Clinics in Oncology

Review Article
Published: 16 May, 2017

Current Clinical Challenges and Emerging Multi-Modal Approaches for the Treatment of Pancreatic Cancer

Fiona Haxho[1#], Manpreet Sambi[1#], Bessi Qorri[1#], William Harless[2] and Myron R. Szewczuk[1]**

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

[2]ENCYT Technologies, Inc. Membertou, NS, Canada

[#]These authors have contributed equally

Abstract

Today, emerging therapies must effectively shut down multiple enabling characteristics that drive pancreatic cancer invasion and progression. These therapies include the concomitant suppression of growth factor signaling and anti-apoptotic pathways, immune-derived promoters of tumorigenesis, mechanisms of acquired drug resistance, as well as pro-metastatic signals that facilitate cancer cell migration and successful homing of disseminated tumor cells. Here, we provide an overview of some of the current treatment options available for patients with pancreatic cancer, as well as their limitations. Finally, we review some alternative multi-target strategies that may provide increased efficacy in cancer therapy.

Keywords: Pancreatic cancer; PDAC; Cancer therapy; Drug resistance; Cancer stem cells; Alternative therapy

OPEN ACCESS

Correspondence:
Myron R Szewczuk, Department of Biomedical and Molecular Sciences, Queen's University, Canada, Fax: +1 613 533 6796; Tel: +1 613 533 2457; E-mail: szewczuk@queensu.ca
William Harless, MD, PhD, ENCYT Technologies, Inc. Membertou, NS B1S 0H1, Canada, Tel +1 902 539 3365; Fax +1 902 539 3363; E-mail: williamharless@ymail.com

Received Date: *13 Mar 2017*
Accepted Date: *13 Apr 2017*
Published Date: *16 May 2017*

Citation:
Haxho F, Sambi M, Qorri B, Harless W, Szewczuk MR. Current Clinical Challenges and Emerging Multi-Modal Approaches for the Treatment of Pancreatic Cancer. Clin Oncol. 2017; 2: 1296.

Introduction

Despite major advancements in cancer treatment, survival rates for patients with pancreatic cancer have shown minimal improvement over the last forty years [1]. Pancreatic ductal adenocarcinoma (PDAC) accounts for the majority of pancreatic cancers and is one of the most lethal malignancies worldwide [2]. The difficulty in diagnosing PDAC at early stages further contributes to low survival rates. Located in the retro-peritoneum of patients who present with non-specific symptoms, PDAC is not diagnosed until it has reached an advanced clinical stage in over 80% of patients [3], with only a 5% five year survival rate [4]. Furthermore, lack of effective screening and early biomarker detection has prevented clinicians from identifying this cancer in a pre-malignant stage.

Once diagnosed, a number of different interventions are used to treat disease progression, including surgical resection, neoadjuvant and adjuvant chemotherapy and radiation. Unfortunately, the inherent and acquired heterogeneity of primary tumors and secondary metastases renders them highly malignant and gradually resistant to the majority of such therapies. Today, it is understood that in order to overcome these treatment challenges, emerging therapies must effectively shut down multiple enabling characteristics that drive pancreatic cancer invasion and progression. These include the concomitant suppression of growth factor signaling and anti-apoptotic pathways, immune-derived promoters of tumorigenesis, mechanisms of acquired drug resistance, as well as pro-metastatic signals that facilitate cancer cell migration and successful homing of disseminated tumor cells. Here, we provide an overview of some of the current treatment options available for patients with pancreatic cancer, as well as their limitations. Finally, we review some alternative multi-target strategies that may provide increased efficacy in cancer therapy.

Current Treatment Strategies and Limitations

Although surgery offers hope for curative therapy, less than 20% of patients present with potentially operable tumors [5]. A number of poor predictors for successful resection has been identified, including lymph node involvement [6], high tumor grade [7], large tumor size [8], elevated CA 19-9 levels [9], and positive tumor margins post-surgery [5]. Unfortunately, surgical patients with pancreatic cancer remain at a high risk for relapse. On average, surgery has been shown to prolong patient survival by only 10 months [10]. Patients with advanced disease do not meet the criteria for surgery as their cancer can demonstrate distant metastasis, pancreatic lymph node involvement, encasement or occlusion of the superior mesenteric vein (SMV) or SMV/portal

vein confluence and/or involvement of the celiac axis, aorta, inferior vena cava or superior mesenteric artery [5]. As a result, radiation and chemotherapy is recommended for these patients.

Radiation therapy is used to eliminate rapidly dividing cells located in a specific area of the body. It can be delivered as high-energy rays or as a radioactive agent and is able to selectively induce DNA damage in proliferating tumor cells. Neoadjuvant radiation therapy may be used prior to surgery to shrink an operable tumor, or used after surgery (adjuvant radiation) to treat residual disease. However, the efficacy of radiation therapy is suboptimal due to its limited tolerance in normal tissue.

Neoadjuvant chemotherapy is used as an induction treatment to shrink a tumor before the primary treatment which is usually surgery. Pre-operative chemotherapy with radiation has also been shown to improve survival, but not the cancer stage of locally invasive tumors [11]. Post-operative chemotherapy and/or chemo-radiation are often incorporated in the therapeutic regimen. Indeed, adjuvant chemo-radiation has become the most frequently used adjuvant treatment for resectable pancreatic cancer in the United States [5]. Since 1997, gemcitabine (20,20-difluoro-20-deoxycytidine or dFdC) has been the first-line therapy for patients with PDAC [12]. This is due to its lower toxicity when compared to other chemotherapeutic agents and increased progression-free survival. Gemcitabine is a nucleoside pyrimidine analog with multiple modes of action inside cancer cells, the most important being the inhibition of DNA synthesis [13]. When gemcitabine triphosphate (dFdCTP) is incorporated into DNA, only a single deoxynucleotide can be incorporated afterwards, ultimately preventing chain elongation [14]. The induction of apoptosis through caspase signalling is another important mechanism of action, in which gemcitabine activates p38 mitogen-activated protein kinase (MAPK) to trigger apoptosis in response to cellular stress in tumor cells, but not in normal cells [15-18]. Indeed, activity of MAPK-activated protein kinase (MK2), a p38-MAPK effector, was shown to be required for gemcitabine-induced cell death *in vitro*. However, in these patients, gemcitabine treatment has been shown to prolong the average survival rate by only 4 months and is primarily used in palliative care. In phase II and III studies, gemcitabine has also been administered in combination with platinum analogues, including cisplatin [19,20] and oxaliplatin [21]. Other effective chemotherapy agents used today in the neoadjuvant and adjuvant setting include folfirinox (5-fluorouracil, leucovorin, irinotecan, oxaliplatin), or a combination of gemcitabine with nanoparticle-bound paclitaxel (abraxane). Paclitaxel is a chemotherapeutic agent which interacts with microtubules, specifically the β-subunit on tubulin to prevent the formation of functional mitotic spindles during cell division [22]. In one phase III trial, combined treatment with gemcitabine and paclitaxel demonstrated significantly improved patient outcomes, when compared to treatment with either agent alone [23].

These regimens can demonstrate minor tumor shrinkage in 20-30% of patients and can slow the progression of the disease for approximately six months in patients with metastatic cancer [24]. Developments of other complementary agents to enhance chemotherapeutic effects are currently under review in pre-clinical and clinical trials [5]. Conceptually, any treatments that are better able to shrink primary tumors from borderline operable to potentially curative, or to eradicate remaining micrometastatic disease after surgery, would represent a huge advancement in our ability to treat pancreatic cancer. Such treatments would also be predicted to improve progression-free survival in patients with metastatic disease, most of whom will die within one year of diagnosis.

Ongoing Challenges for Drug Development

Acquired drug resistance

One of the greatest difficulties in curing metastatic disease is the inability to prevent or reverse the acquired resistance to drug therapy. The high frequency of acquired chemoresistance in PDAC has been linked to an accumulation of highly penetrant genetic mutations at various loci, including K-ras, p53, cdkn2a and smad4/DPC4 [25]. Originating in the ductal epithelium, pancreatic cancer can quickly evolve from a pre-malignant lesion to an aggressive and invasive metastatic disease [25]. 90% of PDACs have point mutations within the KRAS2 oncogene, resulting in constitutive expression of Ras [26]. These genetic alterations can sustain the malignant phenotype because once activated, Ras initiates a signal transduction cascade that activates proliferation and cell survival pathways and increases cancer cell invasion [27]. These point mutations are of clinical interest because they may result in the expression of pancreatic tumor-specific neo-antigens, capable of being recognized by helper and cytotoxic T lymphocytes, and may therefore represent novel vaccine targets [27].

In contrast to the constitutive expression of the KRAS2 oncogene, the p53 tumor suppressor gene is inactivated in approximately 80% of pancreatic tumors [28]. As a result, there is impaired DNA damage recognition and repair in pancreatic epithelial cells, impaired apoptosis and deregulated cell cycle control [29]. Two other tumor suppressor genes, p16Ink4a and p15ARF are encoded by the cdkn2a locus. Inactivation mutations in these genes are present in about 90% of human pancreatic cancers [30], and are implicated in the drug-resistant mechanisms of the cancer. The number and combination of these mutations correlates with patient prognosis, such that patients with 3-4 mutations will have a poorer diagnosis than those with only 1-2 mutations [30].

The upregulated or downregulated activities of specific drug transporters also play crucial roles in the efficacy of chemotherapy. For example, human equilibrative nucleoside transporter-1 (hENT1) is a membrane facilitative transporter responsible for the direct entry of gemcitabine into cancer cells [31]. Gemcitabine is a hydrophilic drug, and its rate of cellular entry through the hydrophobic plasma membrane is negligible without hENT1 transporter activity. Indeed, a lower expression level of hENT1 has been correlated with gemcitabine resistance [31]. As major drivers of drug resistance, these genetic and cellular components represent important targets for drug development as well as patient-specific predictors of treatment response.

Pancreatic tumor heterogeneity and cancer stem cells

In an effort to improve personalized cancer therapy, studies have begun to elucidate the genetic heterogeneity among pancreatic cancer patients. Tumor heterogeneity, a concept proposed over 30 years ago, refers to the presence of multiple subpopulations within a single neoplasm each of which are postulated to originate from a unique lineage [32]. These lineages may differentiate subpopulations by their ability to metastasize, self-renew, proliferate and acquire chemoresistance, among other processes observed in tumorigenesis [32]. As described above, current cytotoxic therapies for PDAC are designed with the intention of arresting cell proliferation and target

processes in cell division such as DNA synthesis [12]. Although single agent and combination therapies show some efficacy in increasing patient survivorship, cancerous lesions continue to be treated as homogenous growths. As a result, almost no treatment options have been developed to target this intratumoral heterogeneity.

The concept of clonal expansion and subsequent development of unique subpopulations within tumors was first described by Peter Nowell [33], and its clinical implications have been extensively described for over four decades [34,35]. In order to effectively cure PDAC, emerging therapies must target the variation in tumor cell composition and their unique susceptibility to different anti-cancer agents [36]. This variation can include differences at the genetic level (i.e. different mutations that subpopulations may possess) and/or at the phenotypic level (i.e. the unique cell surface protein targets on a subpopulation of cells that can be therapeutically exploited). Therefore, PDAC must be conceptualized as a dynamic growth that is influenced by the surrounding microenvironment and is made up of a diverse population of cells. Two different subpopulations can co-exist or they can be separated by a physical barrier, such as blood vessels, or by a difference in their microenvironment. Both of these factors may generate differences in how these subpopulations respond to therapy and difficulties arise in detecting these mosaic phenotypes in heterogeneous tumors, further complicating the development of a personalized treatment regimen [37]. For example, inflammatory cells have been shown to secrete both pro-angiogenic and anti-angiogenic cytokines [38]. Therefore, the tumor cells that are able to respond to pro-angiogenic cytokines, as opposed to those that do not, would be able to promote tumor neovascularization and comprise a distinct subpopulation within the tumor. This has been shown in bladder cancer, in which a subpopulation of cells with increased expression of CD14, a glycoprotein involved in the signaling pathways of Toll-like receptors (TLRs) were able to facilitate neovascularization of bladder tumors by recruiting endothelial cells with greater efficiency [39]. Interestingly, cells that expressed low levels of CD14 had greater proliferative capacity and represented another distinct subpopulation. Notably, both of these cell populations were postulated to behave synergistically in promoting overall tumor growth [39]. This suggests that targeting one particular cancer cell subpopulation might not be effective as other surrounding subpopulations can compensate to facilitate their regrowth. Moreover, one study performed a comprehensive genome assessment on 24 different pancreatic cancers [40]. Results revealed an average of 63 genetic mutations per cancer, spanning 12 separate signal transduction pathways. This study supports the notion of pancreatic cancer being a genetically heterogeneous malignancy, partially accounting for its notable resistance to therapy as well as varied responses to treatment. Moreover, this finding likely explains why no candidate gene has yet been identified. This cancer cell heterogeneity will likely dictate an individualized, unique approach for each particular case.

Another major obstacle in the treatment of pancreatic cancer is the selective targeting and killing of cancer stem-like cells (CSCs). Tumor initiating populations, or CSCs, have been identified in a number of cancers, all of which began with the seminal discovery of these tumor progenitor cells in leukemia [41], followed by verification of these populations in breast [42], brain [43] and pancreatic cancer [44]. In PDAC, tumor initiating cells (TICs) are characterized as being $CD44^+/CD24^+/ESA^+$ [44]. 0.2-0.8% of pancreatic cancer cells possess this unique phenotype and have the capacity to re-establish progeny with a nearly identical phenotype when compared with the primary tumor [44]. Not only do these cells possess unlimited self-renewal potential, they are also capable of giving rise to differentiated progeny [44]. The identification and targeting of this subpopulation is of particular clinical importance as these cells are also resistant to chemotherapy [45].

Hypoxia is another important hallmark of tumor growth and can select for a unique subset of cancer cells that are capable of survival in an oxygen-depleted environment, including CSCs [46]. These cells can upregulate their expression of cytokeratin 19 (an epithelial stem cell marker for breast cancer) [47] and/or CD34 (a hematopoietic stem cell marker expressed in leukemic cancer). Unless these cells are activated to proliferate, conventional chemotherapeutic drugs will not affect TIC expansion. Thus, while it may appear that a tumor is shrinking, in reality chemotherapeutic drugs are targeting the differentiated cell population within the tumor allowing for the tumor to regenerate itself and potentially metastasize and home to distant organs [48]. Recent findings demonstrate that these cells are capable of epithelial-to-mesenchymal transition (EMT), contributing to their motility and metastatic behavior due to phenotypic changes [49]. Current therapies have already been designed to target CSC-specific antigens in order to inhibit their roles in cell survival, adhesion, self-renewal and invasion.

A greater understanding of individual CSC populations and how they interact with one another will advance progress in the treatment of pancreatic cancer. Therapeutic targets of pancreatic CSCs include genes located in developmental pathways such as hedgehog, Wnt, Notch, CXCR4 and Met. In addition, targeting apoptotic pathways such as DR5 and nodal-activin may also provide significant therapeutic benefit [44,49].

Desmoplasia, the tumor microenvironment and immune-regulated tumorigenesis

Paracrine signals from pancreatic cancer cells stimulate the extracellular proliferation of leukocytes, fibroblasts, endothelial cells, neuronal cells, collagen type I and hyaluron. This extracellular proliferation of cells is known as a desmoplastic reaction forming a thick stromal environment around the pancreatic cancer cells [50], providing a mechanical barrier to the tumor cells and also thought to contribute to the anti-angiogenic environment that is characteristic of PDAC. Both properties directly affect therapeutic efficacy as the dense microenvironment limits drug delivery to the primary tumor. Furthermore, the increased deposition of collagen and fibronectin results in decreased elasticity of tumor tissue accompanied with an increase in tumor interstitial fluid pressure (IFP). Increased IFP results in a lower perfusion rate of therapeutic agents, ultimately diminishing their efficacy [51]. Studies have demonstrated that the signals that influence the proliferation of the desmoplastic reaction originate from the K-ras mutant oncogene in the epithelium of the tumor [52]. Sonic hedgehog (SHH) functions similarly to the K-ras mutant. Although it is overexpressed in pancreatic cancer cells during the early stages of their development, SHH does not act on the SHH pathway in these cells [52]. Instead, it acts in a paracrine fashion in extracellular fibroblasts, resulting in their growth and differentiation. The key players in the formation and turnover of this dense stroma are pancreatic stellate cells. Certain growth factors, including TGF-β1, PDGF and FGF, are able to activate these cells into myofibroblasts which can then secrete components of the extracellular matrix to further reduce the vascularization of the primary PDAC tumor [53].

In addition to forming a mechanical barrier around the pancreatic cancer cells, the stroma has an important role in tumor formation, progression and metastasis [54]. Many proteins expressed by stromal cells have been directly correlated with poor prognosis and resistance to current therapies, including COX-2, PDGF receptor, VEGF, stromal-derived factor, chemokines, integrins, secreted protein acidic and rich in cysteine (SPARC) and SHH elements.

Notably, the dense stroma is characterized by a tumor-promoting immunosuppressive environment. Using a CD40 antibody combined with gemcitabine chemotherapy, researchers have attempted to reverse immune suppression and drive anti-tumor T cell responses in patients with non-resectable pancreatic cancer. Studies have shown that this dual combination results in tumor regression by stimulating tumor-associated macrophages (TAMs) to attack and deplete the pancreatic cancer stroma [55]. To date, treatment of PDAC has proved most effective in patients with locally advanced disease, especially in patients with tumors characterized by wild-type tumor suppressor Smad4 (DPC4). These tumors are known to be less prone to metastasis and possess higher stromal content. However, primary tumors that have already metastasized cannot be effectively treated with current stromal-targeting agents. This is due to the fact that although PDAC has a rich and hypovascularized stroma, metastases arising from this cancer do not, making them more similar to other highly vascular tumors [56]. Other studies have also suggested a role for the tumor stroma in the T cell-depleted microenvironment of pancreatic cancers [57]. Several cell types found in the desmoplastic reaction are involved in localized tumorigenesis, including TAMs, cancer associated fibroblasts, regulatory T-cells (Tregs) and myeloid derived suppressor cells. In addition, K-ras dependent signaling molecules have been shown to upregulate granulocyte-macrophage colony stimulating factor (GM-CSF) when activated, thus promoting the maturation of immature myeloid progenitor cells into myeloid derived suppressor cells.

Unlike the immunosuppressive nature of the stroma, the primary pancreatic tumor and/or distant micrometastases can be exposed to a highly inflammatory microenvironment. Tumor-derived pro-inflammatory signalling can contribute to chemoresistance , selection of cancer stem-like cells and the desmoplastic reaction. Nuclear factor kappa B (NF-κB) signaling, critical for the inducible expression of cellular and viral genes involved in inflammation, has been found to be constitutively activated in pancreatic cancer. Tumor-derived inflammation is also associated with cyclooxygenase (COX) activity. COX-1 is constitutively expressed in most tissues, while COX-2, the inducible form, is not normally expressed, but upregulated by cytokines, growth factors, and tumor-promoter genes. COX-2 has been found to be upregulated in PDAC, localized to the cytoplasm of the tumor cells and not in the surrounding stromal or inflammatory cells [58]. COX-2 is one of the downstream target genes of NF-κB, and is involved in mechanisms such as prostaglandin synthesis, promotion of angiogenesis, inhibition of immune surveillance and inhibition of apoptosis.

Cancer-Associated Hypercoagulation and Angiogenesis

Thromboembolic disease is a common complication and can be the presenting feature of pancreatic cancer, usually associated with a poorer prognosis [59]. Pancreatic cancer cells activate platelets and express several pro-coagulant factors, including tissue factor and thrombin [59]. Tissue factor, a transmembrane-receptor protein that initiates the extrinsic pathway of coagulation, can promote an angiogenic phenotype by upregulation of vascular endothelial growth factor (VEGF) and downregulation of the angiogenic inhibitor thrombospondin [60,61]. Tissue factor also seems to control angiogenic tumor signals through the production of growth-regulatory molecules from the endothelium [61]. Furthermore, mutated or activated KRAS2 (found in 95% of pancreatic cancers) can directly or indirectly affect angiogenesis (through increased VEGF expression), thrombosis (through increased expression of urokinase plasminogen activator), and metastasis (through increased expression of matrix metalloproteinases) [62]. Thrombin is another key enzyme involved in coagulation, and can convert circulating fibrinogen to fibrin, activate platelets, and amplify initial signals in the coagulation cascade. Thrombin generates a fibrin scaffold that attracts endothelial cells, activates various protease-activated receptors on endothelial cells, increases expression of VEGF receptors, and activates hypoxia inducible factor 1α (HIF-1α), leading to the production of several angiogenic molecules [63]. Functional thrombin receptors have been identified in human pancreatic cancer cells [64,65], but not in healthy pancreatic cells [66]. Thrombin enhances adhesion of PDAC cells to ECM proteins and to endothelial cells, suggesting an important role for tumor growth and invasion [67]. Fibrin, the end product of the coagulation cascade, also plays an important role in the prothrombotic and proangiogenic state of cancer, especially in pancreatic cancer. The fibrin matrix functions as a scaffold and as a reservoir for proangiogenic growth factors such as heparin binding growth factor-2 and VEGF. It enhances the activity of heparin binding growth factor-2 [68], and sequesters several growth factors from proteolytic degradation [69].

The activation of coagulation is not simply a phenomenon, but has also been shown to enhance tumor growth, angiogenesis and metastasis. Treatment options include warfarin and low-molecular-weight heparins (LMWH); however, studies over the past decade indicate that the use of LMWH in the prevention of venous thromboembolic disease improves outcomes for cancer patients, in comparison with warfarin and other anticoagulants [59]. A review by Khorana and Fine summarizes the promising clinical studies employing anti-coagulant therapy in cancer [59]. Strong preclinical data suggest that heparin, or LMWH, offer advantages over warfarin in terms of efficacy of anti-coagulation, as well as anti-cancer effects (including inhibition of angiogenesis). Emerging prospective clinical data support this finding by showing improved outcomes with protracted use of LMWH [59].

A Multi-Modal Approach To Optimizing Treatment

Suppression of cancer cell metabolism and growth

In addition to the use of proliferation-targeted interventions such as chemotherapy and radiation, major metabolic pathways in cancer cells may also be exploited. This may include: 1) the disabled/reduced supply of glucose and glutamine to the tumor; 2) interruption of the mechanisms that enable survival in a hypoxic environment [70]; and/or 3) prevention of the cancer cell's ability to digest intracellular organelles for energy [71]. Since aberrant metabolic pathways have become a hallmark of cancer, investigators have identified several key metabolic enzymes to target, including hexokinase, pyruvate kinase, lactate dehydrogenase A (LDHA) and ampicillin-activated proteinkinase (AMPK). Several pre-clinical trials have demonstrated the anti-tumor effects of agents directed against these enzymes. Two

of these drugs, rapamycin and metformin, have shown promising results when used alone, or in combination with other anti-cancer therapies. Rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), is able to decrease glucose uptake by reducing levels of Glut1 in pancreatic cancer [72]. Metformin, an oral hypoglycemic agent for the treatment of type 2 diabetes, has a glucose-lowering effect and is able to reduce hyperinsulinemia by improving insulin sensitivity in peripheral tissues [73,74]. Metformin reduces gluconeogenesis in the liver, an effect mediated by AMP-activated serine-threonine protein kinase (AMPK). AMPK is an intracellular sensor of energy and nutrient levels, and is a regulator of cell ATP and lipid, cholesterol and glucose metabolism and homeostasis [75,76]. Recent studies have shown that diabetic patients treated with metformin have a lower incidence of cancer. The phosphorylation of the AMPK catalytic subunit is regulated by liver kinase B1 (LKB1). Notably, LKB1 is the protein product of a corresponding tumor suppressor gene. The activation of the LKB1–AMPK pathway inhibits the mammalian target of rapamycin complex-1 (mTORC1), a kinase activated in the majority of human cancers [77]. In prostate cancer cell lines, metformin demonstrated an anti-proliferative effect via the induction of a p53 target gene (REDD1) [78]. Notably, metformin was also found to play a role in NF-κB signaling, a pro-inflammatory pathway implicated in the enhanced proliferation, anti-apoptotic mechanisms, and invasiveness of cancer cells, as well as in the immune surveillance of tumors [79]. Hattori and colleagues showed that metformin blocked NF-κB activation induced by TNF-α in vascular endothelial cells [80]. In smooth muscle cells, metformin was found to suppress the phosphorylation of key signaling molecules involved in NF-κB activity, including p38, JNK, Erk and Akt [81]. Metformin-mediated inhibition of NF-κB activity in mouse pancreatic tumors was also found to downregulate the mRNA expression of MCP-1, TGF-β1, TNF-α, and IL-1β, each of which play unique roles in tumor development [82]. Metformin has also been shown to inhibit the TNF-α-induced secretion of CXCL8, a chemokine with well-established pro-tumorigenic actions [83].

A number of other studies have demonstrated an anti-cancer effect of metformin on the cell cycle, apoptosis and glioblastoma [86], colon [87], ovary [88], pancreas [89], lung [90], and prostate tumors [91]. Metformin also seems to have an affect on CSCs. Bao et al. showed that metformin attenuates CSC phenotypes, functions and mediators [92]. The drug reduces the expansions of CSC clones by inducing apoptosis and by inhibiting CSC mediators and markers. Other lines of research suggest that metformin regulates the EMT status, an essential differentiation program in early embryonic development that is modified in cancer to mediate acquisition of malignant and stem-like cell properties [93]. Metformin decreases the expression of key drivers of EMT including the transcription factors ZEB1, TWIST1 and SNAI2 (Slug), and the pleiotropic cytokines TGFβs in several cell types [94]. The inhibition of these components of EMT by metformin causes an inhibition of cell invasiveness without affecting cell migration [95]. Metformin was also shown to reduce the expression of miR-34a and its direct EMT targets Notch, Slug, and Snail [96].

Targeting Tumor-Associated Inflammation and Apoptosis-Resistant Cancer Cells

Due to the inflammatory nature of the disease, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin have been proposed to provide therapeutic effects to manage inflammation and sensitize malignant cells to chemotherapy [97]. NSAIDs such as aspirin inhibit NF-κB activation by binding to IKK-2. Inhibition of NF-κB in PANC-1 cells by aspirin was found to be dose-dependent. Notably, MTT assays on PANC-1 cells treated with 18 mM aspirin resulted in no significant inhibition in the growth of cells [98]. A different study also investigated the effect of aspirin on the proliferation of four pancreatic cancer cell lines. It found a negative correlation between the intensity of COX-2 expression and the IC_{50} of aspirin [99]. A dose-dependent growth inhibition was seen across all cell lines following 72 hours of aspirin treatment. However, cell lines with low COX-2 expression (KP-2 and PNS-1) demonstrated a significantly lower IC_{50} than those with high COX-2 expression (MiaPaca-2 and PANC-1).

Anti-inflammatory agents have also been proposed to counteract acquired resistance to drug therapy. One study used four chemoresistant PDAC cell lines and treated the cells with gemcitabine, different concentrations of aspirin, or a combination of these drugs [97]. Aspirin was found to significantly induce apoptosis *in vitro* and reduce the viability, self-renewal potential, expression of inflammatory mediators and CSC signaling. Specifically, treatment with aspirin for 48 hours decreased the expression of TNF-α and downregulated the self-renewal stem cell markers Oct4, Nanog and SOX2. Aspirin also blocked the growth and invasion of orthotopic pancreatic tumor xenografts and significantly prolonged the survival of mice when co-administered with gemcitabine. Furthermore, aspirin treatment resulted in decreased inflammation and desmoplasia *in vivo,* as well as reduced expression of tumor progression markers Ki67, c-Met, CSCR4, CD44 and TNF-α [97]. Due to its well-established safety profile, as well as its promising application in pre-clinical cancer studies, aspirin represents a novel and well-tolerated chemo-sensitizing agent for the treatment of pancreatic cancer.

Celecoxib is another COX-2 inhibitor that has demonstrated potent anti-tumor activity in a wide variety of tumor types, including prostate [100], colorectal [101], breast [102], and non-small cell lung cancers [103]. Today, several pre-clinical trials are assessing the use of celecoxib in the prevention and treatment of pancreatic, breast, ovarian, non-small cell lung cancer and other advanced human epithelial cancers [104]. Among the COXIB-family members, celecoxib has the unique capacity to induce apoptotic cell death in tumor and endothelial cells. Although inhibition of COX-2 can contribute to its cytotoxic effects, celecoxib is a prototype of drugs that induce cell death independently from COX-2 mainly by activation of an intrinsic, mitochondria-dependent apoptosis pathway [104]. COX-2-independent drug targets include the survival kinase Akt, the Ca^{2+} ATPase SERCA, GSK-3b/b-catenin, and anti-apoptotic proteins of the IAP and the Bcl-2 families [104]. Studies have also shown that celecoxib can significantly trigger cell death in Bcl-2 overexpressing cells and downregulate the anti-apoptotic factors Mcl-1 and survivin [104]. Thus, the pro-apoptotic activity of celecoxib differs from that of standard chemo-radiation and provides promising evidence for the use of celecoxib in apoptosis-resistant tumors. Furthermore, neoplastic disease that depends on Bcl-2, Mcl-1 or survivin for cell survival seems to be an ideal target for the use of celecoxib alone or in combination with chemotherapy, radiation or other anti-cancer agents.

Blocking Upregulated Receptor Signaling via Glycan Modification

The abnormal expression of cell surface glycosylation has become a key hallmark of cancer and provides a new dimension for targeting

tumor cells. Specifically, the terminal sialylation of several receptors that are upregulated or constitutively active in cancer cells is known to regulate their structure, ligand affinities, as well as downstream signaling cascades. The sialidase activity of neuraminidase-1 (Neu1) has been previously shown to regulate the activation of EGFR, insulin receptor (IR), and a number of TLRs [105]. Notably, these receptors each play unique and profound roles in tumor development via promotion of cell proliferation and survival pathways, cell growth and metabolism, and immune-mediated tumorigenesis, respectively [106]. It is important to note that the regulatory role of Neu1 is dependent upon a cell-surface signaling platform that induces neuromedin B G protein-coupled receptor (NMBR) activation and MMP9 activity. Abdulkhalek et al. [105], have reported an extensive review describing this cell-surface molecular mechanism and its role in regulating receptor activation and downstream signaling.

We have previously shown that Neu1 inhibitor oseltamivir phosphate (OP) demonstrates potent anti-cancer effects *in vitro* and in mouse models of pancreatic [107-109], breast [110], and ovarian cancer [111]. Briefly, Neu1 inhibition by OP is able to suppress oncogenic downstream cellular pathways that are associated with EGF and insulin signaling [109,112], as well as TLR-mediated pro-inflammatory signaling [113-115]. We have previously reported that OP treatment in mice bearing PANC-1 and MiaPaCa-2 tumor xenografts significantly improved animal health and survival, decreased tumor volume and angiogenesis, and prevented metastasis to the liver and lungs [107-109]. Tumor neovascularization was significantly decreased in OP-treated mice, as indicated by H&E analysis and immunostaining for tumor CD31 (murine endothelial marker). It is proposed that the ability of OP to downregulate a number of signaling pathways simultaneously may be responsible for its broad therapeutic effect.

Gilmour et al. [109] found that OP could directly target Neu1 desialylation of EGFR, and prevent receptor activation and subsequent auto-phosphorylation *in vitro*. In mouse models, immunohistochemistry and western blot analysis showed that OP-treated mice expressed significantly decreased phospho-EGFR levels from intact pancreatic tumor xenografts and lysed tumor samples, when compared to the untreated cohort. One study by O'Shea et al. [108], analyzed the effect of OP on chemoresistant pancreatic cancer cell lines. They found that these cells, although resistant to gemcitabine, cisplatin, tamoxifen and other chemotherapy agents, were sensitive to OP treatment which resulted in reduced cell proliferation and viability. Other markers of tumor progression were also analyzed in these studies, including the relative levels of cell adhesion molecules, E- and N-cadherin. Normally, more malignant cancer cell phenotypes will display an increase in their surface expression of N-cadherin and a corresponding decrease in relative E-cadherin. This expression paradigm will later dictate EMT pathways that facilitate cancer cell motility and metastasis. Like most membrane glycoproteins, cadherins are terminally sialylated and may act as Neu1 substrates. O'Shea et al. [108], showed that OP-treatment of parental and chemoresistant pancreatic cancer cells was able to modulate the expression levels of E- *vs.* N-cadherin, such that OP-treated cells demonstrated relatively higher expression levels of E-cadherin and reduced expression of N-cadherin, when compared to the respective expression levels of untreated cells. These findings suggest that OP may be exerting its effects by targeting the glycan modification of adhesion molecules that play critical roles in cancer cell migration and invasion. This is consistent with the well-established positive regulatory role of Neu1 on the structure and function of cell surface integrins, other major cell surface recognition and adhesion molecules [116]. Future studies should build upon these promising findings and aim to improve OP-based protocols for the treatment of pancreatic cancer.

Conclusion

Several clinical challenges remain in the treatment of pancreatic cancer. In addition to its late detection, there have been no significant advancements in patient outcomes and drug development. Today, it is understood that in order to face these challenges, future studies must not rely on targeting a single oncogenic pathway, but must suppress the multiple enabling hallmark capabilities of pancreatic tumor cells. This is due to the fact that the cancer cell program is adaptive and invasive, such that more aggressive phenotypes will survive and metastasize, despite therapeutic intervention. Future studies should investigate the potential of multi-modal regimens that can suppress tumor growth and malignancy, immune-regulated tumorigenesis, stromal-derived promoters of tumor progression and desmoplasia, as well as the genetic and cellular components that drive drug resistance.

Acknowledgment

This work was supported F Haxho was the recipient of the Queen's Graduate Award (QGA), the Graduate Entrance Tuition Award (GETA), the Natural Sciences and Engineering Research Council of Canada (NSERC) Alexander Graham Bell Canada Graduate Scholarship-Master's (CGS M), and now the Vanier Canada Graduate Scholarship. M Sambi is a recipient of the QGA. B Qorri is the recipient of the QGA and the 2017 Terry Fox Research Institute Transdisciplinary Training Program in Cancer Research. The authors report no other conflicts of interest in this work.

Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

References

1. Sener SF, Fremgen A, Menck HR, Winchester DP. Pancreatic cancer: a report of treatment and survival trends for 100,313 patients diagnosed from 1985-1995, using the National Cancer Database. J Am Coll Surg. 1999; 189(1): 1-7.

2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61(2): 69-90.

3. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. Nat Rev Clin Oncol. 2010; 7(3): 163-72.

4. Matsuda T, Matsuda A. Five-year relative survival rate of pancreas cancer in the USA, Europe and Japan. Jpn J Clin Oncol. 2014; 44(4): 398-399.

5. Rossi ML, Rehman AA, Gondi CS. Therapeutic options for the management of pancreatic cancer. World J Gastroenterol. 2014; 20(32): 11142-11159.

6. Riediger H, Keck T, Wellner U, zur Hausen A, Adam U, Hopt UT, et al. The lymph node ratio is the strongest prognostic factor after resection of pancreatic cancer. J Gastrointest Surg. 2009; 13(7): 1337-44.

7. Wasif N, Ko CY, Farrell J, Wainberg Z, Hines OJ, Reber H, et al. Impact of tumor grade on prognosis in pancreatic cancer: should we include grade in AJCC staging? Ann Surg Oncol. 2010; 17(9): 2312-2320.

8. Fortner JG, Klimstra DS, Senie RT, Maclean BJ. Tumor size is the primary prognosticator for pancreatic cancer after regional pancreatectomy. Ann

Surg. 1996; 223(2): 147-153.

9. Goonetilleke KS, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. Eur J Surg Oncol. 2007; 33(3): 266-270.

10. Bilimoria KY, Bentrem DJ, Ko CY, Ritchey J, Stewart AK, Winchester DP, et al. Validation of the 6th edition AJCC Pancreatic Cancer Staging System: report from the National Cancer Database. Cancer. 2007; 110(4): 738-744.

11. Massucco P, Capussotti L, Magnino A, Sperti E, Gatti M, Muratore A, et al. Pancreatic resections after chemoradiotherapy for locally advanced ductal adenocarcinoma: analysis of perioperative outcome and survival. Ann Surg Oncol. 2006; 13(9): 1201-1208.

12. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol. 1997; 15(6): 2403-2413.

13. Huang MT1, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH. Inhibitory effects of curcumin on in vitro lipoxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res. 1991; 51(3): 813-819.

14. Gandhi V, Legha J, Chen F, Hertel LW, Plunkett W. Excision of 2',2'-difluorodeoxycytidine (gemcitabine) monophosphate residues from DNA. Cancer Res. 1996; 56(19): 4453-4459.

15. Ferreira CG, Span SW, Peters GJ, Kruyt FA, Giaccone G. Chemotherapy triggers apoptosis in a caspase-8-dependent and mitochondria-controlled manner in the non-small cell lung cancer cell line NCI-H460. Cancer Res. 2000; 60(24): 7133-7141.

16. Habiro A, Tanno S, Koizumi K, Izawa T, Nakano Y, Osanai M, et al. Involvement of p38 mitogen-activated protein kinase in gemcitabine-induced apoptosis in human pancreatic cancer cells. Biochem Biophys Res Commun. 2004; 316(1): 71-77.

17. Chandler NM, Canete JJ, Callery MP. Caspase-3 drives apoptosis in pancreatic cancer cells after treatment with gemcitabine. J Gastrointest Surg. 2004; 8(8): 1072-1078.

18. Kummer JL, Rao PK, Heidenreich KA. Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. J Biol Chem. 1997; 272(33): 20490-20494.

19. Colucci G, Giuliani F, Gebbia V, Biglietto M, Rabitti P, Uomo G, et al. Gemcitabine alone or with cisplatin for the treatment of patients with locally advanced and/or metastatic pancreatic carcinoma: a prospective, randomized phase III study of the Gruppo Oncologia dell'Italia Meridionale. Cancer. 2002; 94(4): 902-910.

20. Inal A, Kos FT, Algin E, Yildiz R, Dikiltas M, Unek IT, et al. Gemcitabine alone versus combination of gemcitabine and cisplatin for the treatment of patients with locally advanced and/or metastatic pancreatic carcinoma: a retrospective analysis of multicenter study. Neoplasma. 2012; 59(3): 297-301.

21. Louvet C, Labianca R, Hammel P, Lledo G, Zampino MG, André T, et al. Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. J Clin Oncol. 2005; 23(15): 3509-3516.

22. Horwitz SB. Taxol (paclitaxel): mechanisms of action. Ann Oncol. 1994; 6: S3-S6.

23. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013; 369(18): 1691-703.

24. Marsh Rde W, Talamonti MS, Katz MH, Herman JM. Pancreatic cancer and FOLFIRINOX: a new era and new questions. Cancer Med. 2015; 4(6): 853-863.

25. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell. 1988; 53(4): 549-554.

26. Hruban RH, Maitra A, Goggins M. Update on pancreatic intraepithelial neoplasia. International journal of clinical and experimental pathology. 2008; 1(4): 306-316.

27. Gedde-Dahl T, 3rd, Eriksen JA, Thorsby E, Gaudernack G. T-cell responses against products of oncogenes: generation and characterization of human T-cell clones specific for p21 ras-derived synthetic peptides. Hum Immunol. 1992; 33(4): 266-274.

28. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell. 2004; 119(6): 847-860.

29. Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, et al. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat Genet. 1994; 8(1): 27-32.

30. Hahn SA, Hoque AT, Moskaluk CA, da Costa LT, Schutte M, Rozenblum E, et al. Homozygous deletion map at 18q21.1 in pancreatic cancer. Cancer Res. 1996; 56(3): 490-494.

31. Nordh S, Ansari D, Andersson R. hENT1 expression is predictive of gemcitabine outcome in pancreatic cancer: a systematic review. World J Gastroenterol. 2014; 20(26): 8482-8490.

32. Heppner GH. Tumor heterogeneity. Cancer Res. 1984; 44(6): 2259-2265.

33. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976; 194(4260): 23-28.

34. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi P. Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res. 1978; 38: 3758-3763.

35. Heppner G, Yamashina K, Miller B, Miller F. Tumor heterogeneity in metastasis. Prog Clin Biol Res. 1986; 212: 45-59.

36. Samuel N, Hudson TJ. The molecular and cellular heterogeneity of pancreatic ductal adenocarcinoma. Nat Rev Gastroenterol Hepatol. 2011; 9(2): 77-87.

37. Burrell RA, McGranahan N, Bartek J, Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature. 2013; 501(7467): 338-345.

38. Lorusso G, Ruegg C. The tumor microenvironment and its contribution to tumor evolution toward metastasis. Histochem Cell Biol. 2008; 130(6): 1091-1103.

39. Cheah MT, Chen JY, Sahoo D, Contreras-Trujillo H, Volkmer AK, Scheeren FA, et al. CD14-expressing cancer cells establish the inflammatory and proliferative tumor microenvironment in bladder cancer. Proc Natl Acad Sci U S A. 2015; 112(15): 4725-4730.

40. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008; 321(5897): 1801-1806.

41. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997; 3(7): 730-737.

42. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100(7):3983-8.

43. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003; 63(18): 5821-5828.

44. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007; 67(3): 1030-1037.

45. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nature Rev Cancer. 2005; 5(4): 275-284.

46. Kim Y, Lin Q, Glazer PM, Yun Z. Hypoxic tumor microenvironment and cancer cell differentiation. Curr Mol Med. 2009; 9: 425-434.

47. Helczynska K, Kronblad A, Jögi A, Nilsson E, Beckman S, Landberg G, et al. Hypoxia promotes a dedifferentiated phenotype in ductal breast carcinoma in situ. Cancer Res. 2003; 63(7): 1441-1444.

48. Kreso A, van Galen P, Pedley NM, Lima-Fernandes E, Frelin C, Davis T, et al. Self-renewal as a therapeutic target in human colorectal cancer. Nat Med. 2014; 20(1): 29.

49. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, et al. EMT and dissemination precede pancreatic tumor formation. Cell. 2012; 148(1-2): 349-361.

50. Neesse A, Algül H, Tuveson DA3, Gress TM4. Stromal biology and therapy in pancreatic cancer: a changing paradigm. Gut. 2015; 64(9): 1476-1484.

51. Whatcott CJ, Posner RG, Von Hoff DD, Han H. Desmoplasia and chemoresistance in pancreatic cancer. In: Grippo PJ, Munshi HG, editors. Pancreatic Cancer and Tumor Microenvironment. Trivandrum (India). 2012.

52. Tian H, Callahan CA, DuPree KJ, Darbonne WC, Ahn CP, Scales SJ, et al. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. Proc Natl Acad Sci U S A. 2009; 106(11): 4254-4259.

53. Jimeno A, Feldmann G, Suarez-Gauthier A, Rasheed Z, Solomon A, Zou GM, et al. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. Mol Cancer Ther. 2009; 8(2): 310-314.

54. Zhang GN, Liang Y, Zhou LJ, Chen SP, Chen G, Zhang TP, et al. Combination of salinomycin and gemcitabine eliminates pancreatic cancer cells. Cancer Lett. 2011; 313(2): 137-144.

55. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011; 331(6024): 1612-1616.

56. Schrader H, Wiese M, Ellrichmann M, Belyaev O, Uhl W, Tannapfel A, et al. Diagnostic value of quantitative EUS elastography for malignant pancreatic tumors: relationship with pancreatic fibrosis. Ultraschall in der Medizin. 2012; 33(7): E196-201.

57. Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ, et al. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. Cancer cell. 2012; 21(6): 822-835.

58. Tucker ON1, Dannenberg AJ, Yang EK, Zhang F, Teng L, Daly JM, et al. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res. 1999; 59(5): 987-990.

59. Khorana AA, Fine RL. Pancreatic cancer and thromboembolic disease. The Lancet Oncology. 2004; 5(11): 655-663.

60. Rao LV, Pendurthi UR. Factor VIIIa-induced gene expression: potential implications in pathophysiology. Trends in cardiovascular medicine. 2001; 11(1): 14-21.

61. Zhang Y, Deng Y, Luther T, Müller M, Ziegler R, Waldherr R, et al. Tissue factor controls the balance of angiogenic and antiangiogenic properties of tumor cells in mice. J Clin Invest. 1994; 94(3): 1320-1327.

62. Kranenburg O, Gebbink MF, Voest EE. Stimulation of angiogenesis by Ras proteins. Biochim Biophys Acta. 2004; 1654(1): 23-37.

63. Maragoudakis ME, Tsopanoglou NE, Andriopoulou P. Mechanism of thrombin-induced angiogenesis. Biochem Soc Trans. 2002; 30(2): 173-177.

64. Ikeda O, Egami H, Ishiko T, Ishikawa S, Kamohara H, Hidaka H, et al. Expression of proteinase-activated receptor-2 in human pancreatic cancer: a possible relation to cancer invasion and induction of fibrosis. Int J Oncol. 2003; 22(2): 295-300.

65. Rudroff C, Schafberg H, Nowak G, Weinel R, Scheele J, Kaufmann R. Characterization of functional thrombin receptors in human pancreatic tumor cells (MIA PACA-2). Pancreas. 1998; 16(2): 189-194.

66. Rudroff C, Seibold S, Kaufmann R, Zetina CC, Reise K, Schafer U, et al. Expression of the thrombin receptor PAR-1 correlates with tumour cell differentiation of pancreatic adenocarcinoma *in vitro*. Clin Exp Metastasis. 2002; 19(2): 181-189.

67. Rudroff C, Striegler S, Schilli M, Scheele J. Thrombin enhances adhesion in pancreatic cancer in vitro through the activation of the thrombin receptor PAR 1. Eur J Surg Oncol. 2001; 27(5): 472-476.

68. Sahni A, Sporn LA, Francis CW. Potentiation of endothelial cell proliferation by fibrin(ogen)-bound fibroblast growth factor-2. J Biol Chem. 1999; 274(21): 14936-14941.

69. Sahni A1, Baker CA, Sporn LA, Francis CW. Fibrinogen and fibrin protect fibroblast growth factor-2 from proteolytic degradation. Thromb Haemost. 2000; 83(5): 736-741.

70. Von Hoff DD, Korn R, Mousses S. Pancreatic cancer--could it be that simple? A different context of vulnerability. Cancer cell. 2009; 16(1): 7-8.

71. Le A, Rajeshkumar NV, Maitra A, Dang CV. Conceptual framework for cutting the pancreatic cancer fuel supply. Clin Cancer Res. 2012; 18(16): 4285-4290.

72. Ma WW, Jacene H, Song D, Vilardell F, Messersmith WA, Laheru D, et al. [18F]fluorodeoxyglucose positron emission tomography correlates with Akt pathway activity but is not predictive of clinical outcome during mTOR inhibitor therapy. J Clin Oncol. 2009; 27(16): 2697-2704.

73. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. N Engl J Med. 1995; 333(9): 550-554.

74. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, et al. Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. N Engl J Med. 1998; 338(13): 867-872.

75. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001; 108(8): 1167-1174.

76. Shackelford DB1, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer. 2009; 9(8): 563-575.

77. Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. Trends Mol Med. 2007; 13(10): 433-442.

78. Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P, et al. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer Res. 2011; 71(13): 4366-4372.

79. Pacifico F, Leonardi A. NF-kappaB in solid tumors. Biochem Pharmacol. 2006; 72(9): 1142-1152.

80. Hattori Y, Suzuki K, Hattori S, Kasai K. Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. Hypertension. 2006; 47(6): 1183-1188.

81. Isoda K, Young JL, Zirlik A, MacFarlane LA, Tsuboi N, Gerdes N, et al. Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. Arterioscler Thromb Vasc Biol. 2006; 26(3): 611-617.

82. Tan XL, Bhattacharyya KK, Dutta SK, Bamlet WR, Rabe KG, Wang E,

et al. Metformin suppresses pancreatic tumor growth with inhibition of NFÎºB/STAT3 inflammatory signaling. Pancreas. 2015; 44(4): 636-647.

83. Rotondi M, Coperchini F, Pignatti P, Magri F, Chiovato L. Metformin reverts the secretion of CXCL8 induced by TNF-Î± in primary cultures of human thyroid cells: an additional indirect anti-tumor effect of the drug. J Clin Endocrinol Metab. 2015; 100(3): E427-432.

84. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res. 2006; 66(21): 10269-10273.

85. Queiroz EA, Puukila S, Eichler R, Sampaio SC, Forsyth HL, Lees SJ, et al. Metformin induces apoptosis and cell cycle arrest mediated by oxidative stress, AMPK and FOXO3a in MCF-7 breast cancer cells. Plos One. 2014; 9(5): e98207.

86. Carmignani M, Volpe AR, Aldea M, Soritau O, Irimie A, Florian IS, et al. Glioblastoma stem cells: a new target for metformin and arsenic trioxide. Journal of biological regulators and homeostatic agents. 2014; 28(1): 1-15.

87. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res. 2007; 67(14): 6745-6752.

88. Erices R, Bravo ML, Gonzalez P, Oliva B, Racordon D, Garrido M, et al. Metformin, at concentrations corresponding to the treatment of diabetes, potentiates the cytotoxic effects of carboplatin in cultures of ovarian cancer cells. Reproductive sciences. 2013; 20(12): 1433-1446.

89. Wang LW, Li ZS, Zou DW, Jin ZD, Gao J, Xu GM. Metformin induces apoptosis of pancreatic cancer cells. World J Gastroenterol. 2008; 14(47): 7192-718.

90. Ashinuma H, Takiguchi Y, Kitazono S, Kitazono-Saitoh M, Kitamura A, Chiba T, et al. Antiproliferative action of metformin in human lung cancer cell lines. Oncol Rep. 2012; 28(1): 8-14.

91. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene. 2008; 27(25): 3576-86.

92. Bao B, Wang Z, Ali S, Ahmad A, Azmi AS, Sarkar SH, et al. Metformin inhibits cell proliferation, migration and invasion by attenuating CSC function mediated by deregulating miRNAs in pancreatic cancer cells. Cancer Prev Res (Phila). 2012; 5(3): 355-364.

93. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008; 133(4): 704-715.

94. Vazquez-Martin A, Oliveras-Ferraros C, Cufi S, Del Barco S. Martin-Castillo B, Menendez JA. Metformin regulates breast cancer stem cell ontogeny by transcriptional regulation of the epithelial-mesenchymal transition (EMT) status. Cell Cycle. 2010; 9(18): 3807-3814.

95. Cerezo M, Tichet M, Abbe P, Ohanna M, Lehraiki A, Rouaud F, et al. Metformin blocks melanoma invasion and metastasis development in AMPK/p53-dependent manner. Mol Cancer Ther. 2013; 12(8): 1605-1615.

96. Cifarelli V, Lashinger LM, Devlin KL, Dunlap SM, Huang J, Kaaks R, et al. Metformin and Rapamycin Reduce Pancreatic Cancer Growth in Obese Prediabetic Mice by Distinct MicroRNA-Regulated Mechanisms. Diabetes. 2015; 64(5): 1632-1642.

97. Yiyao Zhang LL, Pei Fan, Nathalie Bauer, Jury Gladkich, Eduard Ryschich, Alexandr V,et al. Aspirin counteracts cancer stem cell features, desmoplasia and gemcitabine resistance in pancreatic cancer. Oncotarget. 2015; 6(12): 9999-10015.

98. Sclabas GM, Uwagawa T, Schmidt C, Hess KR, Evans DB, Abbruzzese JL, et al. Nuclear factor kappa B activation is a potential target for preventing pancreatic carcinoma by aspirin. Cancer. 2005; 103(12): 2485-2490.

99. Kokawa A, Kondo H, Gotoda T, Ono H, Saito D, Nakadaira S, et al. Increased expression of cyclooxygenase-2 in human pancreatic neoplasms and potential for chemoprevention by cyclooxygenase inhibitors. Cancer. 2001; 91(2): 333-338.

100. Mathew P. Inflammatory pathogenesis of prostate cancer and celecoxib. J Clin Oncol. 2010;28(12):e197.

101. Atari-Hajipirloo S, Nikanfar S, Heydari A, Noori F, Kheradmand F. The effect of celecoxib and its combination with imatinib on human HT-29 colorectal cancer cells: Involvement of COX-2, Caspase-3, VEGF and NF-kappaB genes expression. Cell Mol Biol (Noisy-le-grand). 2016; 62(2): 68-74.

102. Dai ZJ, Ma XB, Kang HF, Gao J, Min WL, Guan HT, et al. Antitumor activity of the selective cyclooxygenase-2 inhibitor, celecoxib, on breast cancer *in Vitro* and *in Vivo*. Cancer Cell Int. 2012; 12(1): 53.

103. Hou LC, Huang F, Xu HB. Does celecoxib improve the efficacy of chemotherapy for advanced non-small cell lung cancer? British journal of clinical pharmacology. 2016; 81(1): 23-32.

104. Jendrossek V. Targeting apoptosis pathways by Celecoxib in cancer. Cancer Lett. 2013; 332(2): 313-324.

105. Abdulkhalek S, Hrynyk M, Szewczuk MR. A novel G-protein-coupled receptor-signaling platform and its targeted translation in human disease. Research and Reports in Biochemistry. 2013; 3: 17-30.

106. Haxho F, Neufeld RJ, Szewczuk MR1. Neuraminidase-1: a novel therapeutic target in multistage tumorigenesis. Oncotarget. 2016; 7(26): 40860-40881.

107. Hrynyk M, Ellis JP, Haxho F, Allison S, Steele JA, Abdulkhalek S, et al. Therapeutic designed poly (lactic-co-glycolic acid) cylindrical oseltamivir phosphate-loaded implants impede tumor neovascularization, growth and metastasis in mouse model of human pancreatic carcinoma. Drug design, development and therapy. 2015; 9: 4573-4586.

108. O'Shea LK, Abdulkhalek S, Allison S, Neufeld RJ, Szewczuk MR. Therapeutic targeting of Neu1 sialidase with oseltamivir phosphate (Tamiflu(R)) disables cancer cell survival in human pancreatic cancer with acquired chemoresistance. OncoTargets and therapy. 2014; 7: 117-134.

109. Gilmour AM, Abdulkhalek S, Cheng TS, Alghamdi F, Jayanth P, O'Shea LK, et al. A novel epidermal growth factor receptor-signaling platform and its targeted translation in pancreatic cancer. Cell Signal. 2013; 25(12): 2587-2603.

110. Haxho F, Allison S, Alghamdi F, Brodhagen L, Kuta VE, Abdulkhalek S, et al. Oseltamivir phosphate monotherapy ablates tumor neovascularization, growth, and metastasis in mouse model of human triple-negative breast adenocarcinoma. Breast cancer. 2014; 6: 191-203.

111. Abdulkhalek S, Geen OD, Brodhagen L, Haxho F, Alghamdi F, Allison S, et al. Transcriptional factor snail controls tumor neovascularization, growth and metastasis in mouse model of human ovarian carcinoma. Clin Transl Med. 2014; 3(1): 28.

112. Alghamdi F, Guo M, Abdulkhalek S, Crawford N, Amith SR, Szewczuk MR. A novel insulin receptor-signaling platform and its link to insulin resistance and type 2 diabetes. Cellular Signalling. 2014; 26(6): 1355-1368.

113. Abdulkhalek S, Szewczuk MR. Neu1 sialidase and matrix metalloproteinase-9 cross-talk regulates nucleic acid-induced endosomal TOLL-like receptor-7 and -9 activation, cellular signaling and pro-inflammatory responses. Cellular Signalling. 2013; 25(11): 2093-2105.

114. Abdulkhalek S, Guo M, Amith SR, Jayanth P, Szewczuk MR. G-protein coupled receptor agonists mediate Neu1 sialidase and matrix metalloproteinase-9 cross-talk to induce transactivation of TOLL-like receptors and cellular signaling. Cellular signalling. 2012; 24(11): 2035-2042.

115. Abdulkhalek S, Amith SR, Franchuk SL, Jayanth P, Guo M, Finlay T, et al. Neu1 sialidase and matrix metalloproteinase-9 cross-talk is essential for Toll-like receptor activation and cellular signaling. J Biol Chem. 2011; 286(42): 36532-36549.

116. Pshezhetsky AV, Ashmarina LI. Desialylation of surface receptors as a new dimension in cell signaling. Biochemistry (Mosc). 2013; 78(7): 736-745.