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Novel therapeutic targets for pancreatic cancer

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Abstract

Pancreatic cancer has become the fourth leading cause of cancer death in the last two decades. Only 3%-15% of patients diagnosed with pancreatic cancer had 5 year survival rate. Drug resistance, high metastasis, poor prognosis and tumour relapse contributed to the malignancies and difficulties in treating pancreatic cancer. The current standard chemotherapy for pancreatic cancer is gemcitabine, however its efficacy is far from satisfactory, one of the reasons is due to the complex tumour microenvironment which decreases effective drug delivery to target cancer cell. Studies of the molecular pathology of pancreatic cancer have revealed that activation of KRAS, overexpression of cyclooxygenase-2, inactivation of p16[INK4A] and loss of p53 activities occurred in pancreatic cancer. Co-administration of gemcitabine and targeting the molecular pathological events happened in pancreatic cancer has brought an enhanced therapeutic effectiveness of gemcitabine. Therefore, studies looking for novel targets in hindering pancreatic tumour growth are emerging rapidly. In order to give a better understanding of the current findings and to seek the direction in future pancreatic cancer research; in this review we will focus on targets suppressing tumour metastatsis and progression, KRAS activated downstream effectors, the relationship of Notch signaling and Nodal/Activin signaling with pancreatic cancer cells, the current findings of non-coding RNAs in inhibiting pancreatic cancer cell proliferation, brief discussion in transcription remodeling by epigenetic modifiers (*e.g.*, HDAC, BMI1, EZH2) and the plausible therapeutic applications of cancer stem cell and hyaluronan in tumour environment.

Core tip: Some of the targets discussed here have been discovered to enhance the effectiveness of gemcitabine upon co-administration of the corresponding agents, for instance, hyaluronidase can deplete hyaluronan in stromal region to enhance gemcitabine delivery. Besides, some signaling molecules, *e.g.*, RalGEF-RAl, Rac1, and PAR2 are being targeted to suppress metastasis. Tumour proliferation is limited upon DR5 activated apoptosis and others promising therapeutic areas like epigenetic modifiers; IAP, miR, lncRNA, and cancer stem cells-tumour microenvironment will also be discussed.

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer death in the last two decades because of various obsta-

cles in its treatment[1]. Late and poor prognosis are two of the causes of high fatality rate[2]. Patients diagnosed having pancreatic cancer are usually at their very late stage and spreading of the highly metastatic pancreatic cancer cell into the lymphatic system and vicinal organs limited the choices of effective treatments[3].

Gemcitabine is the current standard chemotherapy for pancreatic cancer[4], however, due to the complex tumour microenvironment[5] and high metastatic property of pancreatic cancer. The effectiveness of gemcitabine in treating pancreatic cancer is unsatisfactory. Studies of targeting the molecular pathology have been carried out to quest for more potential targets; for instance, activation of oncoprotein V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS)[6], overexpression of cyclo-oxygenase-2[1], inactivation of p16$^{INK4A[6]}$ and loss of p53 activities[6] mark the onset of pancreatic cancer. Because, the treatments of targeting these molecules can enhance the efficacy of gemcitabine in pancreatic cancer[3], these imply that combinatorial therapies may be the future direction in treating pancreatic cancer[7]. Therefore, in the following context of this review we are going to briefly evaluate plausible therapeutic targets, in terms of the molecular and cellular level which covers the roles of several signal transducers, signaling pathways, surface proteins, receptor proteins, non-coding RNA, epigenetic modifiers and tumour microenvironment in driving pancreatic cancer and to explore any possibilities of combinatorial therapy among them.

SIGNAL TRANSDUCERS

CTHRC1

Collagen Triple Helix Repeat Containing-1 (CTHRC1) is a secretory protein[8], which participates in vascular remodulation through limiting collagen matrix deposition[9], and also morphogenesis but most importantly enhancing cell migratory ability and adhesiveness in tumour cells[8]. CTHRC1 is found expressed in a wide spectrum of human cancer cells, and is in particular found highly expressed in pancreatic cells[10]. The CTHRC1 protein is found highly expressed in invasive melanoma but weakly expressed or absent in benign nevi or non-invasive melanoma[8]. Over expression of CTHRC1 in pancreatic cancer has enhanced the tumour cells migration and metastatic properties; studies of using induced hyper CTHRC1 expressed pancreatic cancer cell, MiaPaCa-2-CTHRC1 and shRNA-CTHRC1 suppressed pancreatic cancer cells, BxPC3 and Panc1, are used to evaluate CTHRC1 on pancreatic cancer cells metastatic in *in vivo* mice model[10]. The result has revealed a wider metastatic spread of hyper CTHRC1 expressed pancreatic cancer cell to secondary organs while the hypo CTHRC1 expressed pancreatic tumour cells has reduced tumour cells spreading to neighboring organs when compared with the tumour cells transfected with control shRNA[10]. The phosphorylation of Focal adhesion kinase (FAK)-steroid receptor coactivator (Src) cascade and extracel-

lular signal-regulated kinases (Erk) are the causes of the enhanced metastasis[10], it is found that the binding of CTHRC1 onto the wingless-type MMTV integration site family protein, member 5A (Wnt5a) can stabilize the Wnt receptor complex[11] and the facilitated binding of the Wnt5a into its Wnt receptor complex will activate paxillin which leads to phosphorylation of Src-FAK signaling cascade and Erk[10], as both Src and Erk signaling pathways could lead to tumour progression and enhanced motility[12], overexpression of CTHRC1 has increased the phosphorylation of Src and Erk, and vice versa[10], these indicating the CTHRC1 plays a critical role in controlling pancreatic tumour cell adhesiveness and metastasis. Besides, activating the fore mentioned kinases, CTHRC1 is reported to repress the production of collagen I into the stromal environment of pancreatic cancer[8], supporting of its role as a cancer metastasis enhancing gene.

As suppressing CTHRC1 can reduce the metastatic and motility of pancreatic cancer cell, future studies can investigate on the feasibility of combining CTHRC1 targeted therapy with current anti pancreatic cancer drugs. CTHRC1 appears as a promising target in sequestering pancreatic cancer from spreading to neighboring organs, however, whether it could sensitize the tumour cells to current anti-cancer treatments in pancreatic cancer is not yet published. CTHRC1 would be a more promising target if it is proved to sequester pancreatic cancer during chemotherapy, providing a higher chance in elimination of tumour cells in the patient.

RAC1

RAS-related C3 botulinum toxin substrate 1 (Rac1)[13] is found to be an important factor in regulating pancreatic islet morphogenesis[14], failure of cell spreading has been reported on gelatin-coated culture by blocking Rac1 in isolated islet cells[14]. Apart from its vital role in directing organogenesis, Rac1 is one of the Rat sarcoma (Ras) effectors[15] and is being overexpressed in pancreatic cancer[16]. It has been found diminishing the formation of acinar-ductal metaplasia (ADM), pancreatic intraepithelial neoplasia (PanIN) and tumours when its expression is ablated in K-Ras^{G12D} induced pancreatic ductual adenocarcinoma (PDAC) mice model[15]. In cancer biology, Rac1 is found to promote tumour migration and metastasis through lamellipodia production[17]. Studies of targeting Rac1 may be beneficial in slowing down the spreading of pancreatic cancer cells.

Two guanine nucleotide exchange factors (GEFs) have been reported activating Rac1, dynamin 2 (Dyn2) has been reported regulating Rac1 in an undefined mechanism[15]; Dyn2 is found associated with vav 1 guanine nucleotide exchange factor (Vav1) in coimmunopreciptation, an onco-protein acts as a guanine nucleotide exchange factor (GEF) in Rac1 activation, and Vav1 is stabilized by the degradation of lyzozyme and heat shock cognate 70 upon binding with Dyn2[15]. Truncated form of Dyn2 has found unable to associate with Vav1 and

leading to reduced activation of Rac1 by 50%[15]. However, cell lines deficit in Vav1 expression (*e.g.*, Panc1) would undermine this therapeutic direction[15]. Another GEF, T lymphoma invasion and metastasis 1 (Tiam1), which is reported as an oncogene and associated with various cancers, Tiam1 directs Rac1 to enhance tumour proliferation and metastasis through the Wnt signaling pathway, however, suppressions of Tiam1 and Rac1 will lead to the activation of another oncoprotein, RhoA, which also promotes pancreatic tumour cells aggressiveness and metastasis[17]. The tumour growth and long term survival are significantly suppressed and enhanced respectively, upon simultaneous inhibition of Rac1 and RhoA[17]. These revealed the invasiveness and tumour migration of pancreatic tumour cells are under complex controls, balanced Rac1 and RhoA expression level is suggested to be one of those[17], and the possibility of the participation of Dyn2 in between Rac1 and RhoA, as the effect of reduction of activated Rac1 in truncated Dyn2 experiment on RhoA is unknown.

However, when shifting to microRNA research, microRNA-124 (miR-124) is found able to suppress Rac1 mRNA and protein levels in pancreatic cancer cells, through the binding onto 3'-UTR of the Rac1 mRNA[16]. Although the suppression of RhoA by miR-124 is yet to be determined; miR-143 is found able to suppress Rac1 and RhoA at the same time, producing a decreased tumour migration result in a pancreatic tumour cell xenograft model[18].

From the recent findings, Rac1 is difficult to target and obtain therapeutic value, owing to switching on another oncoprotein RhoA, however, the discovery of miR-143 is exemplifying; microRNA could be the way out in tackling target that is similar to Rac1 which has an antagonist carries the similar tumour proliferative and metastasis function. It is worth to investigate on how miR-143 suppressing this "double fused" system in pancreatic tumour metastasis enhancement and its effect on long term survival.

RalGEF-Ral effector signaling network

Ras-like guanine nucleotide exchange factors (RalGEFs) and Ras-like (Ral) protein (which is also named as Ral small GTPase) have drawn increasing attention in cancers mediated by Ras, because RalGEFs are one of the direct effectors of activated Ras[19] and the discoveries of the important roles of Ral proteins in tumourigenesis and metastasis[20], however, the exact mechanism of the signaling network requires further studies to complete. There are more than four kinds of RalGEFs (*e.g.*, Ral-GDS, Rgl1, Rgl2, and Rgl3) and two homologues of Ral are found, Ral-A and Ral-B, in which they share same nucleotide sequence but differ in 82% of amino acid sequence[19-21]. It is known that activated Ras will activate RalGEFs and in turn the activated RalGEF will convert the GDP bound Ral into GTP bound Ral, the activated Ral GTPase will then activate its downstream targets, for instance RalBP1, filamin, PLCδ1, PLD1, *etc.*, bringing out the corresponding biological responses[19]. Although the general mechanism is elucidated nowadays, the exact RalGEFs activating RalA and RalB are remain unknown, so do the identity of the exact Ras proteins in activating a particular RalGEFs[19].

There are two homologues of Ral small GTPase which are named RalA and RalB, their roles are distinct in tumourigenesis[19], but are seemingly overlapped in metastasis and invasiveness[21], ubiquitinated form of RalA and RalB have been found and it is in a non-degradative manner for selective localization modulation and functional regulations of Ral[22]. Studies have shown that mutated RalA in a constitutively active state can cause transformation of human cells but not in the same mutant of RalB[23], stable suppression of RalA in pancreatic cancer cells has brought a significant inhibition in the anchorage-independent growth[19], and inhibition of RalA can delay the tumourigenesis K-Ras mutants PDAC in mouse model[19], and the binding of RalA onto RalBP1 or Sec5 is found crucial in Ras - mediated transformation[23]. Aurora A kinase (AAK) is a kind of RalA inhibitors which prevents RalA phosphorylation, in fact an AAK, MLN8237 has been entered phase III clinical trials, and such targeting is not effective in suppressing RalA signaling[19].

On the other hand, suppression of RalB alone does not reduce tumourigenesis and transformation but bringing a more pronounced effect in metastatic tumour growth suppression when compared to RalA inhibited alone pancreatic cell lines. In addition, enhanced apoptosis in RalB suppressed cells in suspension state[24]; these are suggesting RalB has a more significant role in the control of metastatic growth of cancer cells than RalA[21]. However, when abrogating the expression of either RalA or RalB in pancreatic cell lines, reduced invasiveness is observed in some pancreatic cancer cells with RalA or RalB suppression but not in all kinds of pancreatic cancer cells, *e.g.*, reduced invasiveness is observed in RalA and RalB suppressed Capan-1 cell line, while in Panc-1 cell line suppressed RalA boosted the cancer cell invasiveness and RalB can bring a reduced invasiveness, and in T3M4 cell line RalB suppression cannot bring down the cancer cell invasiveness but RalA suppression can bring a reduced invasiveness[21]. Thus, RalA and RalB may participate in the control of the invasiveness of pancreatic cancer cells, but there should be some other signaling pathways in control to this tumour malignancy, as the invasiveness reduction cannot be observed in all types of pancreatic cancer cell lines[21]. In regard to the observations, RalB has been suggested in maintaining the viability of the cancer cells in the circulatory system and ensuring tumour cells invasiveness to other organs[21].

Nevertheless, the localization of Ral proteins may also have their roles in the control of the cancer malignancies and is in relation to their ubiquitination and phosphorylation status, as de - ubiquitination of RalA in lipid raft microdomains is reported at the loss of cell-matrix interactions, and ubiquitination of RalA promotes lipid raft microdomains exposure on the cell membrane

when the cell got re-adhered[22]. Since lipid raft microdomains served as the platform for various signaling pathways, when cancer cell is in detached state, its growth is inhibited due to the loss of related signaling cascade in the lipid raft microdomains[24,25]. Therefore, the prevention of the re - exposure of the lipid raft microdomains onto the membrane can be a direction in the RalGEF - Ral signaling cascade for inhibiting the cancer metastasis by targeting the ubiquitination and activation of RalA.

All in all, the Ral proteins in the RalGEF - Ral signaling cascade play important roles in the control of cancer malignancies, targeting the RalGEF would seem to be efficient in shutting down the transduction of the signaling cascade, however, the question of the availability of inhibitors to RalGEF is concerning, as it is a Ras like signaling molecules, the design of an effective inhibitor to RalGEF may not be easy, and the effectors downstream of this signaling cascade should be closely investigated to aid the discovery of inhibitors that can block the signal transduction downstream of this pathway.

SIGNALING PATHWAYS

Notch signaling pathway

Notch signaling is found to be an important pathway in pancreas development, however the exact mechanism of how Notch regulates pancreatic development and the effectors it recruits are not fully understood[26]. Notch signaling pathway has been reported to maintain a pool of pancreatic progenitor cells at the early stage of pancreatic development, and governs pancreatic ductal cell differentiation which found to be triggered by the intensity of the Notch activation[26]. Implying Notch signaling mediates different effectors depends on cell type, and the stage of organogenesis.

In the pancreatic cancer, Notch signaling molecules are over-expressed[26] and could produce oncogenic, anti-tumour, and drug resistance[27] activities basing on the cellular context[26]. In an ADM study using mouse PDAC model has shown that subject carrying mutant KRAS[28], Notch is constitutively activated and up-regulated in the absence of EGFR[28], while in wild type KRAS carrier, Notch activation requires EGFR activation to induce ADM[29]. Implying the mutation of Ras could alter the activation pathway of Notch. Moreover, the anti-tumour activity of Notch signaling is brought out by Notch2 receptor deletion in mutant KRAS carrier[29], which showed PanIN development is inhibited and subject survival is risen[29]. For the same model, deletion of Notch1 resulted an opposite effect, PanIN development is accelerated and subject median survival is decreased[28,30]. As these two Notch receptors are localized in different compartment of a pancreatic cell, and the exact location of them is not yet concluded[26]. Thus, studying the distribution of Notch1 and Notch2 in pancreatic cell may help to understand their roles in pancreatic cancer and the effectors downstream of this signaling pathway.

As the functions of the Notch1 and Notch2 receptors appeared to be distinct and the involvement of EGFR for activation, the roles of Notch receptors may act as the decision maker in deciding how the cell behave according to the external environment. Due to the complex environment during cancer development, figuring out the roles of Notch at each stage of the pancreatic cancer development will definitely help sorting out targets this signaling pathway that can compromise pancreatic cancer.

Nodal/Activin signaling pathway

Nodal and Activin are morphogens which are being secreted into extracellular region[31,32] to mediate gene expression in target cell through phosphorylating the transcription factor mothers against decapentaplegic homolog 2, 3 and 4 (Smad2, Smad3, and Smad4)[33], and the signal intensity is found to be able to determine the cell fate decision that the target cell would execute[33]. Thus it is an important switch in deciding cell differentiation, self-renewal and pluripotency maintenance[33], the decision of the cell fate control is found to be related to the signal intensity and signal gradient generated by this pathway[33].

It is found that these two morphogens are over-expressed in pancreatic stem cells and pancreatic stellate cells, their expression levels are barely detectable in highly differentiated pancreatic cancer cell and normal pancreas or other developed tissues[34]. Moreover, it has been suggested that a small population of cancer stem cell is encompassed in pancreatic carcinomas[34], therefore, taking these two characteristics together this signaling pathway can be a specific therapeutic target for pancreatic cancer.

The common receptors of Nodal and Activin which are named Activin-like type I receptor 4 and 7 (Alk4 and Alk7, also written as Alk4/7), are being targeted by the inhibitor SB431542[34]. Targeting Alk4/7 is to abrogate the signal transduction from Nodal/Activin receptors to the transcription factors Smad 2, Smad 3, and Smad 4; and preventing the downstream genes transcription which favor tumour malignancies expression[34]. Under *in vivo* condition, pancreatic cancer cell L3.6pl pre-treated with co-administration of SB431542 and gemcitabine before implanting onto immunocompromised mice, have resulted a significant increase in apoptosis of cell carrying CD133^{+} surface marker, implying such regimen can deplete the population of cancer stem cell in pancreatic cancer, and prevented the tumorigenicity of the cancer cell in this xenograft model; while such observations cannot be obtained in either single treatment alone[34].

However, such regimen is challenged by the abundant stroma in the xenograft model employing primary pancreatic cancer tissue, co-administration of SB431542 and gemcitabine cannot inhibit the tumour growth in such model, overcoming the sheltering effect of stroma to the pancreatic cancer cell is vital for efficient drug delivery to the tumour cell[35]. The triple-administration of SB431542, gemcitabine and CUR199691 resulted in an enhanced depletion of cancer stem cell population, as CUR199691 is an inhibitor targets hedgehog signaling pathway and ultimately depletes the stroma[34].

Besides, pancreatic tumour cells with certain mutations on Smad 4 gene have showed to be less responsive towards the regimen[34]. As Smad 4 is one of the factors for the signal transduction in the Notch/Activin signaling cascade[34], thus it is essential for the future studies to identify others up-stream targets controlling mutated Smad 4, so as to provide regimen for pancreatic cancer patients with mutations in Smad 4.

Nodal/Activin signaling is a promising target in elimination of pancreatic cancer stem cell from the studies presented here, despite its limitation in pancreatic cancer patients with mutations in Smad 4 gene, its effectiveness in wild type Smad 4 still makes it an attractive target in primary pancreatic cancer tissue model with the use of hedgehog inhibitor.

Metastatic suppressor-N-myc downstream-regulated gene-1

The N-myc downstream-regulated gene-1 (NDRG1) has recently been identified as a metastasis suppressor in several human cancer types[36], including human pancreatic cancer[37]. NDRG1 is found to increase the expression of tumour suppressor gene Smad4, which further inhibits the phosphatidylinositol-3 kinase (PI3K)/phosphorylated protein kinase B (AKT) signaling and extracellular signal-regulated kinase (ERK) pathway[36], besides, NDRG1 inhibits broad signaling molecules in nuclear factor - kappa B (NF-κB) signaling pathway, which resulted in reduced cancer metastasis[37]. As these three signaling pathways contribute to cancer cell proliferation and metastasis promotion, and cross-talk activities among them[36], therefore, NDRG1 is playing a role of modulator in orchestrating the signals in this triad networks.

The regulation of NDRG1 is debatable; numerous of studies have found out that hypoxia condition[38], epigenetic regulation[39] and iron depletion[40] can up-regulate NDRG1 expression level and such up-regulation seems to correlate with the differentiation status of the cancer cell.

It is worth to note that in a human PDAC model, under 2% of oxygen supply the NDRG1 mRNA and protein levels are elevated in differentiated pancreatic cancer cells but there are no change in the mRNA and protein levels in poorly differentiated cell lines[38]. Suggesting NDRG1 expression depends on both hypoxic condition and differentiation status of the tumour cell. The main focus would be on the rationale behind this phenomenon, as undifferentiated pancreatic cancer cell is comparatively more invasive and metastatic than highly differentiated counterpart[38]. In light of this, poorly differentiated pancreatic cancer cell (*e.g.*, Panc1) would have its NDRG1 level being suppressed in order to maintain high CXC chemokines[37] and high pro-angiogenic factor vascular endothelial growth factor (VEGF) expression[38] to direct cancer cell proliferation and angiogenesis. While NDRG1 over - expression has found down-regulating of these two signaling molecules and leading to suppression of tumour growth and angiogenesis[37].

The low NDRG1 expression in undifferentiated pancreatic cancer cells is related to the epigenetic regulation, as treating the undifferentiated pancreatic cancer cells with methyltransferase inhibitor 5-aza-2'-deoxycytidine has enhanced NDRG1 protein expression level, however, the epigenetic control on NDRG1 is not directly acting on the NDGR1 promoter, as there is no significant DNA methylation in the NDRG1 promoter region; suggesting other genes being silenced are essential for the NDRG1 expression[39].

As NDRG1 expression is affected by numerous factors, studies of targeting the molecular events downstream of NDRG1 are carried out, for instance an novel synthetic derivative of curcumin (CDF) has shown its inhibitory effect of the expression of VEGF, hypoxia inducible factor-1 α (HIF-1α), miR-210 and cancer stem cell self-renewal properties under hypoxia condition and are crucial for pancreatic cancer cell to promote tumour angiogenesis[41].

Although the exact mechanism of controlling the NDRG1 remains unclear, the current findings have suggested maintaining a high NDRG1 expression level in undifferentiated cell is able to suppress the tumour malignancies. Therefore, studies in finding enhancing NDRG1 expression genes is important in suppressing pancreatic cancer growth and metastasis.

Energy metabolism

As mentioned in the previous sections, the low vascularity structure of pancreatic tumour leading to a hypoxic environment and the adaptation of pancreatic cancer cells in hypoxic conditions through enhanced proliferation, angiogenesis and metastasis have been described. However, the primary element for cell survival is energy source which normally generated in glycolysis and Kreb's Cycle, as pancreatic cancer cells have an oxygen scarcity issue[42]; metabolic changes in pancreatic cancer cells allow them to cope with hypoxia.

First, the utilization of glucose would rely heavily on TCA-independent pathways, for example, there is up-regulation of pentose-phosphate pathway, anaerobic respiration for ATP production in hypoxic environment[43]. Secondly, glutamine metabolism is also elevated in hexosamine biosynthetic pathway which is crucial for the production of UDP-N-acetylglucosamine, and it is used for glycosylating proteins in proteins modification[44]. Glutaminolysis is also employed by hypoxia pancreatic cancer cell which metabolizing glutamine to generate glutamate and can be further metabolized in TCA cycle to produce pyruvate and lactate for further ATP production[44]. Lactate production is important for tumour cell invasiveness and neighboring cell proliferation, as inhibition of the enzyme glutamine fructose-6-phosphate amidotransferase by azaserine can cause significant reduction in hypoxic pancreatic cell proliferation[42]. Apart from targeting glutaminolysis, cannabinoids are found to suppress TCA cycle and induce the reactive oxygen species which leads to AMP-activated protein kinase

level increase to mediate autophagy in pancreatic cancer cells[45-47]. The ROS signaling activation could also abrogate the electron transport chain in mitochondria with unclear mechanism[48], leading to depletion of ATP in the cell and the AMPK dependent autophagy would be mediated[45].

By considering the founding in the energy metabolism of PDAC, targeting glutamine, glucose metabolism and increase the ROS production in hypoxic region in pancreatic tumour can elicit autophagy in PDAC. It is important to evaluate the effects of targeting them in *in vivo* model, as blocking major metabolic pathways is very likely to damage normal tissues, specific targeting the metabolic pathways in PDAC would minimize such drawback and enhancing the therapeutic value of targeting the energy metabolism pathway.

RECEPTOR PROTEINS

DR5
The death receptor 5 (DR5), is found to be frequently expressed in pancreatic cancer stem cell[49] and mediates cancer cells apoptosis *via* caspase 8 recruitment upon interacting with another receptor, Tumour necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL)[50,51], forming the death-inducing signal complex (DISC) to induce apoptosis, thus this enables the elimination of pancreatic cancer stem cell specifically, and reduces the occurrence of tumour relapse and overcoming the chemoresistance of pancreatic cancer stem cell[52].

Several studies on targeting activation of Apo2L/TRAIL induced apoptosis have been carried out, by combing with chemotherapies to obtain a synergistic effect in shrinking cancer stem cell population in pancreatic cancer[53]. Co-administration of DR5 agonist Tigatuzumab and gemcitabine has recorded more tumour regression on PDA xenografts than administrating either agent alone[49]. Moreover the up-regulation of several signaling molecules, for instance, cell surface death receptor Fas, Fas-associated death domain, and tumour necrosis factor receptor 1-associated death domain in the apoptotic pathway are also recorded[49]. Indicating the co-administration of Tigatuzumab and gemcitabine can result in cell growth inhibition and apoptosis for cell expressing DR5[49].

Another study has showed that dihydroartemisinin can increase intracellular ROS concentration and would lead to DR5 expression elevation and in turn mediate apoptosis *via* Apo2L/topoisomerase, TNF-α-related apoptosis-inducing ligand (TRAIL)[52]. Revealing the apoptotic pathway activation through DR5 requires high intracellular ROS[52]. Therefore, eliciting apoptosis in DR5 over-expressed cancer cell is a promising therapy for pancreatic cancer[50].

PAR2
The Protease-activated Receptor-2 (PAR2) is a member of the G-protein coupled receptor family and is activated by trypsin[54]. PAR2 is able to promote angiogenesis through two distinct pathways. The first one is *via* the activation of the mitogen-activated protein kinase (MAPK) to mediate VEGF release[55], another pathway involves the tissue factor to bind with integrin-linked kinase to up-regulate HIF-1α expression *via* AKT phophorylation and eventually enhanced VEGF expression[56]. Hence, PAR2 is essential for tumour survival under hypoxic condition in the micro environment, as PAR2 maintains a constitutive high level of HIF-1α for angiogenesis promotion and this also explains the high metastatic property of pancreatic cancer cell in hypoxia region.

Besides, the role of PAR2 in pancreatic cancer cell migration is also being reported, PAR2 is found to mediate MAPK-epidermal growth factor receptor 1/2 (EGF1/2) signaling pathway with the utilization of extracellular ATP, blocking the cross talk between PAR2 and extracellular ATP can be a target in reducing pancreatic cancer metastasis[57].

HER3
The Human Epidermal Growth Factor Receptor (HER) family consists of four members in which they are all type 1 transmembrane receptor with tyrosine kinase properties[58], except HER3[59], a member of HER which is found overexpressed 41% in pancreatic cancer[60]. Because of lacking tyrosine kinase activity in HER3, it requires phosphorylation by another HER receptor to activate PI3K/AKT signaling pathway to mediate cell angiogenesis and metastasis[61]. The expression level of HER3 has been correlated with tumour progression[59].

Therefore, HER3 has been an important target for suppressing tumour angiogenesis and metastasis by using humanized monoclonal antibodies, *e.g.* U3-1287 which has gone through phase 1 clinical trials with well tolerance in solid tumour patients , MM-121 and tyrosine kinase inhibitors[62,63]. The anti - HER3 agents block the activation site on the HER3 receptor, preventing the activation of HER3 during heterodimerization with HER2 receptor[59] and promoting receptor internalization upon binding onto its extracellular domain[58]. This reduces the activation of PI3K/AKT signaling pathway and its downstream effectors activation, resulting tumour growth suppression[59].

In view of this, because of HER3 over-expression in PDAC and its crucial role in activating the signaling pathway essential for cell growth, it is a valuable and specific therapeutic target upon co-administration of anti - HER3 agent and gemcitabine has resulted an enhanced anti-tumour effect[64], confirming the therapeutic value of anti - HER3 agents in PDAC and is worth investing in more clinical studies.

All in all, we have described three receptor proteins which carry out apoptosis, tumour proliferation and metastasis. Current studies are focusing on how to trigger the signaling molecule that could induce apoptosis, inhibit the receptors that favor cancer proliferation and metastasis, so as to reduce tumour progression. How-

ever, the possibility of combining these two approaches in the same model is not yet published, in which the total effect on tumour clearance is expecting to be more efficient.

CELL SURFACE PROTEIN

E-Cadherin

E-Cadherin is a transmembrane protein[65] and is a member of cadherins family in which its expression in epithelial cells is controlled by intracellular signaling molecules[66]. E-Cadherin directs the positioning of the cell during morphogenesis, controlling cell migration and tissue structure maintenance[67]. It is reported that during epithelial-mesenchymal transition (EMT) the E-Cadherin level in neoplastic epithelial cells is down-regulated, suggesting triggering the dedifferentiation of neoplastic epithelial cells into mesenchymal cell with higher motility[65].

The fading E-cadherin expression is frequently reported in undifferentiated, noncohesive pancreatic cancers[68], and it is found that the silencing of E-cadherin is mediated by Snail/ histone deacetylase 1 (HDAC1)/histone deacetylase 2 (HDAC2) complex[65] and Enhancer of Zeste Homolog 2 (EZH2)[69] through hypermethylation of its promoter region[68]. Inhibition of Snail and HDAC2 are also carried out to validate the E-cadherin expression is under such complex governance[70].

Since the absence of E-Cadherin marks the onset of metastasis and PDAC progression, a study targeting E-Cadherin restoration by using microRNA 101, has inhibited the EZH2 binding on E-Cadherin promoter region in PANC1 preventing E-Cadherin silencing and in turn inhibited its tumorigenicity xenograft[69].

The key for targeting E-Cadherin to obtain therapeutic value in PDAC is to up-hold the E-Cadherin expression by down-regulating its inhibitor, as described above, inhibiting EZH2, and Snail/HDAC1/HDAC2 can reduce E-Cadherin depression and suppresses the tumorigenicity of pancreatic cancer, in the future studies, discovering targets that suppress E-Cadherin expression is important for therapy involving E-Cadherin.

Galectin-4

Galectin-4 (Gal-4) is a glycan binding proteins which belongs to the galectin family. Gal-4 is found over-expressed in cystic tumours of the human pancreas, PDAC and cancer stromal cell[71]. Activated galectins carry out several functions; include cell-cell adhesion, cell proliferation[72], mediation of intracellular signaling[73] and tumour metastasis[74], *etc.*, However the mechanisms behind are remain unknown.

A study has evaluated the inhibition effect of Gal-4 in a pancreatic cancer cell, PaTu-S cell, can lead to enhanced tumour migration[74]. The exact reason is yet to be elucidated, but it is speculated that the reduction of Gal-4 on the cell membrane would destabilize cell-cell interaction, allowing tumour cells escape[75]. Another important implication suggests Gal-4 expression may be dependent on the tumour development stage, and is vital for tumorigenesis as it promotes cell-cell adhesion[76].

Because of Gal-4 multi-roles in expressing tumour malignancies, and the little knowledge on how Gal-4 control cell migration and tumour metastasis, it is worth to investigate its related signaling pathways and identifying possible inhibitors so as to enable targeting Gal-4 in treating pancreatic cancer.

TMPRS S4

Transmembrane Protease, Serine 4 (TMPRSS4) is found highly expressed in several cancer cells, including pancreatic cancer cell[77]. However its regulation mechanism is poorly known[78], several studies have shown that TMPRSS4 can promote EMT, metastasis and invasiveness in human epithelial pancreatic[79], lung and colon cancer[77].

It is reported that EMT mediation is not solely rely on TMPRSS4 up-regulated integrin $\alpha 5$ to activate FAK/ERK signaling pathway and enhanced invasiveness[79] but also count on the down-regulation of E-Cadherin in TMPRSS4 up-regulated cancer cells[79].

Another downstream target of TMPRSS4 is the urokinase-type plasminogen activator (uPA) gene[80]. TMPRSS4 would activate the transcription factors of μPA *via* c-Jun N-terminal kinase mechanism before promoting μPA transcription in a cell-type dependent manner[80]. And the increased μPA gene transcription marks the increased tumour cell aggressiveness.

The coupling effects of TMPRSS4 on tumour aggravated invasiveness and metastasis with other signaling molecules (*e.g.*, integrin $\alpha 5$, uPA), moreover, the inverse expression pattern of TMPRSS4 and E-cadherin suggests TMPRSS4 can be suppressed by targeting E-cadherin inhibitors as previously mentioned and should be investigated in future studies. TMPRSS4 expression is affected by various signaling molecules and by considering its important role in expressing tumours malignancies, it is a target with multiple approaches for suppression.

IAP

Inhibitor of apoptosis protein (IAP) is a group of proteins bind to caspases and inhibit caspases apoptotic effect resulting apoptosis abortion[81]. The importance of apoptosis mediation in cancer therapies has an irreplaceable place, and therapies incapable to induce cell death would be meaningless. However, most therapies nowadays involve the elicitation of apoptosis at their end, and resistance of the corresponding therapies developed due to the presence of IAP[82]. Therefore IAP is the obstacle to tackle with, so as to ameliorate the effectiveness of therapies targeting apoptosis induction.

Two of the IAP members would be discussed here which are X-linked IAP (XIAP) and survivin, because of the reports of their close interaction in triggering anti-apoptotic effect[82].

Survivin's action has been controversial in anti-apoptotic activity[82], it is reported that survivin carries out neurogenesis, angiogenesis, cell cycle progression in can-

cer cell[83] and displays caspase inhibitory effect through associating with XIAP and stabilizes XIAP *via* their baculovirus-inhibitor of apoptosis repeat (BIR) domain[84]. Most of the survivin inhibitors that are under clinical trials have improved the effectiveness of chemotherapies *e.g.*, topoisomerase, TRAIL[82]. Revealing survivin's submissive and supporting role in IAP targeted treatment.

XIAP is the most studied IAP, it is found able to suppress caspase-3, caspase-7 and caspase-9 apoptotic activities[85]. Inhibition of its caspase binding domains, which are named, BIR-2 and BIR-3, with the use of phenylurea-based chemical inhibitors of XIAP (XAntags) could make pancreatic cancer cells more vulnerable to apoptosis[85].

Because of the anti-tumour effect in inhibiting XIAP and the supportive role of surviving in apoptotic inhibition, co - suppressing XIAP and survivin has also been performed in Panc-1 cell[86], resulting cell proliferation hindrance, and enhanced gemcitabine effectiveness in XIAP and survivin suppressed model than sole suppression of either IAP[86].

Nevertheless, there is no cell toxicity recorded in XIAP knocked out mouse model and *in vitro* cell model, possibly by the compensatory up-regulation of other cIAPs, and the masking effect of such up - regulation requires further studies[81]. From the recent findings in IAP, inhibiting IAP is a promising therapeutic direction in promoting apoptosis progression in PDAC cells, thus enhancing the effectiveness of current chemotherapies upon co - administration in treating PDAC.

NON-CODING RNA

MicroRNA

MicroRNAs (miRNAs) consist of 18-24 base pair which are small and non-coding-sequence[87]. They execute target gene expression control by binding miRNA 3'UTR on to the target gene mRNA[87], and only when perfect binding of miRNA on to the target mRNA could mediate mRNA cleavage, otherwise, it would result into inhibited protein production[88]. miRNAs which induce over-expression of oncogenes are termed the oncogenic miRs (onco-miRs), on the other hand, miRNAs which suppress cell transformation are named tumour suppressor miRs (TSG-miRs)[89]. The abnormal expression levels of these two kinds of miRNAs are observed in pancreatic cancer[90]. Current studies are either suppressing onco - miRs or reconstituting the TSG-miRs level[91], therefore, in the following we will discuss some TSG-miRs which are promising therapeutic targets in pancreatic cancer.

miR-34: miR-34 is reported to be up-regulated by p53[92], inducing cell cycle arrest in primary and tumour derived cell lines[93]. A significant reduction of miR - 34 expression level in gastric cancer cells with p53 mutation has been observed and reconstituted miR - 34 expression by transfecting pancreatic cancer cells with letivirus carrying vector expressing miR - 34[94], and resulted in decreased

Notch2 and Bcl-2 protein production, reduced tumoursphere formation from cancer stem cell (CSC)[94]. Although the relationship between miR-34 and p53 is still unclear[94], the encouraging results generated by miR-4 in p53 deficient pancreatic cancer cells[93] have make it a worthy therapeutic target.

miR-143: miR-143 has been studied for its anti-metastasis and anti-tumour proliferation in liver undergone metastasis and a pancreatic cancer xenograft in mouse model, respectively[95]. miR-143 expression level in KRAS mutant pancreatic cancer cells is also being ablated[96], re-expressing miR-143 in its deficit cell lines has performed, GEF, RAC1, matrix metalloproteases (MMPs) and KRAS are the inhibition targets for miR-143[95], as described previously lessened RAC1 level can inhibit metastasis and tumorigenesis, while inhibiting KRAS is even more important, which implies a board spectrum of signaling pathways diminishing effect.

Another tumour growth promotion factor that miR-143 targets is the cyclooxygenase (COX-2)[97], COX-2 is reported as an essential factor for prostaglandin synthesis to mediate inflammation and cancer cell growth and survival[98]. In pancreatic cancer cell, miR - 143 was found to be repressed by prostaglandin[99], and restoration of miR-143 level can decrease both mRNA and protein level of COX-2 and inhibited cell growth[98].

miR-200: miR-200 is a family of miRNAs related to EMT[100], reconstituted expression level of miR-200 has restored the phosphatase and tensin homolog (PTEN) expression level[100], as PTEN is widely down regulated in various cancer cell lines and is a tumour suppressor gene in which reduced expression would lead to enhanced tumour aggressiveness[101,102], while membrane type-1-matrix metalloproteinase (MT1-MMP) is up-regulated and lead to aggravated cancer invasion[101-103]. Restoration of miR-200 by using CDF, which is a synthetic analog of curcumin, and a natural compound, BR - DIM are reported and are found able to enhance PTEN expression level and a decreased MT1-MPP promoted invasiveness[100]. Therefore, agents which could enhance miR-200 expression would have promising therapeutic value in curbing pancreatic cancer aggressiveness for enhanced treatments efficiency.

The three TSG-miRs exemplified the diverse roles of miRNAs in anti-tumour activities, up-regulation of TSG-miRs can suppress tumour malignancies expression, however, suppressing onco-miRs that can up-regulate oncogenes also have tumour malignancies suppression effect, therefore screening and studying the agents that can up-regulate TSG-miRs and down-regulate onco-miRs are vital for PDAC therapy development.

Long non-coding RNA

Long non-coding RNAs (lncRNAs) are transcribed from intergenic and intronic regions in human genome[104] by RNA polymerase II[105], which lengths more than 200 bp[106] and their biological functions have been reported,

for instance, epigenetic control, transcription regulation, pre and post-translational regulation[107], cell cycle and differentiation control and even governing the apoptosis process[108]. lncRNA is used as a diagnostic parameter and can be a therapeutic target in cancers[104]. However, the definition and discovery of lncRNAs are expected to keep on changing as very little is known in this emerging area[109]. In the following, two lncRNAs that are highly expressed in pancreatic cancer will be discussed.

HOTAIR: HOX transcript antisense RNA (HOTAIR) is a lncRNA which is highly expressed in a range of primary tumours and metastatic cell[110], in which its expression pattern is variable but in general is also over-expressed pancreatic cancer[111]. HOTAIR carries out tumour supporting effect by inhibiting anti - tumour genes activity, in which the interaction of HOTAIR and a Polycomb-group (PcG) family protein named, EZH2, would promote chromosomal histone protein histone 3 protein at lysine 27 (H3K27) trimethylation, which leads to repressed tran-scription of multiple gene targets[112]. However, there are some genes inhibited in an EZH2-independent manner[113].

Suppressed HOTAIR expression by using RNAi in pancreatic cancer cell has caused retarded cell growth, diminished tumour aggressiveness; altered cell-cycle progression and apoptosis induction[114]. Thus, relieving the repressed transcription of the tumour suppressor genes by suppressing HOTAIR expression is therapeuti-cally valuable in treating PDAC. As the studies of genes activation mechanism and the genes that are targeted by HOTAIR are still ongoing[114], and the mechanism of genes being independently regulated by EZH2 but de-pendent on HOTAIR only, are currently under studies.

Studies of targeting HOTAIR in PDAC cell lines have achieved reduced tumour malignancies expression, indicating the relieved tumour suppressor genes expres-sion by targeting HOTAIR has made HOTAIR an attrac-tive target in pancreatic cancer therapies development. However, cautions should be taken on the over-expressed genes induced by HOTAIR, as HOTAIR induced and suppressed multiple genes at the same time and some of the over-expressed genes expression level do not reduce with HOTAIR suppression[114], suggesting another mecha-nism may exist in down-regulating them. In conclusion, a more thorough understanding on the regulation and the functions of HOTAIR induced and suppressed genes, it could lead to a more rounded target in promoting tumour suppressors genes functions while inhibiting oncogenes activities.

MALAT1: Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1), as known as the Nuclear-En-riched Abundant Transcript 2[115] is found highly expressed in normal pancreatic and lung tissues with high abundance and highly conserved among mammalian[116]. Intensive studies of MALAT1 in non-small-cell lung carcinoma revealed its metastasis and tumorigenicity promotion ac-tivities[117]. Although inadequate studies of MALAT1 in

pancreatic cancer cell model, it has been reported for its promotion of tumour malignancies expression in various cancer types[116]. For instance, in colorectal cancer, a Chi-nese herb extract Resveratrol is shown to down-regulate MALAT1 and cause suppression of Wnt/β signaling *via* decreasing β-catenin nuclear localization and eventually inhibited the invasiveness and metastasis of colorectal cancer[118].

Apart from promoting tumourigenesis, invasiveness and metastasis, MALAT1 also participates in the control of cell cycle progression, oncogenic transcription fac-tor B-MYB is found up-regulated with the expression of MALAT1, silencing of B-MYB in fibroblast model resulted into cell cycle arrest in G1/S and S phase[119], moreover, another transcription factor, E2F transcription factor 1 (E2F1), which is essential for cell cycle progres-sion and is also under the modulation of MALAT1, however down - regulated MALAT1 brought down E2F1 expression has elicited p53 expression enhancement and lead to cell cycle arrest and hence reduced cell prolifera-tion[119]. This implies MALAT1 could induce DNA dam-age response *via* an unknown mechanism[119].

With regard to the findings of MALAT1 in other cancer, MALAT1 expression in PDAC is also very likely to correlate to pancreatic cancer progression. Although MALAT1 expression level is high in normal pancreatic tissue, its expression level in pancreatic cancer is not yet reported and also the role of MALAT1 in pancreatic tu-mour activities. Thus, if MALAT1 has a similar tumour malignancies promotion role in pancreatic cancer as in other cancer types, it would be a promising therapeutic target for PDAC treatment development.

EPIGENETIC MODIFIER

HDAC

Histone Deacetylases (HDACs) are a group of four class-es of deacetylases[120], each class of the enzyme contributes to different tumour malignancies expression, for example, as mentioned previously, HDAC1 is responsible for the acceleration of EMT and metastasis in PDAC[121], while HDAC2 would desensitize the PDAC towards DNA dam-age response and decreased prop - apoptotic proteins[122], however, only the third class, which is named the human hst proteins (SIRTs) did not respond to HDAC inhibitors (HDACIs) under current clinical trials[120], but a HDACI named Sirtinol is able to induce apoptosis with the admin-istration of Sirtinol[121], and its effect is further enhanced with the co - administration with gemcitabine[123].

The exact mechanism of HDACIs in mediating anti-tumour activities remain further elucidation. However, studies have shown that it is not necessary for HDACIs to inhibit the expression of HDACs in mediating tumour suppression, for instance, a class I and II HDACI did not cause changes in the expression level of HDAC1, and other tumour suppressor genes but has shown reduced cell proliferation in cervical tumour cell[124]. Moreover, a class I and II HDACI inhibitor is found able to cause

a changes in the expression profile of class Ⅲ HDAC, SIRTs[121]. These evidences suggest the working mechanism of HDACIs involve complex molecular control. On the other hand, Rel/p65 (NF-κB) is found relating to the expression level of HDAC, for instance, over-expression of class I HDAC in pancreatic cancer cells, their expression level of NF-κB is also high, besides, a class I HDACI valproic acid (VPA) is studied and found to cause a decreased expression in pancreatic cancer cells which leads to enhanced PDAC apoptosis[125], in which over-expression of NF-κB has been reported for enhanced tumour growth, angiogenesis, chemo-resistance and metastasis[125]. Blocking NF-κB activity by VPA can obtain anti-tumour effect in such case[126].

The action of HDAC on gene silencing is mediated by deacetylating the histone proteins in the chromatin leading to chromatin condensation, resulting silenced genes transcription[121]. As the genes being silenced in cancer are mostly related to tumour suppressors, anti - apoptosis, and often resulted in drug resistance, therefore, targeting HDAC by using HDACI is believed to reduce the these tumour malignancies expression by suppressing the related signaling pathways of the PDAC and synergistically enhance the anti - tumour effect of current chemotherapy.

BMI1: B-Cell-specific Moloney murine leukemia virus Insertion site 1 (BMI1), belongs to the polycomb group (PcG) which represses transcriptional activity of various genes[127]. Over-expression of BMI1 in a board spectrum of cancer cells is observed, it strengthens tumour growth by providing anti-apoptotic activities and participate in tumour metastasis by up-regulating PI3K/AKT signaling pathway[127]. In *in vitro* experiment, PDAC with BMI1 suppressed using shRNA has shown enhanced cell death in response to gemcitabine treatment, a significant decrease for its cell surface markers $CD44^+CD24^+ESA^+$, loss of self-renewal ability, reduced tumour sphere formation by CSCs and reduced tumour size in xenograft model[127].

Because of the diversified anti-tumour effects of silencing BMI1 in pancreatic cancer cell, such as reduced invasiveness, tumourigenesis[127], metastasis, CSC phenotypes, cell proliferation[128] and also chemo-resistance[127]. Besides, CSC is reported to be the causes for tumour relapse in pancreatic cancer[129], thus, the diversified roles of BMI1 in pancreatic cancer have made it a very attractive target, future studies targeting BMI1 inhibition and its downstream effectors would benefit PDAC treatment development.

EZH2

The polycomb repressor complex 2 member, Enhancer of Zeste Homolog 2 (EZH2) is a histone methyltransferase which is highly expressed in pancreatic cancer cells[130], EZH2 mediates tumour suppressor genes transcription inhibition through trimethylation of the H3K27[131], such as suppressing Rap1GAP expression in squamous car-

cinoma[132], E-Cadherin in pancreatic cancer[131]. Besides, several reports have suggested EZH2 suppresses miRNAs in contributing to pancreatic cancer progression, *e.g.*, microRNA-218 (miR-218), microRNA-26a[133,134], miR-218 is essential in suppressing tumour proliferation and metastasis in nude mouse model[133], EZH2 is believed to interact with two polycomb repressive complexes (PRCs), PRC1 and PRC2, and promoting the methylation of the target miRNA promoter region to silence the miRNAs expression in pancreatic cancer[133]. It is found that with the administration of EZH2 inhibitor, such as 3-deazaneplanocin A (DZNeP), can reduce EZH2 expression of EZH2 and rescued the expression of miR-218 leading to tumour malignancies reduction[133,135].

Apart from suppressing miRNAs in tumour progression, studies of the role of EZH2 as a tumourigenesis initiator have found that EZH2 also suppresses tumour suppressor gene $p16^{INK4}$, in which it suppresses tumour proliferation and regeneration, enhanced EZH2 expression has caused $p16^{INK4}$ down - regulation, counteracting the suppression effects exerted by $p16^{INK4}$[136]. Such control is crucial for the regeneration of the injured acinar pancreatic cell, in which the injured cell undergone de-differentiation into metaplastic epithelial intermediate, depleted $p16^{INK4}$ allows the cell to re-differentiate into acinar cell from metaplastic epithelial intermediate[136]. Thus in combination with the early appearance of PaIN lesion in pancreatic cell baring KRAS mutation and the loss of $p16^{INK4}$ expression due to enhanced EZH2, invasive and metastatic tumour development are accelerated, demonstrating the linkage between regeneration and tumourigenesis under the influence of mutant KRAS[137].

Further studies of the role of EZH2 in pancreatic CSC has found it is essential in maintaining the CSC population in pancreatic cancer, suppressing EZH2 has decreased the degree of H3K27 methylation, reduced CSC population in pancreatic cancer, enhanced genes expressions for cell differentiation and migration[130]. Since the trimethylation of H3K27 and the expression is correlated with the CSC population, it is suggested that the H3K27 trimethylation by EZH2 can be used as a marker for the CSC population which allows rapid evaluation for the population of CSC when compared to conventional methods, hence, speeding up the studies of the effectiveness of compounds towards pancreatic CSC[130].

From the current findings of suppressing EZH2 in pancreatic cancer, EZH2 has an important role in tumour development initiation and supporting cancer stemness, and co-administration of DZNeP and gemcitabine has achieved promising anti-tumour effects. Nevertheless, EZH2 has also demonstrated its possibility to act as an indicator for CSC population estimation, and CSC elimination is an important factor for researchers to evaluate the efficacy of the compounds under studies, thus EZH2 is a versatile targets that possess both therapeutic and assay values and screening compounds suppressing EZH2 would definitely help speeding up therapies development

in PDAC.

PANCREATIC CANCER STEM CELL (PANCREATIC CSC)

The tumour cell population is reported to encompass a population of cancer stem cell (CSC)[138], and it is reported to give rise to the cancer stemness in various cancers, by carrying out self-renewal, metastasis, invasiveness enhancement[139], and drug resistance for pancreatic tumour[140]. Studies in CSC have led to the discovery of distinguishable cell surface markers presented in various cancer types, and this allowed the isolation of cancer stem cell for various studies[141]. In this section, we will briefly discuss how CSC contributes to enhanced cancer malignancies, and the plausible targets in CSC that have been reported to have therapeutic value.

Signaling pathways in CSC

There are three members of hedgehog proteins in the hedgehog signaling[141], a member of the hedgehog family, sonic hedgehog is found over - expressed in both pancreatic cancer cell and CSC[142]. The up-regulated sonic hedgehog signaling molecules facilitates the development of PanIN and enhanced accumulation of mutations in KRAS while inhibiting the hedgehog signaling pathway by the hedgehog signaling inhibitor cyclopamine has resulted decelerated tumour growth and on set of apoptosis[142], another inhibitor GDC-0449 is reported to produce reduced cell viability, caspase-3 mediated apoptosis, reduced tumour sphere formation in pancreatic CSC[143]. Sonic hedgehog has displayed its critical role in tumourigenesis initiation and tumour proliferation, targeting hedgehog signaling is therefore advantageous in the early development of pancreatic cancer.

In Notch signaling pathway, over-expressed Notch-1 promotes EMT and tumour sphere formation[144], which is confirmed by the increase expression of CD44 and EpCAM cell surface markers on CSC[144], suggesting Notch as a factor in pancreatic tumourigenesis in CSC, but the role of it in CSC self-renewal requires further studies[141]. Notch mediates signaling by nuclear translocation and is modified by γ-secretase before entering the nucleus, thus inhibitors of γ-secretase have been used to study the role of Notch in pancreatic cancer and also pancreatic CSC[145], a Notch inhibitor, PF-03084014 is found able to bring a reduction of CSC population, tumour re-growth and inhibited several cancer malignancies, *e.g.*, tumour growth, angiogenesis in pancreatic cancer xenograft model with the co-administration with gemcitabine[146], therefore its effect on pancreatic cancer is expectable.

The CXCR4 signaling pathway which comprises the ligand, stromal cell - derived factor-1/CXCR chemokine ligand 12 (SDF-1/CXCL12) and the G-protein coupled receptor, CXCR4. This signaling pathway is up - regulated in pancreatic cancer cell due to enhanced expression of CXCR4, and resulting into tumour metastasis promo-

tion; enhanced migration and strengthened stromal adhesion[141]. In pancreatic CSC, co-expression of CD133^{+} and CXCR4^{+} on the CSC signified a highly metastatic characteristic and contributes to tumour metastasis, therefore, disrupting the SDF-1 mediated CXCR4 signaling and depletion of the CD133^{+} CSC can abrogate the metastatic characteristic of pancreatic tumour[146]. Although targeting the CXCR4 in stopping pancreatic tumour metastasis looks promising, CXCR4 inhibitors are found highly toxic and non - specific reaction are the drawbacks that must have to be overcome before translating into clinical trials or practices[147].

Forkhead Box M1 (FoxM1) is a transcription factor found over-expressed in pancreatic cancer in which it promotes the expression of EMT characteristic[141], which is deduced by the increased mesenchymal cell markers expression including, zinc-finger E-box binding homeobox 1 (ZEB1), ZEB2, E-cadherin, and vimentin, and also enhanced tumour sphere formation which marks the strengthened self-renewal ability for CSC[148], as enhanced EMT is having close resemblance to CSC in giving rise to cancer stemness[149]. A natural compound genistein can inhibit the FoxM1 signaling pathway by down-regulating the expression of FoxM1 and its downstream gene targets (*e.g.*, VEGF, MMP-9) leading to reduced EMT and reduced tumour sphere formation and have resulted into reduced tumour growth and enhanced apoptosis[150]. The exact mechanism of the regulation of genistein on FoxM1 and its target genes is not clear yet, however, the application genistein can rescue the microRNA-200 (miR-200) expression by attenuated FoxM1 expression, and enhanced expression of miR-200 can inhibit the EMT progression[147]. Because of the important role for FoxM1 plays in the EMT and CSC progression, and the availability of FoxM1 inhibitor has made FoxM1 an attractive target and should evaluate the anti - tumour effects under co-administration of genistein and gemcitabine.

Cell surface marker on CSC

There are several cell surface markers on CSC which are not only be used to isolate CSC, but also have important functions towards CSC. For instance, expression of CD44^{+}/CD24^{+}/ESA^{+} mark the pancreatic cancer cell that function as CSC, with several signaling pathways (*e.g.*, BMI1, sonic hedgehog) up-regulated and self-renewal and tumorigenesis enhancement are observed[151], while ablated CD133 would lead to loss of CSC self - renewal ability[146]. This demonstrates the markers presented on the CSC can provide some clue on the de-regulated signaling pathways in the tumour which can help deciding the targets of the sub-population of the pancreatic tumour. Nevertheless, a novel CSC marker, c-Met, is found to be essential for tumour growth, tumour sphere formation and metastasis, inhibiting the expression of c-Met have suppressed these tumour malignancies[152], possibly *via* the downstream signaling pathways of c-Met, such as Ras-MAPK, and PI3K-AKT[153]. With the emerging knowl-

edge of the cell surface markers and their downstream signaling, options for targeting signaling transduction in PDAC is ever growing, besides, the surface markers can also act as the reference reflecting the de-regulated signaling pathways, hence, facilitating the therapeutic direction formulation.

TUMOUR MICROENVIRONMENT

Matrix metalloproteinase
MMPs are a group of zinc-dependent endopeptidases which hypothetically can degrade almost all proteins in the extracellular matrix (ECM)[154]. MPPs are over-expressed in pancreatic cancer and the biological roles of MMPs in cancer are to digest the proteins in basement membrane in the ECM which leads to enhanced migration of tumour cell[155], an evidence of migration signal mediation by cleavage of laminin-5 in ECM has been reported[156]. Moreover, cleavage of E-Cadherin by MMP-3, MMP-7[157] and A disintegrin and metalloprotease 10 (ADAM10) are observed, the loss of E-Cadherin not only enhanced cell mobility but also enhanced the tumour invasiveness and migration[158]. In addition, the release of pro-angiogenic inflammatory cytokine (TNF-α)[157] and VEGF[159] are correlated with the MPPs activity and all these confirm the role of MPPs in EMT and tumour metastasis promotion[160].

MMP is found related to the pancreatic stellate cell, which will be described in the next section, the TGF-α up-regulation in pancreatic stellate cell correlates with up-regulated MMP-1, suggesting a possibility of the association of these two molecules overexpression in enhanced tumour cell invasion[161].

As the importance of MMPs in tumour angiogenesis and metastasis is undeniable, studies of formulating MMP inhibitors (MMPIs) are undergoing; a MMPI, SB-3CT is able to reconvert the MMP-2 into its pro-enzyme state and has brought down liver metastasis[162]. However due to the usual late stage discovery of pancreatic cancer in real life[163] the use of MMPIs is limited and also MMPs activities have been observed to be stage dependent[164], therefore MMPs can be targeted for PDAC patients with early detection and can be applied widely when early detection method for PDAC is developed.

Pancreatic stellate cell
Pancreatic stellate cell (PaSC) resides in the exocrine of the pancreas and has dual roles in normal pancreatic tissue[165], first it acts as a storage of vitamin A[166], secondly upon pancreatic injury, the PaSC would be activated to acquire a myo-fibroblast-like phenotype which is called activated PaSC[167-169], activated PaSC will secret proteins into the ECM[170] resulting into pancreatic fibrosis[165] and on setting chronic pancreatitis which could lead to high risk of PDAC development. As mentioned in 10.1, high TGF-α expression level correlates with the high MMP-1 expression level in inducing PaSC migration, inhibition of MMP-1 has showed such migration induction is curbed by using MMP-1 tissue inhibitor and siRNA of

MMP-1[161], indicating PaSC activity can be modulated *via* MMPs.

Other studies by targeting PaSC proliferation and migration have been carried out, transgelin has been reported to be over-expressed in activated PaSC but not in normal acinar cell which could cause pancreatic fibrosis[171]. Moreover, knocking down transgelin expression has reduced cell proliferation and migration abilities in *in vitro* experiment[171], providing a biomarker for specific therapeutic target in knocking down PaSC population in the future.

Hedgehog signaling pathway
The Hedgehog (hh) signaling pathway, involves the secreted signaling molecules hedgehog proteins, which is classified into 3 subcategories, namely, Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh)[172]. Among these 3 hh, Shh is found over-expressed in 70% of primary PDAC[173]. Hedgehog biological roles have been described as an essential factor in embryonic development and regulate cell proliferation[172].

The mediation of hedgehog signaling is triggered upon the binding of Shh to the Patched 12-transmembrane domain receptor (Ptch) which further activates another transmembrane signal transducer, smoothened (Smo), that would lead to localization of transcription factors in the nucleus and initiate transcription of downstream effectors[174], for instance, Cyclin D2, FoxM1, jagged 2 (JAG2), *etc*[175].

It is reported that tumourigenesis and tumour proliferation requires constitutive activated hedgehog signaling, and in pancreatic stromal cell in PDAC, Smo is over-expressed and direct tumour cell growth in the vicinity of stromal cell, leading to a therapy targeting hedgehog signaling in tumour-stromal interaction[172]. Besides, report of Shh activation in CSC is crucial for CSC proliferation[176], and it has been discussed for the CSC in aggravating pancreatic cancer treatment, such as heightened drug resistance and tumour relapse. Therefore, studying hedgehog signaling inhibitors is beneficial for pancreatic cancer treatments. A hh signaling inhibitor, Sulforaphane has been found to inhibit self-renewal capacity in CSC *via* Shh signaling inhibition leading to downstream effectors *e.g.*, Nanog and Oct-4 suppression[176]. Moreover, inhibition of hedgehog in pancreatic cancer cells and tissue are performed and it is found that a marked decrease in EMT with EMT related transcription factors (Snail and Slug) down - regulation and had suppressed PI3K/AKT signaling, which is downstream of hedgehog signaling with an association of decreased cell proliferation[177].

Because of diversified roles of hedgehog signaling in tumour malignancies and CSC population maintenance, and the cross talk among other signaling pathways, *e.g.* FoxM1, Notch (*via* JAG2), targeting hedgehog may have a centralized effect in weakening the malignancies of pancreatic tumour.

Stromal environment-hyaluronan
The microenvironment of pancreatic cancer, has been

accused to be the major challenge in drug delivery because of its highly dense ECM, the penetration of even small drug molecules gemcitabine is prevented[178]. Besides, stromal cells which are the activated fibroblasts and PaSC, inflammatory cells[178]; and distorted vascular structure of blood and lymphatic vessel composed the stromal environment[179]. And the production of stroma is mediated by various factors involved in numerous signaling pathways in an autocrine and paracrine action, TGF-β, insulin growth factor 1, and EGF are the examples[180].

Among the molecules in ECM, hyaluronan or hyaluronic acid (HA) is secreted by PDAC[181], and is a repeat of N-acetylglucosamin/glucuronic acid disaccharide[170]. It is able to interact with a hyaladherin, CD44, to regulate tyrosine kinase receptor and to facilitate angiogenesis, EMT, and chemoresistance[182]. It is also one of the main components that contributes to high intra-tumoral fluidic pressure (IFP) through solvating with water molecule, hence, impeded the diffusion of drug molecules into the target tumour cell[183]. It is found that co-administration of hyaluronidase with gemcitabine or other drugs prolonged the localization of the accompanied drug in the tumour[183]. Therefore, it would become a trend for future development of drug target, *e.g.*, well incorporated with hyaluronidase to facilitate drug delivery, or even HA can be a target to disintegrate the condensed ECM, enhancing the responsiveness of the tumour cell to the treatment.

CONCLUSION

A range of therapeutic targets in PDAC have been briefly described in this article, in which their anti - tumour and oncogenic activities are characterized through various experiments and can be taken as potential target for PDAC therapies development.

Nevertheless, numerous of the targets are found overlapped with each other in producing certain kinds of tumour malignancies, *e.g.*, over-expression of CXCR4, Rac1, BMI1, and *etc.*, in pancreatic tumour cell have observed a metastasis enhancement. In light of this, and hypothetically, in order to prevent metastasis, suppressing these targets should have a more pronounced effect in metastasis inhibition. Moreover, such outflanked approach may also prevent the tumour cell from switching into other signaling pathways producing the same tumour malignancies, and achieving elimination ultimately. Besides, the current knowledge of each of these targets is insufficient, categorizing these targets by the tumour malignancies produced and identify if there is any relationship between them and understand the mechanism behind, would allow the discovery of linkages among them in terms of proteins and mRNA expression levels and mechanistic studies.

Last but not least, screening of suitable inhibitors for these targets is crucial in putting these targets into practice. Toxicity of some of the inhibitors mentioned is reported, while, traditional Chinese medicine (TCM) may be a good source for screening inhibitors that are less or non-toxic compounds, *e.g.*, an EZH2 inhibitor, davidian, is extracted from TCM *Polygonum capitatum* without toxicity observed in xenograft model[184].

Single effort from one side is far from enough in pancreatic tumour elimination due to its high malignancy and complex tumour microenvironment, multiple targets have to be considered in developing PDAC therapies, therefore, the way of applying these targets and which targets should be applied require further effort.

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