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Molecular signature of pancreatic adenocarcinoma: an insight from genotype to phenotype and challenges for targeted therapy

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Abstract

Introduction—Pancreatic adenocarcinoma remains one of the most clinically challenging cancers despite an in-depth characterization of the molecular underpinnings and biology of this disease. Recent whole-genome-wide studies have elucidated the diverse and complex genetic alterations which generate a unique oncogenic signature for an individual pancreatic cancer patient and which may explain diverse disease behavior in a clinical setting.

Areas covered—In this review article, we discuss the key oncogenic pathways of pancreatic cancer including RAS-MAPK, PI3KCA and TGF-β signaling, as well as the impact of these pathways on the disease behavior and their potential targetability. The role of tumor suppressors particularly BRCA1 and BRCA2 genes and their role in pancreatic cancer treatment are elaborated upon. We further review recent genomic studies and their impact on future pancreatic cancer treatment.

Expert opinion—Targeted therapies inhibiting pro-survival pathways have limited impact on pancreatic cancer outcomes. Activation of pro-apoptotic pathways along with suppression of cancer-stem-related pathways may reverse treatment resistance in pancreatic cancer. While targeted therapy or a 'precision medicine' approach in pancreatic adenocarcinoma remains an elusive challenge for the majority of patients, there is a real sense of optimism that the strides made in understanding the molecular underpinnings of this disease will translate into improved outcomes.

Keywords

cancer stem cells; expression signature; K-Ras pathway; molecular pathways; notch signaling; p53; pancreatic cancer; targeted treatment; Wnt signaling

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Declaration of interest

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1. Introduction

Pancreatic adenocarcinoma harbors one of the most aggressive tumor behaviors. The five-year survival rate in patients who undergo resection is 23.4% while this rate decreases to 6% for all stage patients [1,2]. A recent study reported that by 2020, pancreatic cancer-related mortality will catch up with the mortality rate of colon cancer, and by 2030 it will be the second most common cancer-related death after lung carcinoma although its incidence rate will remain out of the five most common cancers [3]. These observations demonstrate that advances in cancer research thus far haven't had substantial impact on the natural course of pancreatic cancer. Therefore, a focus on the molecular biology of pancreas adenocarcinoma is herein emphasized to elucidate mechanisms for therapeutic opportunity and understand resistance to available therapies. We summarize the molecular pathways which play key roles in development and progression of pancreatic adenocarcinoma and discuss potential targetable molecular pathways and biomarkers.

1.1 Core pathways in pancreatic adenocarcinoma

1.1.1 Ras-MAPK pathway—K-Ras (Kristen rat sarcoma viral oncogene homolog) mutation was one of the earliest discoveries in pancreatic cancer and has been reported in as high as 90% of pancreatic adenocarcinoma patients [4]. K-Ras is a small GTPase, and functions in one of the downstream signaling pathway receptor tyrosine kinases such as EGFR. Mutation in the K-Ras oncogene results in gain-of-function and constitutively activates extracellular signal regulated kinase pathway (MAPK; ERKs) [5]. Once ERK is activated, it translocates to the nucleus and promotes transcription activity for target genes that are involved in cell survival, growth and proliferation [6]. The Ras oncogene also interacts with other essential signaling molecules and functions as the maestro of survival pathways. In the early 1990s phosphatidylinositol-3-OH kinase (PIK3CA), an important signal transducing molecule for protein synthesis at the G1 phase of the cell cycle, was shown to be a target of the Ras oncogene [7]. Activation of PIK3CA by K-Ras and other growth stimuli receptors, such as EGFR, promotes cell tumor cell growth and prepares the cell for the next phase of cell cycle progression [8]. The K-Ras oncogene also interacts and activates other signaling molecules found to be related to stress response and cell growth such as c-Jun N-terminal kinase (JNK) and protein kinase C (PKC) [9] suggesting that the signaling network in pancreatic cancer cells is very complex and once a growth stimulus-related survival pathway is activated, simultaneously other cellular responses are also turned on to address cellular stress and altered microenvironment such as hypoxia and free radicals (Figure 1).

The occurrence of a K-Ras mutation has been shown to be evident at an early phase of pancreatic carcinogenesis [10]. K-Ras mutations can be detected in intraductal precancerous lesions (PanIN's) suggesting that they confer a survival and growth advantage for mutant clones compared to wild-type ductal cells [11]. More strikingly, studies also suggest that K-Ras takes the initiative of cellular reprogramming processes to induce transition of the acinar cell into a malignant clone [12]. However, there is also strong evidence that in the presence of intact tumor suppressor response, it may induce cellular senescence [13]. For example, activation of oncogenic K-Ras also results in increased expression of p53 and p16, both of

which promote premature cell senescence [14], indicating that accumulation of other mutations specifically in tumor suppressor genes are required to for acquisition of the features of a completely transformed cancer cell (Figure 2).

Given that K-Ras has a crucial role in pancreatic carcinogenesis, MEK, which is a downstream signal mediator of K-Ras signaling, was investigated as a therapeutic target. Initial preclinical studies with MEK inhibitors reported inhibition of tumor growth in xenograft models as a promising target for pancreatic cancer treatment [15]. However, clinical trials with MEK inhibitors have been conducted in multiple advanced stage tumor types and results have not been sufficiently promising to warrant further development as single agents [16,17]. Although K-Ras mutation is involved in pancreatic carcinogenesis inhibition of the K-Ras pathway has limited impact on pancreatic cancer cells. For example, a back-to-bench study observed a rebound activation of PIK3CA-mTOR pathway as a compensatory mechanism to MEK inhibition (Figure 3) [18]. Overall, the current evidence suggests that KRAS pathway suppression is overridden either by changing expression level of other prosurvival pathways or by activating other signal transducers that will be further discussed below.

1.1.2 EGFR signaling in pancreatic cancer—The EGFR family (EGFR also known as HER) is a subgroup of receptor tyrosine kinases which have been shown to be active in many epithelial cancers such as colorectal, breast and lung cancers [19]. EGFRs are known to be activators of K-RAS, PIK3CA, Signal Transducer and Activator of Transcription (STATs), and phospholipase C (PLC) [20]. These pathways promote cell growth, proliferation and invasion via modifying gene expression profiles and creating a unique signature for each cancer cell.

Overexpression of EGFR has been identified in tissue studies of pancreatic adenocarcinoma [21]. Simultaneous upregulation of EGF along with EGFR overexpression [22] suggests that there might be a closed circuit in regulation of both receptor and ligand. Moreover clinical data has suggested that upregulated EGFR might be associated with more aggressive tumor behavior [23]. Increased EGFR activity has been also be related to higher rates of disease recurrence following surgery in pancreatic cancer [24]. Therefore, studies to investigate the role of EGFR signaling inhibitors in pancreatic cancers have been conducted.

In an early preclinical study, EGFR receptor blockage with cetuximab, a monoclonal antibody against EGFR, showed promising results and induced tumor regressions in orthotopic models of pancreatic cancer [25]. Additional work identified the potential benefit of the small molecule EGFR inhibitor, erlotinib, and suggested promise for combination with gemcitabine [26]. While initial clinical studies of cetuximab reported a modest benefit, a subsequent Phase III trial did not result in any survival advantage for the addition of cetuximab to gemcitabine (HR = 1.06) [27]. A study of the small tyrosine kinase inhibitor, erlotinib, along with gemcitabine, in 519 patients with locally advanced and metastatic pancreas adenocarcinoma, met statistical significance (HR of 0.82 [95% CI; 0.69 – 0.99]) but showed a borderline clinical benefit with less than one month increased overall survival in the experimental arm (Table 1) [28]. Considering the high rate of K-RAS mutations (>

90%) targeting EGFR alone does not appear to be a promising future strategy for pancreatic cancer treatment.

1.1.3 GNAS and PIK3CA signaling pathways—GNAS is a very complex gene that encodes multiple proteins such as the G protein α-subunit ($G_s\alpha$) which functions in signal transduction utilizing seven transmembrane receptors [29]. The activated $G_s\alpha$ induces an enzyme called adenyl cyclase which produces cyclic AMP (cAMP). Then, protein kinase A (PKA) which is an important signal transducer for cell growth and proliferation is activated by cAMP [30]. $G_s\alpha$ pathway also can interact and activate other signaling pathways such as Wnt and the Ras-MAPK pathways [31] and boost the growth of the malignant cell clones.

The presence of GNAS mutations in pancreatic cancer development appears to be more significant at an early stage of carcinogenesis and is more often seen in precursor lesions of pancreatic cancer termed intraductal papillary mucinous neoplasms (IPMNs) [32]. Activated GNAS signaling along with a K-RAS mutation induces IPMN, indicating a dual interaction in these two oncogenic pathway [32]. A study reported distinct disease behavior in pancreatic adenocarcinoma derived from IPMN based on their pathological features such as presence of concurrent IPMN indicating a distinct pathway for pancreatic adenocarcinogenesis in the setting of GNAS mutation [33]. Current evidence also indicates relatively frequent GNAS mutations in pancreatic carcinoma derived from IPMN while there was no GNAS mutation in *de nova* pancreatic adenocarcinoma suggesting a close relation between GNAS mutations and pancreatic carcinogenesis limited to the ones arising from premalignant mucinous lesions [32]. Together these findings suggest that GNAS is a key signaling molecule in pancreatic adenocarcinoma, and perhaps more specifically pertaining to the cancers that arise from IPMNs (Figure 4).

PIK3CA, another downstream signal mediator of receptor tyrosine kinases, initiates the Akt-mTOR pathway. PIK3CA-activated Akt-mTOR fuels cell growth and proliferation in cancer cells [34]. Mutant PIK3CA stimulates downstream signaling which turns on transcription activity and creates cancer cells that can survive in a nutrition-deprived microenvironment and invade the stroma [35]. PIK3CA-activated Akt-mTOR pathway also inhibits apoptosis by increasing the expression of anti-apoptotic Bcl-2 family proteins [36]. Moreover, overactive PIK3CA signaling abolishes K-Ras-induced senescence in the early phases of carcinogenesis [37] suggesting that PIK3CA-mTOR cascade boost Ras-MAPK mediated cell growth and abrogates activation of rebound growth control mechanisms by suppressing tumor suppressor gene activation.

Studies suggest that activation of PIK3CA occurs during the early phase of pancreatic carcinogenesis and is detectable in IPMN lesions [38]. Given the potential anti-apoptotic effect along with growth stimulus potential, the impact of PIK3CA on pancreatic carcinogenesis might be more significant [35]. For example, an *in vitro* study suggested successful apoptosis induction upon inhibition of PIK3CA upstream and downstream pathway [39]. A xenograft model of human pancreatic cancer also showed promising tumor growth suppression by mTOR inhibitors [40]. While preclinical studies provided promising results following inhibition of the PIK3CA/Akt/mTOR pathway, clinical trials have not shown a significant benefit with the oral mTOR inhibitors [41], as assessed in a Phase II

clinical trial where no partial or complete responses were observed. Another Phase II clinical trial confirmed the lack of activity in that 15 of 16 (93.7%) patients enrolled in the study had progressive disease and one patient was reported as non evaluable [42]. After these disappointing clinical outcomes, studies have been conducted to investigate mechanisms explaining this resistance. A study on pancreatic cancer cells elucidated upregulation of Ras-MAPK pathway upon inhibition of PIK3CA-mTOR signaling (Figure 3) [43]. Another back-to-bench study reported activation of EGFR signaling as a rebound response to mTOR inhibition [44]. Furthermore, preclinical work identified stromal cell-derived factor-1 and the CXCR4 signaling loop as a resistance mechanism to mTOR pathway inhibition [45] suggesting that microenvironment factors may also mediate this resistance (Figure 3). Overall, the lack of response to mTOR inhibition in pancreatic cancer indicates a very dynamic signaling network operating in real time in pancreatic cancer cells that confers genetic plasticity and an ability to bypass or compensate for a defective growth signaling pathway.

1.1.4 Loss of PTEN function and dysregulation of PIK3CA pathway—

Phosphotase tensin homolog (PTEN), a negative regulator of the PIK3CA pathway, has been shown to be involved in pancreatic carcinogenesis [46]. Loss of heterozygosity (LOH) in PTEN has been demonstrated in approximately 40% of pancreatic cancers [47]. In an animal model, loss of PTEN induced highly proliferative ductal cells [48] suggesting that PTEN loss may play an important role in early pancreatic carcinogenesis due to loss of a negative control mechanism on PIK3CA [49]. Furthermore, PTEN loss in the setting of a K-RAS mutation further fuels cell growth and enhances K-RAS-related carcinogenesis process [46]. An animal model of PTEN/KRAS mutant cell lines suggested that PTEN mutant tumors may respond to mTOR inhibitors, which was not observed in KRAS/p53 mutant cell lines [50]. However, the clinical utility of a PTEN mutation as a biomarker of PIK3CA pathway inhibitor treatment needs to be further studied.

1.1.5 TP53 mediated cell cycle control and tumor suppression—

In a very early study from 1981, Jay *et al.* [51] discovered a phosphorylated 53 000 dalton protein, p53, in transformed cells while its expression was almost undetectable in resting cells and they hypothesized that p53 might be involved in cell cycle control which was later confirmed by other studies. p53 is capable of inducing expression of another small nuclear protein called p21 that shuts down the cell cycle via inhibition of cyclin dependent kinases (CDKs), more specifically CDK2 and arrests the cell cycle at the G1 phase [52] p53 is also involved in DNA damage response which is turned on by ataxia-telengectesia mutated (ATM) and Breast Cancer susceptibility gene 1 (BRCA1) [53]. The BRCA complex initiates the DNA repair process and if the repair process fails, downstream activation of p53 occurs via check point kinase 2 (CHK2 or CHEK2) which ultimately induces cell cycle arrest [54]. It is believed that cancer cells bearing mutant p53 or loss of both chromosomal copies, bypass this check point, gain mutations in DNA and become more tolerant and resistant to DNA damage-mediated cell cycle arrest [55]. The physiologic function of p53 is not limited to cell cycle control and DNA damage response. If cell cycle arrest occurs successfully, p53 also initiates an apoptotic process by activating redox pathway in mitochondria, generating reactive oxygen species and more importantly, inducing the expression of pro-apoptotic

proteins such as Noxa [56]. Studies also suggest that p53-induced apoptosis mediates the cytotoxic effect of anticancer treatments in cancer cells [57]. Mutations that impair p53 signaling result in resistance to chemotherapeutic agents and treatment failure [58]. Furthermore, p53 also suppresses reprogramming processes, which are an important step for cancer stem cell generation via multiple mechanisms such as regulation of transcription factors and DNA damage response [59]. Overall, evidence from preclinical studies suggests that p53 is a very critical decision making protein not only in inhibition of carcinogenesis but also in self-regeneration of tumor cells.

p53 has been identified to be mutated in 40 – 75% of pancreatic adenocarcinoma cases [60]. Germline p53 mutations which result in Li-Fraumeni syndrome, are also associated with an increased risk of pancreatic cancer [61] indicating that inactivation of p53 has an essential role in pancreatic carcinogenesis. Somatic p53 mutations occur at a later stage of tumor development unlike early K-Ras oncogene activation [62]. Given the critical effect of p53-related pathways on pancreatic cancers, reactivation of p53 has been interrogated in preclinical studies. A study on cell lines suggested that the mutant p53 activator, PRIMA-1, accelerated apoptosis and sensitized the tumor cells to chemotherapy [63]. In another study with an animal model treated with nutlin (MDM2 inhibitor, a wild-type p53 activator) analogs also reported significant tumor inhibition in xenografts [64]. However, clinical studies are needed to elucidate the utility of p53 reactivation in pancreatic cancer patients. Collectively, these data point to a strong relationship between development of pancreatic cancer and p53 dysfunction. Moreover, whether resistance to conventional chemotherapy in pancreatic cancer is related to a disabled apoptotic process in the lack of this master tumor suppressor and can be reversed by targeting treatment warrants further investigation.

1.1.6 TGF-β/SMAD signaling pathway—TGF-β signaling is a very complex signaling network and the role of the TGF-β pathway in cellular homeostasis varies by genetic profile of the cancer cell. For example, TGF-β signaling can induce cell differentiation and acts as a tumor suppressor gene in non-malignant clones [65] while it can also promote angiogenesis and epithelial-mesenchymal transition (EMT) in cancer cells [66].

TGF-β functions as a tumor suppressor in multiple ways. For example, once signal transduction is initiated, Mother against decapentaplegic homologs (SMAD) proteins translocate to the nucleus to initiate transcription of target genes that are known to be involved in differentiation [67]. TGF-β signaling is not only involved in cell differentiation, but also has an operational function in cell cycle control as a tumor suppressor. One study demonstrated that TGF-β can inhibit CDK4 and stabilize retinoblastoma (Rb), which inhibits a cell cycle promoting transcription factor, E2K [68]. Furthermore, there are data suggesting that TGF-β can induce p53-independent expression of p21 which is another important cell cycle controlling protein [69]. TGF-β also suppresses expression of oncogenes. For example, myc which is a cell-cycle promoting transcription factor in proliferating cells is downregulated by the TGF-β signaling [70].

However, TGF-β receptor also cross-talks with oncogenic signaling pathways. For example, TGF-β can activate the Ras-MAPK pathway which also further increases the expression of TGF-β along with cell growth [71]. Moreover, both pathways synergistically induce EMT

and the absence of any of these pathways can cause failure to gain mesenchymal plasticity in epithelial cells [72]. Snai1, a member of snail family, which is induced by the PIK3CA-Akt-mTOR pathway appears to function as an effector protein TGF-β-related EMT [73].

The data regarding the role of SMAD4 in pancreatic cancer are controversial. A study of surgically resected pancreatic cancers showed an improved overall survival in patients whose tumor expressed SMAD4 [74]. One postmortem study also suggests that loss of SMAD4 expression might be a marker of a more aggressive pancreatic cancer biology phenotype with higher potential for metastatic disease progression [75]. However, a clinical study in locally advanced pancreatic adenocarcinoma showed a correlation between local disease progression with retained SMAD4 expression rather than distant disease [76]. Another study indicated a correlation with inactivating mutations of SMAD4 and worse survival outcomes [77]. Although these studies suggest a biomarker role for SMAD4 for pancreatic cancer patients, the data are discordant. A recent study in surgically resected pancreatic tumors showed no association with disease recurrence and SMAD4 expression status [78]. Moreover, an early study reported an inverse relation between SMAD4 expression and survival outcomes [79]. An ongoing trial in locally advanced pancreas adenocarcinoma in North America, RTOG 1201, will evaluate the role of loss of SMAD4 as a biomarker for a more aggressive tumor biology and will correlate genotype with clinical outcome and the use of radiation therapies that may enhance loco-regional disease control (NCT01921751).

Given that preclinical observations suggest that TGF-β pathway also mediates EMT progress and disease progression, studies have been conducted with TGF-β pathway inhibitors. A study investigated a chimeric protein composed of extracellular domain of TGF-βR II as a decoy TGF receptor and IL2 in an animal model [80]. The authors reported inhibition of tumor generation in mice and via downregulation TGF signaling and activation of innate immune system. A clinical trial is currently investigating an antisense oligodeoxynucleotide of TGF-β2 called trabedersen and results are pending (NCT00844064). More translational and clinical studies are required to identify potential benefit of TGF-β signal inhibition in pancreatic cancer.

1.1.7 CDKN2A and its downstream pathway—CDKN2A gene, also known as $p16^{INK4}$ and $p14^{ARF}$, has an exceptional feature which allows encoding of two distinct proteins, $p16^{INK4}$ and $p14^{ARF}$, by alternative reading frames. $p16^{INK4}$ is a major inhibitor of CDK4/CDK6 and Cyclin D1 complex, which promotes a signal for cell cycle progression via inhibiting Rb protein and unleashing the transcription factor E2F [81]. $p16^{INK4}$ interferes with CDK4/CDK6 and CyclinD1 complex-mediated phosphorylation of Rb and ultimately induces cell cycle arrest [82]. The other product of CDKN2A gene, $p14^{ARF}$, inhibits the Mdm-2 (Mouse double minute homolog) which is a negative regulator of p53 [83] indicating that the CDKN2A controls the cell cycle via two distinct mechanisms.

The role of CDKN2A has been widely studied in pancreatic cancer. CDKN2A has been shown to be mutated or deleted in more than 50% of pancreatic cancers and very often homozygous allelic loss occurs during late carcinogenesis [84]. There is also evidence suggesting that epigenetic silencing of CDKN2A is also involved in pancreatic carcinogenesis [85] denoting that multiple mechanisms silence CKDN2A during pancreatic

cancer development. Moreover, germline mutations of CDKN2A, which cause the familial atypical multiple mole and melanoma syndrome (FAMMM), are related to an increased risk of pancreatic cancer [86]. Along with activation of oncogenes, loss of CDKN2A activity was found to be involved in gain of metastatic potential due to an unleashed EMT process [87].

Given the frequent mutations in check point tumor suppressor genes in pancreas adenocarcinoma, the role of check point regulating agents to reinstate CDKN2A activity have been investigated. A preclinical study showed suppression of pancreatic cancer cells growth *in vitro* and *in vivo* after being treated by a CDK4/6 inhibitor [88]. On the other hand, another preclinical study in cell lines reported increased invasiveness of pancreatic cancer cells via upregulation of TGF signaling pathway after programmed death (PD)-0332991 therapy, although it slowed growth of pancreatic cancer cells [89]. A Phase II study of this same targeting agent in breast cancer, Palbociclib, which has now been FDA approved in that disease, remains to have its impact on pancreatic cancer defined [90]. Currently, a clinical trial is recruiting patients to investigate the safety and efficacy of this novel agent in advanced stage solid tumors (NCT01522989).

1.1.8 ATM/BRCA2/Wee1 and DNA damage response pathway—ATM and BRCA2 are two well-known tumor suppressor genes that operate in synchronized fashion to facilitate DNA damage response in human cells. Mutations in the ATM gene cause a syndrome called ataxia telengectasia (name also used for nomenclature of gene) which is a multisystem disorder resulting from DNA damage. Mutations in ATM are also associated with certain cancers such as breast, adrenal and colorectal cancer. A recent genome wide study demonstrated that mutations in the ATM gene are associated with pancreatic adenocarcinoma [91]. Mutations in BRCA2/BRCA1 are also related to an increased risk of pancreatic adenocarcinoma [92,93].

ATM protein kinases are present as dimers in the resting state and upon irradiation autophosphorylation occurs and ATM molecules disassociate and are activated [94]. This activation may occur via direct DNA interaction or chromatin structure change [95]. Once ATM is activated it phosphorylates Chk2 [96] which subsequently inhibits Cdc25, a cell cycle promoter, and activates BRCA1 [97] which is another cell cycle regulator. ATM can also directly phosphorylate p53 [98]. In both pathways, the end point is to induce cell cycle arrest to prevent inheritance of mutated DNA in daughter cells. This defense mechanism prevents accumulations of deleterious mutations in next generation cells and induces apoptosis in selected unhealthy clones. As part of DNA damage response, BRCA2 operates downstream of DNA damage response and is activated by BRCA1/Chk2 signaling [99]. All these elements operate together in a very complex network and the fate of the cell is determined based on outcomes of DNA damage severity and reversibility [55].

Current evidence suggests that ATM and BRCA2 mutations occur at a relatively later stage of pancreatic cancer development [100]. Their exact role in pancreatic carcinogene-sis remains to be elucidated. *In vitro* studies suggest it might be related to resistance to chemotherapeutics via the lack of DNA damage response [101]. In contrast, tumors in BRCA1/BRCA2 mutations carriers lose the second copy of BRCA2, and cannot repair DNA damage which sensitizes cancer cells to apoptosis termed as synthetic lethality [102].

Growing evidence denotes that platinum-based chemotherapy approaches and poly-ADP ribose polymerase (PARP) inhibitors may change the course of disease in this subgroup of pancreatic adenocarcinoma patients [103].

Given that impaired DNA repair and check point control both sensitizes tumor cells and induce apoptosis in pancreatic cancer cells, the role of check point inhibitors has thus also been investigated. Wee-1, a check point kinase, phosphorylates Cdc2 and induces cell arrest [104]. Preclinical studies have investigated the role of inhibition of Wee-1 to induce apoptosis via generating damage in DNA during the mitotic phase. An animal model showed a fourfold increased tumor response to the combination of MK-1775, a Wee-1 inhibitor, with gemcitabine compared to gemcitabine alone [105], a concept which is now being tested in an early Phase IB clinical trial (NCT02037230). Similarly, radiation and chemotherapy sensitization in pancreatic cancer cells has been reported using other check point inhibitors [106], and this promising preclinical evidence warrants further clinical investigation (Figure 2).

1.1.9 SWI/SNF and MLL gene complex-mediated chromatin modification—

Growing evidence implicates that the genetic expression profile of cancer cells is not only determined by signal activated transcription factors but also by modification in chromatin and DNA structural changes [107]. Hypermethylation in tumor suppressor gene promoter regions, which suppress transcription and deacetylation of histone structure which loosens the DNA chromatin structure and increases gene expression in protooncogenes, are key examples of epigenetic modification of gene expression.

Recent global genomic analyses have uncovered the impact of epigenetic modification changes and related genes in pancreatic cancer [108]. In one study, Jones *et al*. reported mutations in ARID1A, which is a member of SWI/SNF family, in pancreatic cancer [109]. More recently, another genomic analysis study revealed mutations in another member of this family, ARID2 [91]. This family functions in chromatin modification via helicase and ATPase activity and change the expression of target genes [110]. A recent study suggests that ARID1A might be a new tumor suppressor gene, as inactivating mutations lead to an increase in carcinogenesis and re-expresssion in cancer cells harboring the mutant gene and inhibit tumor growth [111]. In the same study, the authors reported that mutation in this gene results in decreased expression of tumor suppressor genes such as p21 and SMADs. A recent study identified that ARID1B, A homolog of ARID1A, is required for the survival of cancer cells bearing mutant ARID1A suggesting targetability of SWI/SNF pathway [112]. On the other hand, a syndrome resulting from SWI/SNF dysfunction called Coffin-Siris Syndrome [113] is not associated with an increased risk of any cancer suggesting that it is by itself not sufficient to activate a carcinogenesis process; however, it might be a secondary compensatory process in cancer cells permitting adjustment in their genetic signature by modulating their chromatin structures.

Similarly, mutations in another chromatin-modifying transcription coactivator, MLL3, have been identified in pancreatic cancer [91]. MLL3 is a member of the MLL (mixed linage leukemia) gene families, which are known tumor suppressors and function in histone methylation [114]. Histone methylation can modify amino acids in the structure of histone

bodies, and based on the methylated amino acid and location, it can activate or repress expression of target genes [115]. Studies suggest that MLL is a co-activator of p53 and enhances the expression of p53 target genes [116] which may explain its potential tumor suppressor effect. The exact role of MLL3 in pancreatic cancer development remains to be elucidated. Given high gene expression in cancer cells, one plausible explanation is that epigenetic changes aforementioned in cancer cells advance their genetic plasticity to adapt to the dynamic microenvironment changes such as metabolic distress and metastasis. The impact of targeted inhibition of epigenetic modification on pancreatic adenocarcinoma remains to be investigated.

1.1.10 ADAMs (a disintegrin and metalloproteinase) and MMPs (matrix metalloproteinase)—ADAM is a family of metalloproteinases which is composed of 21 genes. Although 13 of the 21 members function as proteases they also have important roles in cell adhesion and migration [117]. During inflammation their expression is up-regulated and they mediate recruitment of activated inflammatory cells [118]. Studies suggest that ADAM8 and ADAM9 are overexpressed in pancreatic cancer and may facilitate invasion and dissemination of pancreatic cancer cells [119]. Up-regulation of ADAM9 was found to be related to poor tumor differentiation and worse survival outcomes [120]. A preclinical study reported anti-inflammatory macrophage-mediated ADAM8 expression [121] suggesting a close interaction between cancer cells and the tumor environment during the invasion of tumor cells. A recent study in an animal model demonstrated decreased tumor burden and metastatic lesions in mice which were treated with an ADAM8 inhibitor [122] and suggested that ADAM8 could be a druggable target.

MMPs are also important regarding tumor inflammation and are involved in matrix degradation. Given that invasion of tumors requires proteolysis in connective tissue, their role in cancer progression has been also studied extensively. Consistent with their physiologic role, their expression was found to be related to increased invasion of cancer cells in tumor stroma [123]. Therefore, in the late 1990s studies were initiated using MMP inhibitors as therapeutic agents. Given initial promising results in preclinical studies [124], an orally available broad spectrum MMP inhibitor, Marimastat, was evaluated in a randomized clinical trial; however, no benefit was observed when compared to and combined with conventional chemotherapy ($p = 0.95$) [125]. Another MMP inhibitor, BAY 12 – 9566, was also evaluated in pancreatic cancer in a randomized trial and gemcitabine alone was found to be superior to the MMP inhibitor alone arm with regard to overall survival ($p < 0.001$) [126]. Collectively, these data indicate that MMPs have limited impact on pancreatic cancer therapeutically. The lack of efficacy of these drugs could be due to multiple pathways involved in matrix degradation such as ADAMs as aforementioned.

1.2 Cancer immune surveillance and immune editing: immune-modulating agents and other novel approaches

The ability of cancer cells to evade immune surveillance has become a major focus of attention in the field of cancer research. Mutations which generate antigenic peptides on cancer cells result in activation of immune system. However, in many cancers, this immune response fails to eradicate the cancer cells and tumor continues to grow in part via immune

editing processes. Recent research identified targetable peptides that are responsible for the failure of an immune response such as PD-1 receptor and ligand (PD-L1). PD-L1 antibody has been assessed in early safety studies with notable activity in solid tumors [127] and is currently being investigated in pancreatic cancer in Phase I study (NCT01693562). Other immune modulating agents targeting PD-1, CTLA-4, CXCR4 and indoleamine 2,3-dioxygenase (IDO) inhibitors are also currently under investigation in pancreatic adenocarcinoma for safety and clinical activity in Phase I and Phase II studies (Table 2). A novel approach to augment tumor suppressor gene activity in nucleus via nuclear transport inhibitors has been also investigated. A study of an orthotopic mice model suggested improved antitumor effect of gemcitabine when combined with KPT-330 (selinexor) in pancreatic cancer [128]. Currently, the safety and efficacy profile of this agent are also being examined in Phase I/II studies. The potential role of immune modulating agents in pancreatic cancer treatment clearly warrants further translational and clinical investigation.

1.3 Pancreatic cancer stem cells and associated signaling pathways

Tumor heterogeneity in cancer in general has been reported in many preclinical and clinical studies and has led to extensive investigation of this entity. Cancer stem cells are a subgroup of the cancer cell population which have a high potency to generate tumors, resist therapies, and are capable of self-renewal and dissemination to distant organs. A study identified a subclone of pancreatic cancer cells characterized with biomarkers including CD44, CD133, and epithelial-specific antigen (ESA) that exhibit cancer stem cell behaviors [129]. Several studies also evaluated these subgroups of cancer cells in pancreatic cancers and reported many active signaling pathways, more significantly active compared to non-stem cancer cells (Figure 5). Given the aggressive behavior observed in pancreatic cancer, studies have also investigated targeting stem cells in pancreatic adenocarcinoma; the data are summarized below.

1.3.1 Wnt signaling pathway—Aberrant activation of the Wnt pathway has been documented in up to 65% of pancreatic cancer patients [130]. The underlying reason behind the activation of Wnt signaling in pancreatic cancer remains to be elucidated. However, given that the severity of dysplasia in IPMN correlates with up-regulated Wnt signaling, it could be a later event in pancreatic carcinogenesis [131]. Consistent with that, the activity of the Wnt pathway may be more significant in pancreatic cancer stem cells [132] suggesting that Wnt signaling has an accelerating-multi-phase role in exocrine pancreatic cancer development (Figure 2).

Wnt genes have been investigated for more than two decades and their role in embryogenesis and carcinogenesis has been well-documented [133]. Once activation occurs in Wnt signaling, CK1ε and DVL (Dishevelled Segment Polarity protein) dissolve the β-catenin (β-catenin) degradation complex which results in removal of the inhibitory signal on β-catenin. In normal cellular homeostasis, β-catenin is inactivated by an inhibitory complex composed of glycogen storage kinase 3 (GSK3). Adenomatous polyposis coli (APC) and Axin proteins enable degradation of β-catenin via the ubiquitin-protesome pathway [134]. If the degradation process is inhibited by upstream Wnt signaling, β-catenin is stabilized in the cytoplasm and subsequently moves to the nucleus to initiate transcription of certain genes

such as c-myc, cyclin D1 and MMP-7 [135]. Multiple mechanisms have been implicated for increased Wnt signaling in pancreatic cancer. For example, one study demonstrated increased hypermethylation in Wnt signal inhibitors genetic locus [136] which results in lack of negative regulation of β-catenin. Another study reported increased expression of Wnt-1 and frizzled receptors along with up-regulated β-catenin in pancreatic adenocarcinoma cells [130]. In the same study, Zeng *et al.* reported on somatic mutations in β-catenin impairing its interaction with inhibitor protein kinase, GSK3.

The consequences of overactive Wnt signaling pathway have been extensively studied in pancreatic cancer. As stated above, activation of β-catenin results in promotion of cell cycle progress [135] via upregulated expression of target genes such as cyclin D1 and c-myc. An early study reported increased β-catenin activity and nuclear accumulation in solid and pseudopapillary neoplasms suggesting involvement of Wnt pathway in development of diverse pancreatic tumors [137]. A recent study investigated circulating pancreatic cancer cells and concluded that enrichment in Wnt signaling resulted in increased metastasis [138]. Increased β-catenin activity has been also shown to be related to reprogramming processes which suggests a potential role for cancer stem development process [139]. Furthermore, there is strong evidence suggesting that the cell renewal capacity of cancer stem cells might be related to Wnt signaling along with activated notch signaling [140]. Treatment resistance in cancer stem cells has been also attributed to activation of Wnt signaling pathway [141]. However, given that many other signaling pathways which are not observed in non-stem cancer cells operate in cancer stem cells, more evidence is required to establish etiology behind treatment resistance in cancer stem cells.

A monoclonal antibody for the frizzled receptor, OMP-18R5, is under investigation and has shown significant activity against pancreatic cancer cells in animal models along with conventional chemotherapy [142]. Currently, a Phase I study is being conducted for the safety profile and preliminary efficacy evaluation of this agent combined with nab-paclitaxel and gemcitabine (NCT02005315). A fusion protein against both frizzled receptor and Wnt ligand, OMP-54F28, is also in Phase I (NCT02050178).

1.3.2 Sonic hedgehog signaling pathway—Sonic Hedgehog (SHH) was first discovered in the 1980s as a segment polarization gene which plays a very critical role in Drosophila embryogenesis and in which mutation of SHH results in embryonic lethality [143]. The function of this important gene was later investigated in vertebrates and a similar critical functioning of SHH was observed in human organogenesis during embryonic development [144]. Given the crucial role of SHH in embryogenesis mediated by pluripotent stem cells, studies have investigated SHH signaling in cancer stem cells. Two transmembrane receptors have been established for hedgehog signaling: patched and smoothened. In the absence of a polypeptide ligand also called sonic hedgehog, patched receptor activation inhibits smoothened, which is a main signal transducing receptor [145]. However, once the ligand binds to patched receptor, the smoothened receptor is released and activates a transcription factor called Gli1 [144]. Thereafter, Gli1 is stabilized in the cytoplasm, and translocates to the nucleus where it induces transcription of target genes such as Wnt, JAG2, Snail and stem cell markers such as CD44 and CD133 and other genes involved in cell growth and the EMT process [146].

Similar to Wnt signaling, the SHH pathway is also functional at different stages of pancreatic carcinogenesis. One study demonstrated that SHH expression is increased both in early PanINs and pancreatic adenocarcinoma and maintains its activity even at later stages of disease [147]. Compatible with these observations, pancreatic cancer stem cells have been shown to express a 46-fold increased hedgehog pathway genes, in contrast to only fourfold increase in non-stem cancer cells [132] suggesting that there is accelerated activation throughout the carcinogenesis. Current thinking suggests that SHH pathway activations also enhance cancer stem cell maintenance and self renewal [148]. Beyond its effect on cancer cells, SSH has been implicated in tumor and stroma in pancreatic cancer. One study demonstrated up-regulated SHH-mediated desmoplasia and suggested that this might be associated with chemotherapy resistance [149]. Consistently, animal models demonstrate enhanced drug delivery with inhibition of the SHH pathway [150]. A case report of medulloblastoma, a disease with frequent SHH mutations, suggested very promising tumor response to an SHH inhibitor and stimulated clinical development [151]. A Phase I study also indicated potential utility of SHH inhibitors in solid tumors such as basal cell carcinomas [152] and showed substantial activity. However, clinical trials investigating the impact of SHH inhibition in pancreatic cancer have observed quite disparate results compared to the preclinical promise. One clinical trial comparing the efficacy of a SHH pathway inhibitor in combination with chemotherapy was terminated early due to increased mortality in the SHH treatment arm [153]. Another clinical trial studied an SHH inhibitor in combination with gemcitabine showed no significant improvement in the experimental arm was observed compared to gemcitabine alone [154]. Moreover, in the same study, the authors reported no change in pancreatic cancer stem cell populations either although significant changes in desmoplasia were observed. Following these disappointing outcomes, a back-to-bench study in animal models elucidated that stromal desmoplasia was functioning as a restraint rather than as a tumor support [155]. Although currently these agents are being investigated with other treatment modalities (NCT01088815, NCT01130142), this experience form bench to clinic and back to bench suggest that SHH's role is very complex and that stromal response might as much act a defense mechanism of the organ to prevent dissemination of local disease (similar to Ghon's complex in tuberculosis). Moreover, pancreatic cancer stem cells may not be addicted to SHH signaling. Although current evidence does not support druggability of the SHH pathway for pancreatic adenocarcinoma, further preclinical and clinical studies will elucidate the role of SHH inhibitors on pancreatic cancer treatment.

1.3.3 SLIT/ROBO and axon guidance pathway—Recent pancreatic cancer whole exome sequencing has highlighted novel genetic alterations that have not been reported previously in pancreatic cancer development: Axon guidance pathway [91]. SLIT/ROBO-mediated axonal guidance pathway alterations have been found to be involved in an important step of development of the CNS that guides axons to cross the midline and which generate a symmetric mirror image and crosstalk between the two brain hemispheres [156]. Following these discoveries, early evidence suggests a potential influence of axonal guidance pathway induction in tumor angiogenesis [157]. However, emerging evidence also implicates the Slit gene, which is a ligand of ROBO receptors, and is frequently silenced in varied cancers and is associated with worse outcomes with increased cancer stem cell

activity [158]. In a recent pancreatic genome analysis study [91], higher expression of ROBO3, a negative regulator of ROBO1/2, is associated with worse outcomes, whereas high expression of ROBO2 expression resulted in improved survival in pancreatic cancers indicating a tumor suppressor role for the SLIT/ROBO pathway in pancreatic cancer. The totality of these observations implicate a dual role for axonal guidance pathways in pancreatic carcinogenesis, in that while it augments the angiogenic effect on the tumor microenvironment and endothelial cells, it also suppresses tumor cell growth via interacting/regulating various growth signaling pathways [159]. To enlighten the precise effect of this novel pathway on cancer stem cells and disease progression along with its targetability, further mechanistic studies are required.

1.3.4 Notch signaling—Notch signaling has essential functions in the development and differentiation of cells and tissues in many organs and is activated by direct cellular surface interaction. The role of Notch signaling in cancer development was initially discovered in T cell lymphoblastic leukemia [160]. Further studies have demonstrated that the Notch pathway has a crucial role in many human malignancies such as lung, breast cancer as well as pancreatic cancer [161]. One study suggested that Notch mediates the EMT process in the setting of TGF-a stimulation in pancreata and advances metastases to distant organs [162]. Although current evidence indicates late stage activation of Notch signaling along with other signaling pathways which facilitate metastasis in pancreatic cancer progression [163], there is also evidence denoting a potential role in a reprogramming stage of normal acinar cells into ductal adenocarcinoma in the setting of K-Ras mutations [12], supporting the hypothesis that constitutively activated growth signaling might be a key step for malignant transformation particularly in the lack of tumor suppressor activity. More strikingly, increased notch activity fuels EMT processes that give rise to CD44-positive pancreatic cancer stem cells [164] which bear significant resistance to conventional chemotherapy and are associated with an aggressive tumor behavior [165]. An animal model investigating druggability of the notch pathway demonstrated attenuated invasiveness of pancreatic cancer cells upon suppression of Notch activity via abolishing the nuclear factor-κB signaling [166]. There is also evidence indicating that chemotherapy resistance might be reversed by targeted inhibition of notch pathway [167]. Collectively, accumulating evidence suggests that targeting the notch signaling is a viable therapeutic strategy and clinical trials have been initiated to assess the value of notch and stem cell inhibitors in the clinic [168].

2. Conclusion

Collectively, the molecular pathways aforementioned denote a complex network functioning behind the scene in pancreatic cancer. Addiction of pancreatic cancer cells to oncogenic pathways such Kras-MAPK signaling appears to be limited as multiple prosurvival pathways compensate a specific inhibited pathway. Enhancing the activity of pro-apoptotic pathways via inducing mitosis by targeting agents such as wee-1 or check point inhibitors may abrogate resistance to conventional chemotherapy particularly platinum-based agents given that recent evidence in BRCA mutant patients denotes better treatment outcomes with platinum-based agents. Moreover, down regulation of cancer stem cell associated signaling networks such as Wnt and Notch pathways which have been related to lack of response to

standard chemotherapeutic agents, may overcome treatment resistance in pancreatic cancer and change the course of disease. Further genomic and mechanistic studies may advance our understanding of the relation between aggressive disease behavior and the genetic fingerprint of pancreatic cancer and may elucidate further targetable pathways.

3. Expert opinion

Current state-of-the-science outlined here-to-fore indicates a strong relation between genetic alteration and clinical outcome in pancreatic cancer. Genome wide analyses of pancreatic cancers have revealed diverse genetic alterations that modify the gene expression profile of cancer cells and create a unique signature for cancer cell subclones that may determine the fate of the disease. These diverse genetic modifications yield varied subclones with different behaviors that incur one of the biggest challenges in modern cancer therapies, the issue of resistance particularly pertinent in the current precision medicine treatment era. New generation cancer treatments, for example, small molecule tyrosine kinase inhibitors, and monoclonal antibodies targeting certain pathways, suppress the clones that are addicted/dependent on the pathway. However, this initial promising response is generally followed by disease progression due to other clones that can bypass such signaling pathways via their genetic plasticity. These resistant clones grow and ultimately give rise to progressive disease, after an initial honeymoon period with decreased tumor bulk due to impact of targeting agent on sensitive tumor clones.

Another obstacle in cancer therapy relates to the role of cancer stem cells, which are more resistant to therapy due to their genetic plasticity, which advances their capability to generate new clones and develop distant organ metastasis. Studies aforementioned denote a potential relation between cancer stem cells and an aggressive disease behavior. Pathways more active in cancer stem cells such as hedgehog signaling have been studied in clinical trials however the results thus far in pancreatic cancer have been disappointing [154]. Therefore, further translational studies are warranted to reveal the exact role of cancer stem cell related signaling pathways on aggressive nature of pancreatic cancer and reversibility of this challenging situation via modulation of these pathways. Currently, trials are investigating the role of other signal pathway inhibitors functioning in cancer stem cells such as the Notch pathway and results are pending at this time.

A recent genome wide study identified a subgroup of disease, which is more sensitive to chemotherapy due to their genetic signature and instability, for example, homologous repair deficient pancreatic cancers with mutations in BRCA 1 or BRCA2 [169]. These observations show the impact of genetic expression profile of the cancer cells on disease behavior and provide a future insight to test utility of targeting BRCAs via small molecule inhibitors or small interfering RNAs (siRNA) with or without wee-1 inhibitors to advance treatment effect on pancreatic cancer. Therefore, further translational and clinical studies are warranted to evaluate this approach in pancreatic cancer era.

The clinical utility of targeted immunotherapeutic agents are currently being actively investigated in pancreatic cancer. Although initial preclinical and early clinical studies

indicate a potential antitumor effect, ongoing trials will clarify the key settings which immune therapy strategies and in what combinations have efficacy in this disease.

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Highlights

- Pancreas adenocarcinoma is a disease with a rising incidence and is a disease characterized by a multiplicity of challenges including late diagnosis, relative treatment resistance and genomic complexities.
- The article herein evaluates the core genomic pathways that are implicated in both pathogenesis and therapeutic application in this disease.
- Particular areas of focus include the RAS pathway, EGFR signaling, homologous repair aberrations, stem cell targeted approaches and the emerging era of immune therapeutics in this disease.
- The data regarding the importance of the relevant pathways are summarized and the clinical/translational aspects are reviewed with regard to completed and ongoing clinical trials.
- While targeted therapy or a ‘precision medicine’ approach for a given patient with pancreas adenocarcinoma remains an elusive challenge for the majority, there is a real sense of optimism that the strides made in understanding the molecular underpinnings of this disease will translate into improved outcomes.

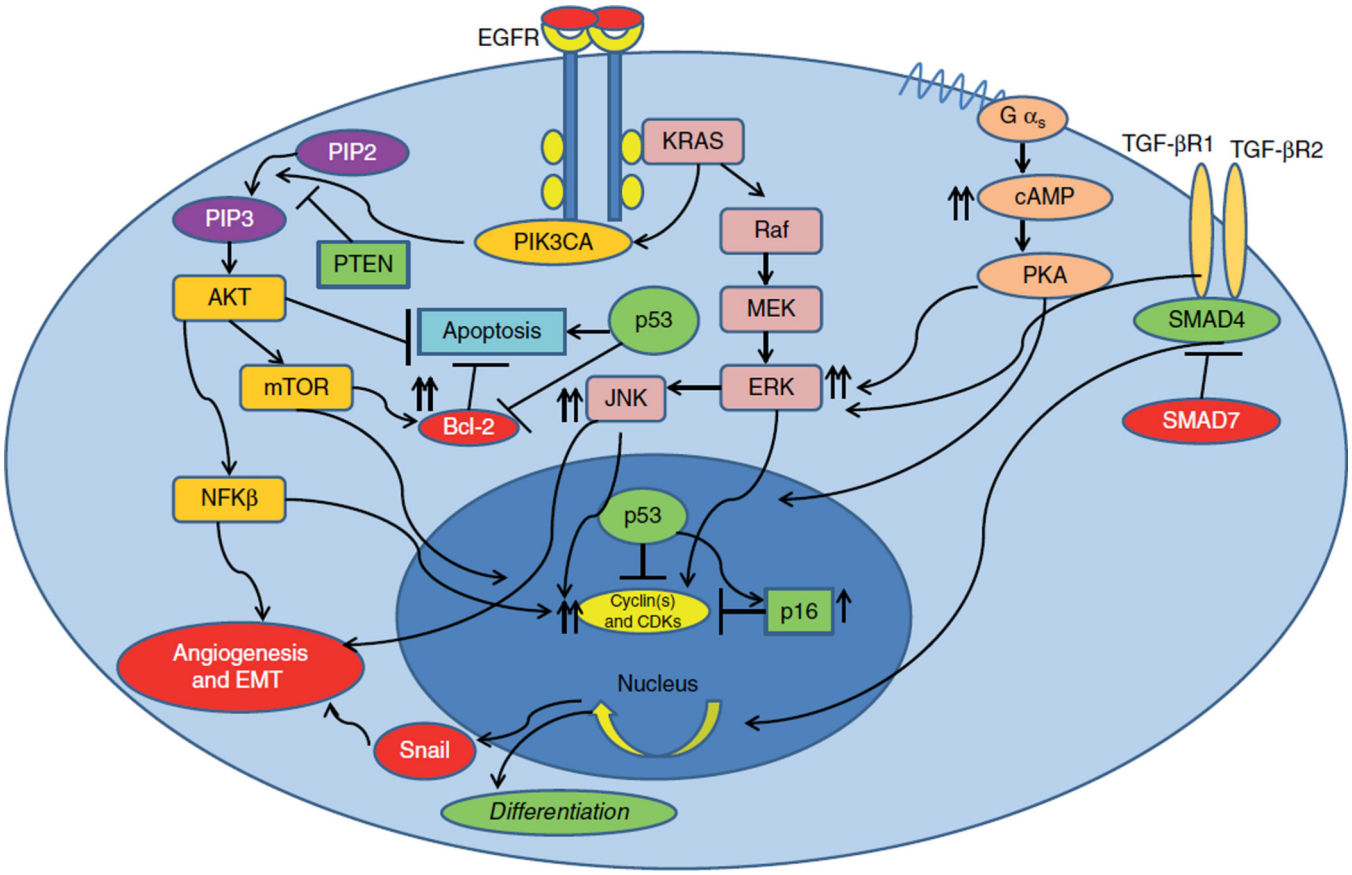

Figure 1. Core oncogenic pathways in pancreatic cancer cells

Activation of diverse pro-survival pathways such as MAPK, PIK3CA-m-TOR and TGF-β in setting of mutant p53 and p16.

EMT: Epithelialmesenchymal transition; ERK: Extracellular signal regulated kinase pathway; JNK: Jun N-terminal kinase; PTEN: Phosphotase tensin homolog; PKA: Protein kinase A.

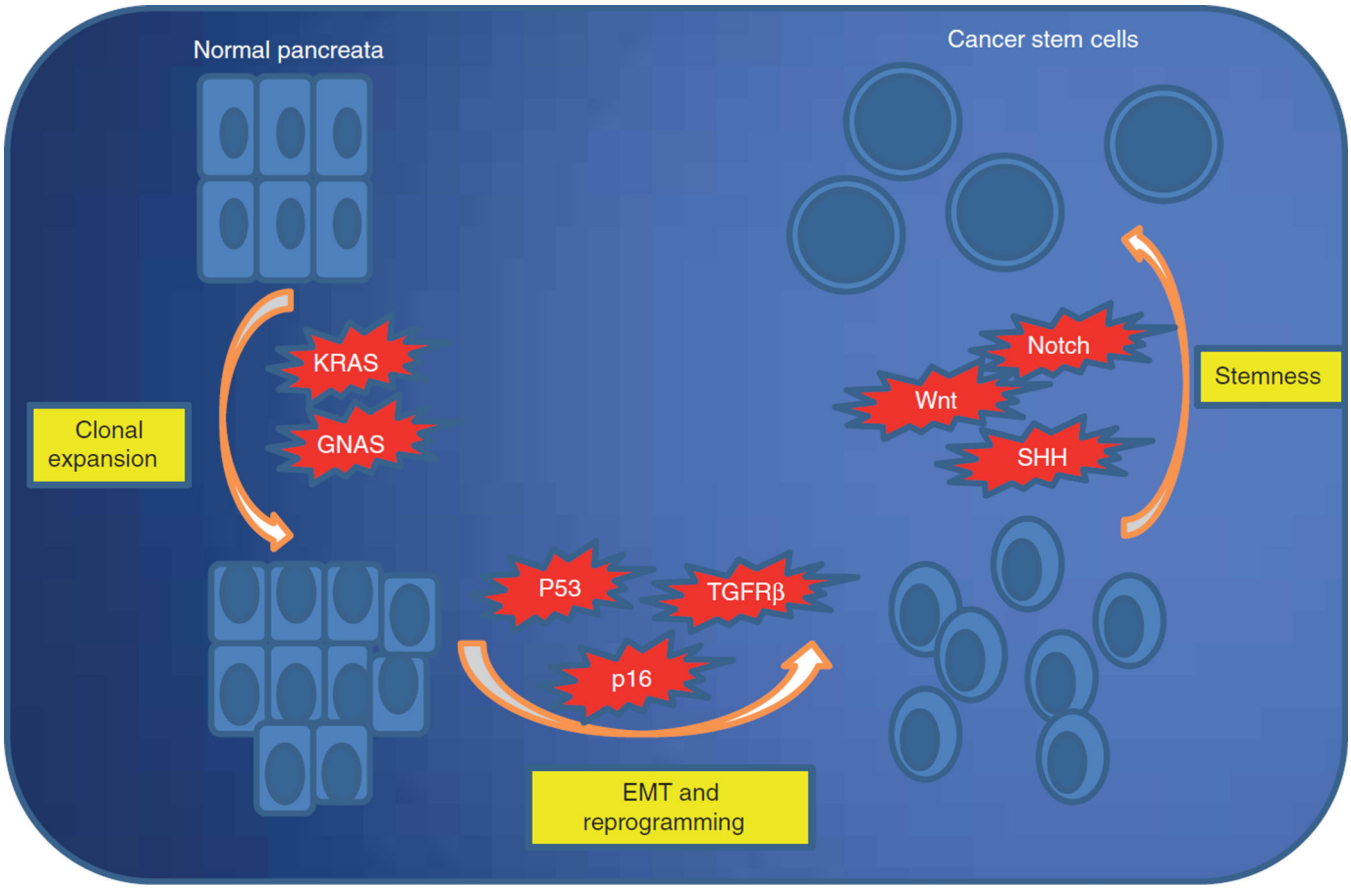

Figure 2. Model progression of pancreatic cancer

Multistep activation of growth pathways from clonal expansion to gain of stemness properties; model progression of pancreatic cancer.

EMT: Epithelialmesenchymal transition.

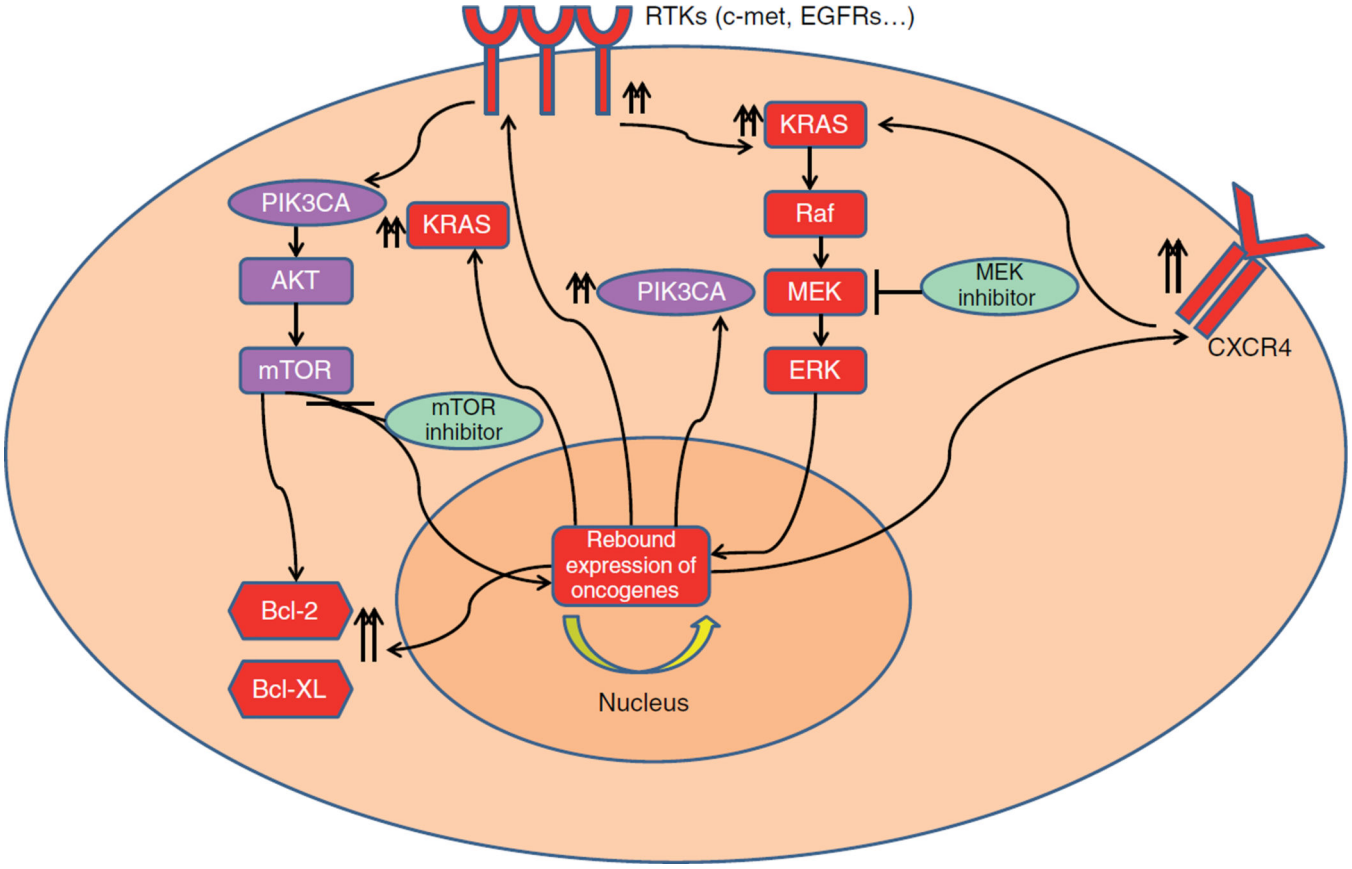

Figure 3. Possible resistance mechanisms

Rebound activation of PI3KCA and K-Ras pathways along with increased RTKs and anti-apoptotic proteins upon treatment with MEK and m-TOR inhibitors.

ERK: Extracellular signal regulated kinase pathway.

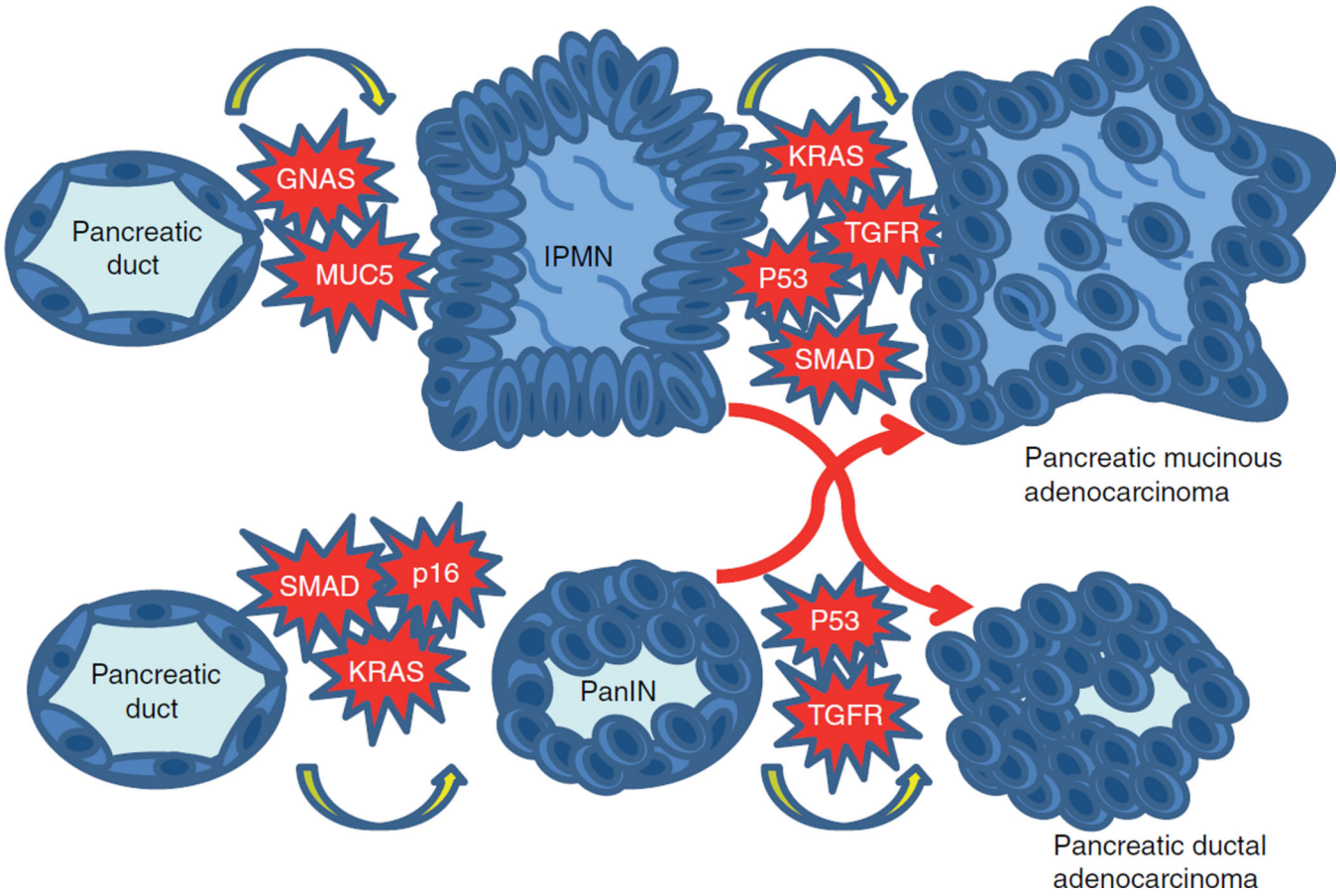

Figure 4. Models of premalignant lesion

Model for development of IPMN and PanIN. Progression for both is not mutually exclusive and both lesions may progress into pancreatic mucinous adenocarcinoma and pancreatic ductal adenocarcinoma (red arrows).

IPMN: Intraductal papillary mucinous neoplasms; PanIN: Pancreatic intraepithelial lesions.

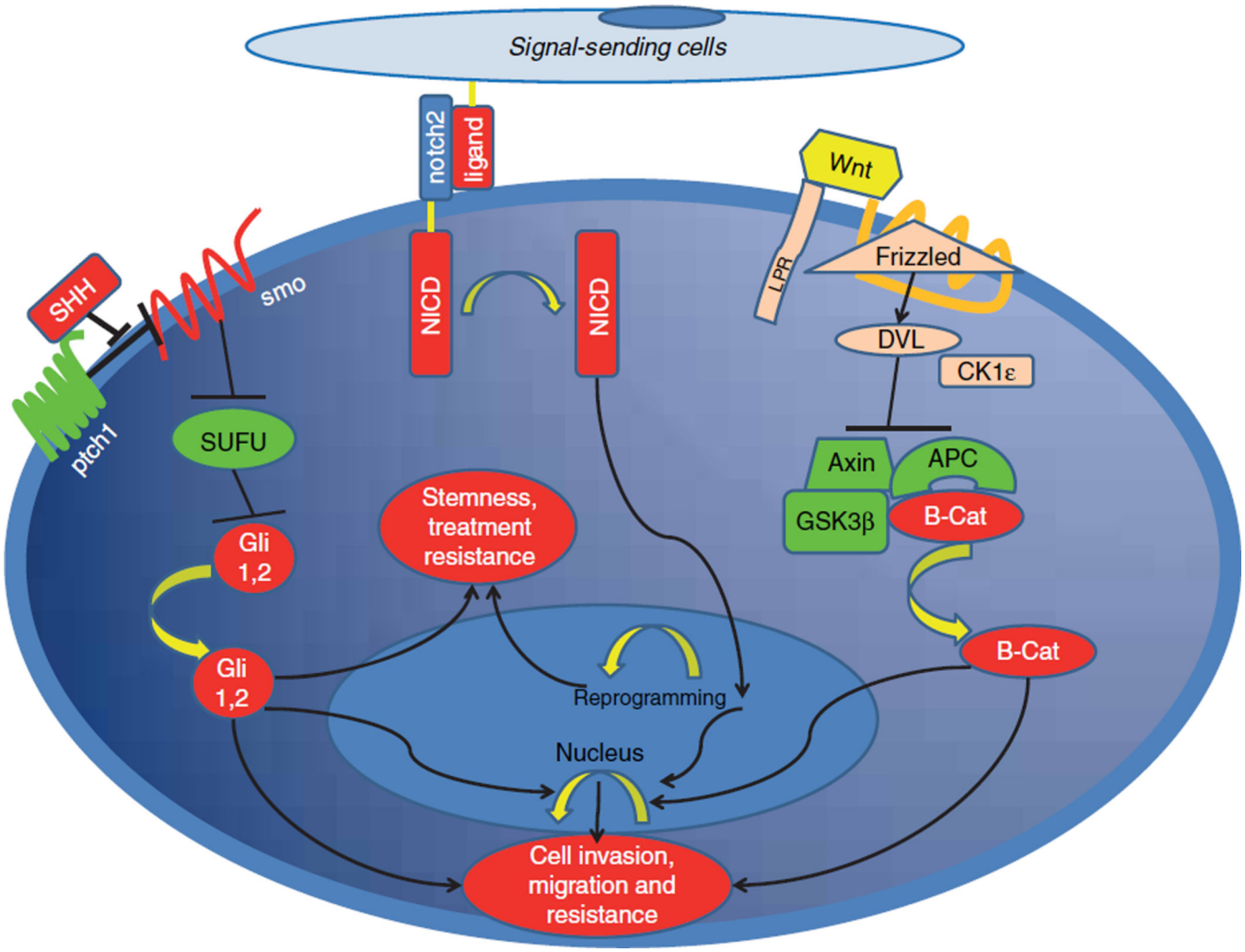

Figure 5. Oncogenic pathways in cancer stem cells

Activation of Sonic Hedgehog, Wnt and Notch pathways confers stemness properties and induce treatment resistance and metastasis.

Table 1

Clinical trials assessing the role of targeted agents in pancreatic cancer.

Study	Study design	Targeting agent	Molecular mechanism	Outcomes
Rinehart *et al.* [16]	Phase II clinical trial in multiple types of advanced cancers	CI-1040 alone	Oral MEK inhibitor	No significant clinical benefit (no objective responses)
Infante *et al.* [17]	Phase II randomized clinical trial in advance stage pancreatic cancer	Trametinib (GSK1120212) combined with gemcitabine	Oral MEK inhibitor	No significant clinical benefit (95% CI, 0.67 – 1.44 p = 0.453)
Philip *et al.* [27]	Phase III randomized clinical trial in advanced stage pancreatic cancer	Cetuximab combined with gemcitabine	EGFR monoclonal antibody	No significant clinical benefit (HR; = 1.06; 95% CI, 0.91 – 1.23; p = 0.23)
Moore *et al.* [28]	Phase III randomized clinical trial in advanced stage pancreatic cancer	Erlotinib combined with gemcitabine	EGFR small molecule tyrosine kinase inhibitor	Superior to gemcitabine alone [HR of 0.77 (95% CI 0.64 – 0.92; p = 0.004)]
Wolpin *et al.* [41]	Phase II trial in gemcitabine-resistant pancreatic cancer	Everolimus	Mammalian target of rapamycin (mTOR) inhibitor	No complete or partial responses
Daud *et al.* [106]	Phase I trial in multiple advanced stage cancers	MK-8776 ± gemcitabine	Chk1 inhibitor	Two PR's and 13 stable disease in N = 30
Bramhall *et al.* [125]	Phase III double blind, placebo-controlled randomized trial in pancreatic cancer	Marimastat combined with gemcitabine	MMP inhibitor	No survival benefit in experimental arm vs gemcitabine alone (165.5 days vs 164 days p = 0.95)
Moore *et al.* [126]	Phase III randomized clinical trial in advanced stage pancreatic cancer	Bay12 – 9566	MMP inhibitor	Gemcitabine superior to Bay12 – 9566 (6.59 months vs 3.74 months p < 0.001)
Kim *et al.* [154]	A single arm pilot clinical trial in advanced stage pancreatic cancer	Vismodegib combined with gemcitabine	SHH inhibitor	Progression free survival and OS; 2.8 and 5.3 months respectively
Ross *et al.* (NCT01130142)	Randomized clinical trial in advanced stage pancreatic cancer	Saridegib combined with gemcitabine	SHH inhibitor	Study suspended due to progressive disease in experimental arm

Table 2

Targeted agents currently being investigated.

Author/NCT #	Study Design	Agent	Target	Observations
Azmi *et al.* [64]	Animal model	Nutlin analogs	MDM2 inhibitor to enhance p53 activity	Decrease tumor burden in xenografts
Penafuerte *et al.* [80]	Animal model	FIST	Decoy TGF-β receptor and IL-2 activator	Abrogated tumor growth and inhibited angiogenesis in mice
NCT00844064	Phase I study	Trabedersen	Antisense oligodeoxynucleotide of TGF-β2	Final results pending
Heilmann *et al.* [88]	Animal model	PD-0332991	CDK4/6 inhibitor	Inhibition of tumor growth
NCT01522989	Phase I study	PD-0332991	CDK4/6 inhibitor	Final results are pending
Rajeshkumar *et al.* [105]	Animal model	MK-1775	Wee-1 inhibitor	Tumor sensitization to gemcitabine in p53 mutant xenograft model
NCT02037230	Phase I study	MK-1775	Wee-1 inhibitor	Final results are pending
NCT00413686	Phase I study	AZD7762	Chk inhibitor	Final results are pending
Gurney *et al.* [142]	Animal model	OMP-18R5	Wnt receptor inhibitor	Anti-cancer stem cell effect and inhibition of tumor growth
NCT02005315	Phase I study	OMP-18R5	Wnt receptor inhibitor	Final results pending
NCT01621243	Phase I/II study	OMP-59R5	Notch 2/3	Final results pending
NCT02050178	Phase I study	OMP-54F28	Wnt receptor and ligand inhibitor	Final results pending
NCT02179970	Phase I study	Plerixafor	CXCR4 inhibitor	Final results pending
NCT02077881	Phase I/II study	Indoximod	IDO inhibitor	Final results pending
NCT01693562	Phase I/II study	MEDI4736	PD-L1 inhibitor	Final results pending
NCT02054806	Phase I study	Pembrolizumab	PD-1 inhibitor	Final results pending
NCT01473940	Phase I study	Ipilimumab	CTLA-4 inhibitor	Final results pending
NCT02301130	Phase I study	Mogamulizumab	CCR4 antibody	Final results pending
Kazim *et al.* [128]	Animal model	KPT-330 (selinexor)	Nuclear export inhibitor	Promote nuclear accumulation of tumor suppressors
NCT02178436	Phase I/II	KPT-330 (selinexor)	Nuclear export inhibitor	Final results pending

IDO: Indoleamine 2,3-dioxygenase; PD: Programmed death.