cancer cells (46). They found that restoration of miR-34 expression in the pancreatic cancer cells down-regulates Notch-1 and -2. They also reported that pancreatic CSCs have high levels of Notch-1 and -2, consistent with the loss of miR-34 expression. These results suggested that miR-34 may be involved in pancreatic CSC self-renewal, potentially via direct modulation of its downstream target Notch (46).

Moreover, alteration of the miR-200 family has been found to be associated with the Notch signaling pathway in pancreatic CSCs. Research from this group has shown that pancreatic cancer cells that are resistant to gemicitabine have molecular characteristics that are reminiscent of CSC features (47). In fact, this is consistent with recent findings in prostate cancer (48). The miR-200a, miR-200b, and miR-200c have been shown to be down-regulated in gemicitabine-resistant pancreatic cancer cells, which show CSC features (49). Furthermore, it was found that overexpression of miR-200 family significantly inhibits Notch pathway in pancreatic cancer cells, suggesting that Notch pathway may be one of the miR-200 targets (50). These results indicate that re-expression of miR-200 may increase drug sensitivity, which indeed may be mediated through the regulation of the Notch signaling pathway and such a strategy would likely be successful in the elimination of CSCs.

Notch regulates epithelial-to-mesenchymal transition (EMT) phenotype and generates pancreatic cancer stem cells. Increasing evidence suggest that EMT may generate CSCs. Epithelial cells are able to acquire a mesenchymal phenotype, leading to increased motility and invasion (51). EMT is characterized by loss of the expression of epithelial markers such as E-cadherin and γ-catenin, and the up-regulation of mesenchymal molecular markers such as zinc-finger E-box binding homeobox (ZEB), snail, slug, vimentin, fibronectin, α-smooth muscle actin (SMA), and N-cadherin (52).

Studies have shown a clear connection between EMT and CSCs. EMT cells have cancer stem-like cell features and CSCs exhibit mesenchymal phenotype. Main et al. showed that breast cancer mesenchymal-like cells acquire breast CSC phenotype (53). Stable knockdown of ZEB1, an EMT inducer, in pancreatic cancer cells resulted in decreased expression of stem cell factors such as Sox2, Bmi1 and p63, leading to a reduction of pancreatic CSCs (54). Similarly, Shah et al. developed two different pancreatic gemicitabine-resistant cancer cell lines with acquired EMT properties (55) and these resistant cells showed high expression of stem cell markers CD24, CD44, and ESA (55). One study has shown that pancreatic CSCs are consistent with gemicitabine-resistant cells (44). It has also been reported that gemicitabine-resistant cells show the acquisition of EMT phenotype, as evidenced by elongated fibroblastoid morphology, lower expression of epithelial marker E-cadherin and higher expression of mesenchymal markers such as ZEB and vimentin, and this was found to be consistent with high levels of Notch-2 and Jagged-1 (56). Moreover, down-regulation of Notch signaling by siRNA partially reversed the EMT phenotype, leading to the mesenchymal-to-epithelial transition (56). Recently, it was further confirmed that pancreatic cancer cells that are resistant to gemicitabine have CSC features, suggesting that the activation of Notch signaling pathways is mechanistically linked with the acquisition of the EMT phenotype and the generation of CSCs in pancreatic cancer. Therefore, targeted inactivation of Notch signaling pathways is likely to be useful for overcoming drug resistance of CSCs and, thereby, may be useful for the eradication of tumor recurrence and metastasis.

Targeting Notch to Eradicate CSCs in Pancreatic Cancer

CSCs are uniquely capable of resisting the effects of conventional chemotherapeutics and thereby survive for a long time, resulting in tumor recurrence and metastases. Thus, eradication of these cells is necessary for the better treatment of pancreatic cancer. This will require targeting of pathways that participate in the formation of CSCs. Indeed, it has been shown that a combination of blocking both sonic
hedgehog and mTOR signaling with standard chemotherapy results in the elimination of pancreatic CSCs (57). Since Notch signaling plays a critical role in CSCs and current cancer therapeutics do not usually target CSCs but instead only kill differentiated tumor cells that make up the bulk of the tumor, the killing of the rare CSC population is of paramount importance, which might be accomplished by Notch-targeted therapy. Therefore, eradication of CSCs by novel approaches is increasingly being recognized as an important goal in curing cancer, which is being tested clinically using Notch-targeted agents and it is hoped that such strategies may be useful for complete eradication of tumors, which will certainly improve the overall survival of patients diagnosed with pancreatic cancer.

The role of GSIs. Notch signaling is activated via the activity of γ-secretase. Therefore, γ-secretase may be a target for cancer therapy. Several forms of GSIs have been found to posses anti-tumor effects. It has been reported that the GSIs inhibit cell growth and induce apoptosis in many human cancer cells including pancreatic cancer cells (58-64). The inhibition of Notch activity by GSIs reduced tumor development in a murine model of pancreatic ductal adenocarcinoma (64). Recently, it was reported that Notch blockade by GSIs depleted stem cells in glioblastoma (61, 65). Inhibition of the Notch pathway by GSIs and the sonic hedgehog pathway enhances the efficacy of temozolomide monotherapy in the context of glioma stem cells (66), suggesting that GSIs may be useful reagents to target CSCs in gliomas. GSIs have the advantage of relative ease of administration, oral bioavailability and low cost. However, GSIs are relatively nonselective drugs due to their ability to block the cleavage of all four Notch ligands and multiple other γ-secretase substrates, causing non-specific effects. Another challenge is to eliminate unwanted toxicity associated with the GSI, especially the cytotoxicity in the gastrointestinal tract (67). In order to overcome such limitations, recent studies have shown that ‘natural agents’, which are typically non-toxic to humans, including sulforaphane, quercetin, curcumin, isoflavone and others, are able to inhibit Notch expression and, thereby, may target pancreatic CSCs.

Sulforaphane. Sulforaphane is a natural compound derived from cruciferous vegetables, such as broccoli or broccoli sprouts, and it has been shown to possess anticancer activity in many human cancer types. A recent study has shown that sulforaphane inhibits breast CSC growth in vitro and in vivo through down-regulation of the Wnt/beta-catenin self-renewal pathway (68). It is important to note that sulforaphane specifically targets the CSC population rather than the bulk tumor cells. Similar to breast cancer, sulforaphane was shown to target the pancreatic tumor-initiating cells (69). A study from the same group also showed the synergistic activity of sulforaphane and sorafenib (a multikinase inhibitor) in eliminating CSCs from pancreatic cancer cells (70). Moreover, sulforaphane increased the sensitivity of cells to several chemotherapeutic agents (cisplatin, gemcitabine, doxorubicin and 5-flourouracil) especially by targeting CSCs, which was, in part, due to targeted inactivation of Notch-1 in pancreatic cancer (71).

Quercetin. Quercetin is a major polyphenol and flavonoid, commonly found in many fruits and vegetables. It has been reported that quercetin decreases the levels of Notch1 protein and its active fragment in a leukemia cell line with constitutive Notch activation (72). Quercetin synergizes with epigallocatechin-3-gallate in inhibiting the self-renewal properties of prostate CSCs, inducing apoptosis and blocking CSC migration and invasion (73). Quercetin was recently reported to target pancreatic CSCs and EMT-phenotypic cells (74). Quercetin also inhibited the growth of pancreatic CSCs in a xenograft model, which was associated with reduced proliferation, angiogenesis, cancer stem cell-marker expression and induction of apoptosis. Importantly, the combination of quercetin with sulforaphane had a synergistic effect in targeting pancreatic CSCs (74).

Curcumin. Curcumin (diferuloylmethane) is an active compound found in the curcuma longa, which is widely used as a flavoring agent in food, and it has been shown to have antitumor activity against many cancer types, including pancreatic cancer in preclinical studies (75). Curcumin has been found to play a critical role in cellular proliferation, growth, survival, apoptosis, migration, invasion, angiogenesis and metastasis in pancreatic cancer (2). Studies from this research group have shown that curcumin inhibits the cell growth and induced apoptosis in pancreatic cancer through inactivation of the Notch pathway (76). Furthermore, it has been reported that curcumin down-regulates miR-21 and up-regulated miR-200 in pancreatic cancer, leading to increased sensitivity to gemcitabine (77). Curcumin has been shown to eliminate CSCs in breast cancer, colon cancer and gliomas (78-80). Recently, it was found that curcumin is able to inhibit significantly the sphere-forming ability (pancreatospheres) of pancreatic cancer cells, which is consistent with increased disintegration of pancreaticospheres and is associated with the attenuation of CSC markers (CD44 and EpCAM), especially in gemcitabine-resistant pancreatic cancer cells containing high proportion of CSCs and increased expression of miR-21 and decreased expression of miR-200 (81). Therefore, curcumin may eliminate pancreatic CSCs partly through inactivation of the Notch signaling pathway.

Genistein. Genistein is a prominent isoflavonoid found in soy products and has been proposed to be responsible for
lowering the rate of pancreatic cancer (82). This research group previously showed that genistein is able to induce apoptosis and inhibit the activation of the NF-κb pathway through the Notch signaling pathway in pancreatic cancer (18, 83). Genistein has been shown to act synergistically with vitamin D to inhibit the growth of prostate cells through the regulation of genes involved in stem cell self-renewal (84-86). It has been demonstrated that Notch pathway was high expressed in pancreatic cancer cells with EMT phenotype consistent with the acquisition of the stem cell features (55, 56). Therefore, genistein could cause reversal of EMT phenotype and eliminated the stem cells. These results support the use of genistein for targeted elimination of pancreatic CSCs.

Considering the relatively non-toxic nature of natural agents, it is interesting that targeting Notch by these natural agents may be a novel approach for targeted elimination of CSCs, especially when combined with conventional chemotherapeutics. Such a strategy may be a novel and safer approach for achieving better treatment outcome for patients diagnosed with pancreatic cancer. However, further in-depth preclinical and clinical studies are warranted in order to appreciate the value of natural agents in overcoming drug resistance, thereby eliminating CSCs that are the primary cause of tumor recurrence and metastasis.

Conclusion

This review article attempts to summarize the role of Notch signaling in pancreatic CSCs. It is concluded that deregulation of the Notch pathway correlates with the development and progression of cancer and the formation of CSCs (Figure 2). Therefore, targeting Notch will, in fact, be useful for targeting CSCs, which may become a novel strategy for achieving treatment benefit in patients diagnosed with pancreatic cancer. To that end, emerging evidence suggests that natural agents may be useful for the inactivation of the Notch pathway, which will result in the elimination of drug-resistant CSCs. In summary, targeting Notch will be useful to target CSCs, especially by natural agents such as curcumin, genistein, sulforaphane and quercetin, which may be a novel strategy towards designing approaches for combination therapy using conventional therapeutics for achieving better treatment outcome for patients diagnosed with pancreatic cancer.

Acknowledgements

The Authors’ work cited in this review was funded by grants from the National Cancer Institute, NIH (1R01CA101870, 5R01CA131151, 5R01CA083695, 1R01CA132794) awarded to F.H.S. We also sincerely thank both Puschelberg and Guido foundations for their generous contributions to our research.

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Received February 10, 2011
Revised March 2, 2011
Accepted March 3, 2011