Review

Seed-in-Soil: Pancreatic Cancer Influenced by Tumor Microenvironment

Huey-Jen Lin 1,* and Jiayuh Lin 2

1 Department of Medical Laboratory Sciences, University of Delaware, Room 305, Willard Hall Education Building, 16 West Main Street, Newark, DE 19716, USA
2 Department of Biochemistry and Molecular Biology, Molecular Medicine Graduate Program, University of Maryland School of Medicine and Comprehensive Cancer Center, 108 N. Greene Street, Baltimore, MD 21201, USA; JLin@som.umaryland.edu
* Correspondence: hlin@udel.edu; Tel.: +1-302-831-7576; Fax: +1-302-831-4180

Academic Editor: Samuel C. Mok
Received: 29 June 2017; Accepted: 18 July 2017; Published: 21 July 2017

Abstract: Pancreatic ductal adenocarcinoma is a fatal malignancy with a five-year survival rate lower than 7%, and most patients dying within six months of diagnosis. The factors that contribute to the aggressiveness of the disease include, but are not limited to: late diagnosis, prompt metastasis to adjacent vital organs, poor response, and resistance to anticancer treatments. This malignancy is uniquely associated with desmoplastic stroma that accounts for 80% of tumor mass. Understanding the biology of stroma can aid the discovery of innovative strategies for eradicating this lethal cancer in the future. This review highlights the critical components in the stroma and how they interact with the cancer cells to convey the devastating tumor progression.

Keywords: pancreatic ductal adenocarcinoma; tumor microenvironment; carcinoma-associated fibroblasts; signaling pathways; immune-suppression and microRNAs

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for >95% of pancreatic cancer, ranking third highest in cancer-related deaths in the US. Genetic analysis of pancreatic cancer has indicated that multiple mutations accumulate over time, including KRAS (about 90%), p16/INK4a/CDKN2A (about 75%), TP53 (about 65%), and SMAD4 (about 50%) [1,2]. Moreover, mutations of KRAS, p16/INK4a/CDKN2A, and TP53 result in cells escaping senescence, which allows the tumors to expand [3]. Lack of early symptoms, routine screenings, and effective treatment options, followed by refractory to conventional therapies and the progression of early metastasis to the neighboring vital organs, have led to <5% of patients surviving for more than five years [4]. Only 10–20% of PDAC patients are candidates for surgery at the time of diagnosis, and merely <20% who undergo curative resection are alive after five years [5]. Hence, understanding the biology of PDAC is important in developing improved and effective treatment regimens.

One of the unique characteristics associated with PDAC is that the malignant epithelial cells account for only approximately 20% of the tumor bulk, while the desmoplastic stroma constitute roughly 80% of tumor mass [6]. Hence, it is reasonable to theorize that the aforementioned malignant features may pertain to the unique roles that stroma plays in the aspects of initiating malignancy, escaping immune surveillance, promoting tumor progression and growth, as well as conveying drug resistance and metastasis [7–9]. This review highlights the impact of these stromal components on PDAC, with an ultimate goal of eradicating such a deadly cancer. Yet, due to the space limitation, authors regret that some of the outstanding findings cannot be mentioned in this report.
2. Cancer-Associated Fibroblasts

PDAC stroma consists of a network that is necessary for supporting tumor growth. The heterogeneous components comprise the carcinoma-associated fibroblasts (CAFs) in an activated state known as pancreatic stellate cells (PSCs), microvasculature, infiltrated immune cells, and the acellular extracellular matrix (ECM) which includes polysaccharides, proteins, cytokines, growth factors, and enzymes [10]. Prior studies illustrated that elevated levels of stroma correlated with poor prognosis, and that ablation of the stromal compartment yielded improved chemotherapy delivery [11,12]. Together, they suggested the tumor-promoting roles that stroma played [11,12]. In supporting this notion, the glycan-binding protein galectin-1 (Gal1) was abundantly expressed in PDAC, and it also plays a stimulating role in the tumor expansion [13]. Genetic ablation of Gal1 in a mouse model of PDAC (E1a-myc tumors) weakened tumor progression by impeding proliferation, angiogenesis, hampering desmoplastic reactions, and by favoring immune surveillance, yielding a 20% improvement in survival duration [13]. Furthermore, cancer-associated mesenchymal stem cells were not only isolated from CAFs, and but also secreted granulocyte macrophage colony-stimulating factor (GM-CSF) for augmenting PDAC growth, survival, invasion, and metastasis [14].

CAFs were identified by their expression of another membrane protein known as fibroblast activation protein-α (FAP), which exerted pleiotropic tumor-promoting effects including blocking immune surveillance, adapting PDAC to the host, enhancing tumor vascular density, and augmenting the desmoplastic growth of the microenvironment [15,16]. The conditional depletion of the FAP in CAFs, hence, restored the immune surveillance (that is, anti-tumor) effect not only of the transplanted tumor, but also of an autochthonous model of PDAC [15]. In CAFs, immune suppression by the FAP is facilitated by CXCL12, a chemokine that excludes cytotoxic CD8⁺ T cells by a mechanism depending on the interaction with its receptor CXCR4 [15]. The inhibition of CXCR4 led to diversified tumor-elimination effects by restoring and enabling the rapid intratumoral accumulation of cytotoxic CD8⁺ T cells [15]. Hence, targeting CXCR4 could lead to immune-mediated anti-tumor effects and develop a potential treatment regimen in the near future.

Moreover, CAFs interacted with cancer cells, in part, by releasing chemical messengers packed into miniature double-membraned, cargo-like structures known as CAF-derived exosomes (CDEs) [17]. CDEs contained intact metabolites including amino acids, lipids, and intermediates for citric acid cycle. Together, CDE can reprogram the metabolic machinery following their intake by the cancer cells. Upon CDEs’ ingestion, the mitochondrial oxidative phosphorylation and the normal oxygen-based energy release were dramatically reduced, whereas glycolysis and sugar consumption was enhanced in the cancer cells [17]. Hence, CDEs reprogrammed the central carbon metabolism in the cancer cells and further promoted tumor growth, even though the tumors were under nutrient-deprivation conditions [17].

Disappointingly enough, the promising experimental findings stated above have not led to satisfactory clinical applications [18–20]. Later studies on stromal biology elicited the discrepancies. For example, Özdemir et al. deleted α-smooth muscle antigen (αSMA) in the myofibroblasts in PtflaCre/+; KrasLSL-G12D/+; Tgfr2flox/flox (PKT) mice, and demonstrated that ablation of myofibroblasts yielded, surprisingly, undifferentiated immune suppression (that is, tumor-promoting) and more invasive PDAC in conjunction with poor prognosis [21]. This finding ignited whether the tumor stroma in PDAC was indeed a double-edged sword, friend, or foe [22]. The mechanisms and functional consequences of the tumor–stroma crosstalk may be more complicated than what was anticipated previously. Single components alone cannot authenticate the biophysical properties or the biochemical complexity around the epithelial cells. Yet, a thorough assessment on crosstalk between multi-factorial parameters in an unbiased manner is required. The sophisticated interactions between the positive and negative growth signals may tip the balance towards tumor suppression or promotion [23–26].
3. Immune Modulation

Tumor-infiltrating immune cells were shown to be vital for tumor progression, metastasis, and chemotherapy resistance \[27–29\]. The mounting of immune-suppressive cells over the course of PDAC included myeloid-derived suppressive cells (MDSCs), T regulatory cells (Tregs), and tumor-associated macrophages (TAMs). Together, they reduced the anti-tumor functionality normally employed by CD8\(^+\) T cells, and thus resulted in an impairment of tumor recognition and elimination. Initially, tumors secreted GM-CSF for recruiting myeloid progenitor cells to the surrounding stroma, which can be further differentiated into MDSCs \[30\]. In tumor stroma, MDSCs further blocked the immune surveillance function naturally exerted by the cytotoxic CD8\(^+\) T cells \[26,27\]. Recent studies indicated that the interactions between ligands and receptors were important for precluding such an immune scrutiny process. The inhibitory receptors, such as programmed cell death 1 receptor (PD-1, on immune cells), can be masked and blunted by its ligand PD-1L secreted from the tumor cells. Binding of PD-1 to PD-1L abolished the tumor-eradication function that should have been employed by the normal cytotoxic CD8\(^+\) T cells or by the nature killer cells \[31,32\]. The outcome of escaping immune surveillance conveyed a permissive tumor microenvironment for cultivating PDAC expansion.

On the other hand, the immune-inhibitory modes ascribed to Tregs involved with the secretion of suppressive cytokines such as interleukin 10 (IL-10), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and transforming growth factor \(\beta\) (TGF\(\beta\)) \[33\]. A subpopulation of CD4\(^+\) T cells can be influenced by TGF\(\beta\) stimulation and then be differentiated into interleukin 17 (IL-17)-secreting CD4\(^+\) T cells (known as Th17) that acquired additional immune-suppressive (that is, tumor-promoting) function \[34–36\]. Interestingly enough, infiltration of Th17 was shown to be aided by oncogenic Kras\(^{G12D}\) \[37\].

Wu et al. elicited that one of the IL-17 cytokine family, IL-17B, played important roles in regulating inflammation, and that delivering neutralizing antibodies reduced tumor burden along with enhanced survival in a mouse xenograft model, manifested by the inhibited tumor proliferation and impeded cancer metastasis \[38\]. The underlying mechanisms account for this phenomenon were revealed. The binding of IL-17 to its receptor induced the expression of REG3\(\beta\), which further promoted cell growth and gained refractory to cell death through activation of the gp130-JAK2-STAT3-dependent pathway \[39\]. Another independent study reported that IL-17B bound to its receptor, IL-17RB, and then induced CCL20/CXCL1/IL-8/TFF1 activation, an event that subsequently rendered noticeable tumor-promoting effects such as the invasion of cancer cells, recruitment of macrophage and endothelial cells at primary sites, as well as resistance of treatments at the distant organs \[38\]. Taken together, IL-17 plays an intricate role in the pathophysiology of cancer, from tumorigenesis, proliferation, metastasis, to confer both immune and chemotherapy resistance.

Regarding the macrophages, TAMs can be divided into two subtypes according to their developmental states and functionalities: the original state M1 (pro-inflammatory), and the tumor-evolved M2 (immune-suppressive and tumor-promoting). The elevated fraction of M2-polarized TAMs was reported to be correlated with an increased risk of lymph node metastasis, neural invasion, chemoresistance, worsening prognosis, and survival \[40,41\]. Moreover, M2-polarized TAMs secrete IL-10, which is known to be associated with immune-suppressive and tumor-promoting functionality \[42\]. The ability of M2-TAMs to enhance tumor invasion and metastasis is not only by preventing tumor cells from being eliminated by CD8\(^+\) cytotoxic T cells or by natural killer cells, but also by promoting cancer cell proliferation, stimulating extracellular matrix breakdown, and augmenting epithelial-mesenchymal transition (EMT) \[43,44\], an event that preludes cancer stem cell phenotypes \[45\]. Under this notion, TAMs were reported to secrete an antimicrobial peptide, hCAP-18/LL-37, which enriched a subpopulation of malignant cells harboring CD133\(^+\) and displaying cancer stem cell phenotypes \[46\]. The pivotal transition from the original state, M1, to the tumor-promoting M2 in PDAC, may be one of the major reasons for the poor prognosis of cancer patients. Mounting evidence suggested that M2-polarization was mediated by Reg3\(\beta\) through the
activation of the STAT3 pathway in an orthotopic mouse model [47], implicating that abrogating the STAT3 pathway could become a promising therapeutic target.

4. Signaling Pathways in PDAC Stroma

4.1. Hyaluronan

Hyaluronan (HA) is a glycosaminoglycan with a high capacity of water retention. A high level of HA in PDAC increased intratumoral fluid pressure, created substantial barriers, and impeded the intratumoral penetration of anti-cancer agents [48,49]. The ablation of stromal HA by using PEGylated human recombinant PH20 hyaluronidase (PEGPH20) led to interstitial fluid pressure normalization and re-expansion of collapsed tumor vasculature, followed by improved prognosis in the KPC (KrasLSL.G12D/+; p53R172H/+; PdxCre+/+) mice model [49]. In favor of this notion, PEGPH20 plus gemcitabine improved therapeutic outcomes in PDAC patients with high HA tumors [50].

4.2. Sonic Hedgehog

Sonic hedgehog (SHh) signaling was recognized as one of the key regulators of tumor epithelia–stromal interaction in PDAC [51]. The SHh ligand, produced by the malignant epithelial cells, signaled to the transmembrane protein Ptc1 on the stromal cells which subsequently relocated Smo to the cell surface [52–54]. This event resulted in the translocation of Gli1 (activator) to the nucleus, followed by a cascade activation of SHh-dependent genes [51–54].

Olive et al. reported that interrupting SHh signaling using the inhibitor IPI-926 (saridegib) ablated stromal CAFs and led to a transient increase in intratumoral vascular density, followed by an enhanced gemcitabine delivery with an improved cytotoxic outcome in the genetic KPC mice model [12]. Despite the aforementioned study providing a promising therapeutic target, another animal model failed to be recapitulated [55]. Similar reports provided paradoxical findings. SHh pathway inhibition suppressed stromal desmoplasia, but accelerated tumor progression of Kras-driven mice; whereas activation of SHh signaling caused stromal hyperplasia and reduced epithelial proliferation, leading to a restraint rather than a supporting effect on tumorigenesis [23]. Likewise, Shh-deficient tumors were shown to be more aggressive and they manifested increased vascularity, indicating ablation of stromal fibroblasts led to poor prognosis [24]. The discrepancies among various studies could be due to global and chronic ablation (by genetic knockout) versus the acute blockade of stromal cells (by SHh inhibitors), studying the initiation phase versus established malignant stages, and dosage-dependent as well as off-target effects.

4.3. Transforming Growth Factor β (TGFβ)

TGFβ-signaling cascade involved the binding of ligands to their receptors, which furthered the recruitment and phosphorylation of the downstream effectors including the SMAD (mothers against decapentaplegic homologs) family of proteins. Upon activation, SMAD underwent phosphorylation and dimerization, followed by translocation to the nucleus for regulating the expression of downstream TGFβ-dependent genes [56]. The role that TGFβ played in pancreatic cancer was complicated, as it was known to inhibit tumor initiation in the early stages, but favor tumor expansion in later phases [57]. Furthermore, TGFβ affected both the stromal and the neoplastic elements, and this aberrant signaling correlated with poor survival [58].

In favoring the tumor-promoting role, elevated levels of TGFβ have been shown to enhance cell proliferation, suppress immune scrutiny and activate PSCs [59–61]. Similarly, Ostapoff et al. demonstrated that introducing a TGFβr2-neutralizing antibody was able to promote a differentiated tumor cell phenotype, and thus inhibit pancreatic cancer metastasis in the orthotopic human tumor xenografts [62]. The underlying mechanisms were involved with targeting the stromal compartment, followed by hampering the activated fibroblasts, collagen deposition, microvessel density, and vascular function [62]. Likewise, the introduction of a TGFβ inactivator (SMAD7) yielded decreased
ECM production, reduced fibrosis, and diminished PSCs activation when using a transgenic mouse model [63]. On the other hand, overly activated TGFβ was demonstrated to augment EMT, an event known to initiate metastasis [64], and to sustain cancer stem cell phenotypes [65]. Taken together, the pleiotropic functionality of TGFβ in cancer shall be further investigated, prior to developing a potentially attractive target for treating PDAC.

4.4. Aberrant Immune Regulators in Tumor Microenvironment

CD40, a cell surface molecule that belongs to the tumor necrosis factor (TNF) receptor family, was reported to participate in immune regulation and mediate tumor apoptosis [66,67]. CD40 was shown to be one of the key regulators conferring T cell-dependent anti-tumor immunity [66,67]. Under normal physiologic conditions, activation of antigen-presenting cells is aided by CD4+ helper cells, which becomes an event that preludes the activation of naïve CD8+ T cells into cytotoxic effector cells. Yet, within the PDAC tumor microenvironment, CD40 could override the demand of the CD4+ helper cells for activating cytotoxic CD8+ T cells. Preclinical studies have evolved the development of CD40-activating antibodies, and they have been tested in clinical trials. One study showed that combination of an agonist CD40 antibody plus gemcitabine resulted in tumor regression in patients who were not eligible for tumor resection [68].

4.5. Constitutively Activated Kras Pathway

KrasG12D is required for both the initiation and maintenance processes of pancreatic cancer in mouse models, and was shown to be the most common oncogenic KRAS mutation presented in more than 90% of human PDAC, leading to a dominant and constitutively active form of GTPase [69,70]. Such an oncogenic Kras often led to a pathological downstream activation of the phosphoinositide 3-kinase (PI3K) pathway [71]. The factors secreted by Kras for maintaining neoplasm and for promoting stroma appear to include SHh and IL-6 [51,72]. The SHh ligand functioned in a paracrine manner to activate signaling in the stroma and to mediate its maintenance [73]. Molecular studies revealed that SHh induced GLI1 binding to the IL-6 promoter and activated IL-6 expression in fibroblasts in a paracrine fashion [74]. This event further maintained the levels of activated STAT3 in the neighboring cancer cells, acting as a transcription factor required for developing premalignant lesions, maintaining tumor stroma, and advancing neoplastic features [74]. Further molecular studies elicited that KrasG12D activated ERK2 and enhanced the invasion of pancreatic cancer cells via MMP-1 [75]. Oncogenic Kras can also augment the tumor microenvironment by infiltrating immune-suppressing cells that impeded the anti-tumor immune responses. This process subsequently promoted permanent inflammation followed by genetic mutations, which ultimately rendered the aggressiveness of PDAC [27].

Gene expression and metabolic flux analyses further elicited the cancer metabolic role that oncogenic Kras played in orchestrating multiple metabolic changes, including stimulating glucose uptake, differential channeling of glucose intermediates into the hexosamine biosynthesis and pentose phosphate pathways, as well as reprogramming glutamine metabolism [76,77]. By rewiring glucose metabolism while maintaining a low level of reactive oxygen species (ROS), oncogenic Kras limited ROS production and ROS-related apoptosis [76]. Together, biomass synthesis (i.e., proteins, nucleic acids etc.) required for cancer cell proliferation can be boosted [76].

5. MicroRNAs

MicroRNAs (miRNAs), the short non-coding RNAs involved in the post-transcriptional suppression of target genes, have been defined as the imperative controllers in tumor proliferations, invasions, and resistance to chemotherapeutic agents. The crosstalk between the malignant epithelia in PDAC and the tumor microenvironment was shown to be interplayed by some miRNAs, such as miR-21 and miR-221 [78]. Ali et al. demonstrated that these miRNAs stimulated the expression of Kras (a target of miR-221) as well as enhanced migration and invasion features leading to advanced PDAC [78]. Moreover, miR-155-secreting pancreatic cancer cells furthered the conversion from
the normal fibroblasts to CAFs, an event preluded the aggressive malignancy by targeting and downregulating p53-induced nuclear protein 1 (TP53INP1) [79]. Conversely, loss of miR-29 was a common occurrence of activated PSCs, and this phenomenon was correlated with a significant increase in ECM [80]. Hence, correcting and sustaining these miRNAs at their normal levels could likely become promising targets for developing innovative medicine.

Valadi et al. denoted that exosomes can shuttle miRNAs between the donor and the recipient cells that subsequently exerted important biological impacts on the recipient cells [81]. For example, Fabbri et al. revealed that the binding of miR-21 and miR-29a in the exosomes secreted from cancer cells to the Toll-like receptors on the immune cells resulted in an inflammatory response that promoted tumor expansion and metastasis [82]. As exosomes acted as a carrier for miRNAs imperative for conveying a tumor microenvironment conducive to metastasis, they shall not be too far away from constituting potential treatment regimens or becoming biomarkers.

6. Cancer Vaccines

Cancer vaccines stimulate the immune system to produce and infiltrate tumor-specific cytotoxic effector T cells by increasing the exposure of tumor-associated antigens to the immune system. The most promising vaccine was GVAX, which was composed of allogeneic PDAC cell lines engineered to secrete GM-CSF [83]. After been administered to the patients with resected or metastatic PDAC, GVAX was able to boost the production of anti-tumor CD8+ T cells in peripheral lymphocytes, with an outcome correlated with an improved survival [84]. Another study testing the combination of GVAX and ipilimumab (an antibody blocking CTLA-4) compared to ipilimumab monotherapy showed an appreciative overall survival in metastatic PDAC patients [85]. Likewise, the combination of GVAX with PD-1/PD-L1 blockade together facilitated effector T cell infiltration into pancreatic tumors in a mouse model [32].

Recently, PDAC was recognized to be one of the “nonimmunogenic” malignancies, due to a shortage of tumor-infiltrating effector lymphocytes. Lutz et al. developed an adjuvant clinical trial by combining GVAX with low-dose cyclophosphamide to deplete Tregs. By inducing the infiltration of T cells, patients demonstrated improved survival, enhanced post-vaccination T-cell responses, and increased intratumoral T effector/Treg ratios [86]. Furthermore, Le et al. developed a chimeric GVAX-based vaccine known as GVAXCRS-207 that comprised not only GVAX, but also live-attenuated Listeria monocytogenes-expressing mesothelin to stimulate innate and adaptive immunity [87]. Mesothelin was reported to be a common antigen expressed in many human cancers, including PDAC [88]. The delivery of GVAXCRS-207 plus cyclophosphamide to the patients yielded encouraging outcomes with extended survival and minimal cytotoxicity [87]. Taken together, GVAX appeared to be very specific, and vaccine therapies were relatively tolerated. Future innovative treatment regimens could adapt the synergistic effect from GVAX along with other target agents.

Another DNA-based vaccine comprised of Mucin 1 plus variable number tandem repeat (MUC1-VNTRn, each repeat of VNTR encodes 20 amino acids GVTSAPDTRPAAPGSTAPPAAH) was recently developed, and was transfected to immature dendritic cells [89]. Upon intake of the plasmid construct pVAX1-MUC1-VNTRn, dendritic cells not only yielded elevated immunogenicity, but their neighboring co-cultured T-cells also gained evident cytotoxicity, which was manifested by their growth inhibitory effect on PDAC in both laboratory cultivation experiments and tumor-bearing animal studies [89].

7. Concluding Remarks and Future Treatment Regimens

In summary, Figure 1 depicted the progression of PDAC can be orchestrated by various tumor microenvironmental elements, and some of them have been utilized for developing targeted therapies (see Table 1). Future improved treatments for PDAC could include combination regimens aiming to normalize desmoplastic reaction, inhibit tumorigenic-signaling pathways, reprogram immune suppression (known as immunotherapy), correct aberrant miRNAs, and implement cancer vaccines.
Table 1. Summary of selected agents targeting microenvironmental factors in pancreatic cancer.

| Target                                      | Clinical Studies                                      | Reference |
|---------------------------------------------|-------------------------------------------------------|-----------|
| Hyaluronan and chemotherapy agent           | Phase 1b PEGPH20 plus Gemcitabine                     | [50]      |
| Transforming Growth Factor β                | Orthotopic human tumor xenografts TGFβr2 neutralizing antibody (2G8) | [62]      |
| Cancer vaccine                              | GVAX. Phase 2                                         | [83,84]   |
| Cancer vaccine and CTLA-4                   | GVAX and ipilimumab                                   | [85]      |
| Cancer vaccine and Treg                     | GVAX and cyclophosphamide                             | [86]      |
| Cancer vaccine, Treg, and mesothelin        | Cyclophosphamide/GVAXCRS-207                          | [87]      |

Figure 1. The pleiotropic influence of the tumor microenvironment on pancreatic ductal adenocarcinoma (PDAC). The progression of (PDAC) (pyramid spheres in the center) is controlled by various molecules and signaling pathways in the tumor microenvironment. While molecules associated with carcinoma-associated fibroblasts or pancreatic stellate cells were displayed in spindles at the left side of the figure, others involved with immune cells are presented in ova at the right side of figure. Moreover, the signaling pathways are presented in rectangles above PDAC, while the microRNAs (miR) are underneath. Tumor-promoting factors are shaded in green, while inhibiting factors are in red. Yet, ones shaded in purple are pleotropic, with both promoting and inhibiting effects, depending on the stage of tumors as well as the research studies. The abbreviations used are CDEs (CAFs-derived exosomes); CTLA-4 (cytotoxic T lymphocyte-associated protein 4); FAPa (fibroblast activation protein-a); Gal1 (glycan-binding protein galectin-1); GM-CSF (granulocyte macrophage colony-stimulating factor); HA (hyaluronan); IL-10 (interleukin 10); IL-17 (interleukin 17); Kras (constitutively oncogenic Kras); miR (microRNA); PD-1 (programmed cell death 1 receptor); PD-1L (ligand for PD-1); and SHh (Sonic hedgehog).
Acknowledgments: This work was supported in part by the Interdisciplinary Collaborative Grants, Comprehensive Cancer Center, the Ohio State University to Huey-Jen Lin, as well as the University of Maryland School of Medicine and Comprehensive Cancer Center start-up fund and an AACR-Pancreatic Cancer Action Network grant to Jiayuh Lin.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Schneider, G.; Schmid, R.M. Genetic alterations in pancreatic carcinoma. Mol. Cancer 2003, 2, 15. [CrossRef] [PubMed]
2. Hezel, A.F.; Kimmelman, A.C.; Stanger, B.Z.; Bardeesy, N.; Depinho, R.A. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2006, 20, 1218–1249. [CrossRef] [PubMed]
3. Moir, J.A.; White, S.A.; Mann, J. Arrested development and the great escape—the role of cellular senescence in pancreatic cancer. Int. J. Biochem. Cell Biol. 2014, 57, 142–148. [CrossRef]
4. Jemal, A.; Siegel, R.; Xu, J.; Ward, E. Cancer statistics, 2010. CA Cancer J. Clin. 2010, 60, 277–300. [CrossRef] [PubMed]
5. Burris, H.A., 3rd; Moore, M.J.; Andersen, J.; Green, M.R.; Rothenberg, M.L.; Modiano, M.R.; Cripps, M.C.; Portenoy, R.K.; Storniolo, A.M.; Tarassoff, P.; 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, 2403–2413. [CrossRef] [PubMed]
6. Farrow, B.; Albo, D.; Berger, D.H. The role of the tumor microenvironment in the progression of pancreatic cancer. J. Surg. Res. 2008, 149, 319–328. [CrossRef] [PubMed]
7. Spector, I.; Zilberstein, Y.; Lavy, A.; Nagler, A.; Genin, O.; Pines, M. Involvement of host stroma cells and tissue fibrosis in pancreatic tumor development in transgenic mice. PLoS ONE 2012, 7, e41833. [CrossRef] [PubMed]
8. Apte, M.V.; Wilson, J.S.; Lugea, A.; Pandol, S.J. A starring role for stellate cells in the pancreatic cancer microenvironment. Gastroenterology 2013, 144, 1210–1219. [CrossRef] [PubMed]
9. Mace, T.A.; Ameen, Z.; Collins, A.; Wojcik, S.; Mair, M.; Young, G.S.; Fuchs, J.R.; Eubank, T.D.; Frankel, W.L.; Bekaii-Saab, T.; et al. Pancreatic cancer-associated stellate cells promote differentiation of myeloid-derived suppressor cells in a STAT3-dependent manner. Cancer Res. 2013, 73, 3007–3018. [CrossRef] [PubMed]
10. Apte, M.V.; Park, S.; Phillips, P.A.; Santucci, N.; Goldstein, D.; Kumar, R.K.; Ramm, G.A.; Buchler, M.; Friess, H.; McCarroll, J.A.; et al. Desmoplastic reaction in pancreatic cancer: Role of pancreatic stellate cells. Pancrea 2004, 29, 179–187. [CrossRef]
11. Erkan, M.; Michalski, C.W.; Rieder, S.; Reiser-Erkan, C.; Abiatar, I.; Kolb, A.; Giese, N.A.; Esposito, I.; Friess, H.; Kleeff, J. The activated stroma index is a novel and independent prognostic marker in pancreatic ductal adenocarcinoma. Clin. Gastroenterol. Hepatol. 2008, 6, 1155–1161. [CrossRef] [PubMed]
12. Olive, K.P.; Jacobetz, M.A.; Davidson, C.J.; Gopinathan, A.; McIntyre, D.; Honess, D.; Madhu, B.; Goldgraben, M.A.; Caldwell, M.E.; Allard, D.; et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 2009, 324, 1457–1461. [CrossRef] [PubMed]
13. Martinez-Bosch, N.; Fernandez-Barrena, M.G.; Moreno, M.; Ortiz-Zapater, E.; Munne-Collado, J.; Iglesias, M.; Andre, S.; Gabius, H.J.; Hwang, R.F.; Poitier, F.; et al. Galectin-1 drives pancreatic carcinogenesis through stroma remodeling and Hedgehog signaling activation. Cancer Res. 2014, 74, 3512–3524. [CrossRef] [PubMed]
14. Waghray, M.; Yalamanchili, M.; Dzubiinski, M.; Zeinali, M.; Erkkinen, M.; Yang, H.; Schradle, K.A.; Urs, S.; Pasca Di Magliano, M.; Welling, T.H.; et al. GM-CSF Mediates Mesenchymal-Epithelial Cross-talk in Pancreatic Cancer. Cancer Discov. 2016, 6, 886–899. [CrossRef] [PubMed]
15. Fearon, D.T. The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance. Cancer Immunol. Res. 2014, 2, 187–193. [CrossRef] [PubMed]
16. Lo, A.; Wang, L.C.; Scholler, J.; Monslow, J.; Avery, D.; Newick, K.; O’Brien, S.; Evans, R.A.; Bajor, D.J.; Clendenin, C.; et al. Tumor-Promoting Desmoplasia Is Disrupted by Depleting FAP-Expressing Stromal Cells. Cancer Res. 2015, 75, 2800–2810. [CrossRef] [PubMed]
17. Zhao, H.; Yang, L.; Baddour, J.; Achreja, A.; Bernard, V.; Moss, T.; Marini, J.C.; Tudawe, T.; Seviour, E.G.; San Lucas, F.A.; et al. Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. Elife 2016, 5, e10250. [CrossRef] [PubMed]
18. Bramhall, S.R.; Rosemurgy, A.; Brown, P.D.; Bowry, C.; Buckels, J.A.; Marimastat Pancreatic Cancer Study Group. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: A randomized trial. *J. Clin. Oncol.* 2001, 19, 3447–3455. [CrossRef] [PubMed]

19. Bramhall, S.R.; Schulz, J.; Nemunaitis, J.; Brown, P.D.; Baillet, M.; Buckels, J.A. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. *Br. J. Cancer* 2002, 87, 161–167. [CrossRef] [PubMed]

20. Moore, M.J.; Hamm, J.; Dancey, J.; Eisenberg, P.D.; Dagenais, M.; Fields, A.; Hagan, K.; Greenberg, B.; Colwell, B.; Zee, B.; et al. Comparison of gemcitabine versus the metalloproteinase inhibitor BAY 12-9566 in patients with advanced or metastatic adenocarcinoma of the pancreas: A phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J. Clin. Oncol.* 2003, 21, 3296–3302. [CrossRef] [PubMed]

21. Ozdemir, B.C.; Pentcheva-Hoang, T.; Carstens, J.L.; Zheng, X.; Wu, C.C.; Simpson, T.R.; Laklai, H.; Sugimoto, H.; Kahlert, C.; Novitskiy, S.V.; et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 2014, 25, 719–734. [CrossRef] [PubMed]

22. Gore, J.; Korc, M. Pancreatic cancer stroma: Friend or foe? *Cancer Cell* 2014, 25, 711–712. [CrossRef] [PubMed]

23. Lee, J.J.; Perera, R.M.; Wang, H.; Wu, D.C.; Liu, X.S.; Han, S.; Fitamant, J.; Jones, P.D.; Ghanta, K.S.; Kawano, S.; et al. Stromal response to Hedgehog signaling represses pancreatic cancer progression. *Proc. Natl. Acad. Sci. USA* 2014, 111, E3091–E3100. [CrossRef] [PubMed]

24. Rhim, A.D.; Oberstein, P.E.; Thomas, D.H.; Mirek, E.T.; Palermo, C.F.; Sastra, S.A.; Dekleva, E.N.; Saunders, T.; Becerra, C.P.; Tattersall, I.W.; et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 2014, 25, 735–747. [CrossRef] [PubMed]

25. Sherman, M.H.; Yu, R.T.; Engle, D.D.; Ding, N.; Atkins, A.R.; Tiriak, H.; Colisson, E.A.; Connor, F.; Van Dyke, T.; Kozlov, S.; et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell* 2014, 159, 80–93. [CrossRef] [PubMed]

26. Neesse, A.; Algul, H.; Tuveson, D.A.; Gress, T.M. Stromal biology and therapy in pancreatic cancer: A changing paradigm. *Gut* 2015, 64, 1476–1484. [CrossRef] [PubMed]

27. Clark, C.E.; Hingorani, S.R.; Mick, R.; Combs, C.; Tuveson, D.A.; Vonderheide, R.H. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res.* 2007, 67, 9518–9527. [CrossRef] [PubMed]

28. Protti, M.P.; De Monte, L. Immune infiltrates as predictive markers of survival in pancreatic cancer patients. *Front. Physiol.* 2013, 4, 210. [CrossRef] [PubMed]

29. Mielgo, A.; Schmid, M.C. Impact of tumour associated macrophages in pancreatic cancer. *BMB Rep.* 2013, 46, 131–138. [CrossRef] [PubMed]

30. Bayne, L.J.; Beatty, G.L.; Jhala, N.; Clark, C.E.; Rhim, A.D.; Stanger, B.Z.; Vonderheide, R.H. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer Cell* 2012, 21, 822–835. [CrossRef] [PubMed]

31. Nomi, T.; Sho, M.; Akahori, T.; Hamada, K.; Kubo, A.; Kanehiro, H.; Nakamura, S.; Enomoto, K.; Yagita, H.; Azuma, M.; et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin. Cancer Res.* 2007, 13, 2151–2157. [CrossRef] [PubMed]

32. Soares, K.C.; Rucki, A.A.; Wu, A.A.; Olinio, K.; Xiao, Q.; Chai, Y.; Wamwea, A.; Bigelow, E.; Lutz, E.; Liu, L.; et al. PD-1/PD-L1 blockade together with vaccine therapy facilitates effector T-cell infiltration into pancreatic tumors. *J. Immunother.* 2015, 38, 1–11. [CrossRef] [PubMed]

33. Beyer, M.; Schultz, J.L. Regulatory T cells in cancer. *Blood* 2006, 108, 804–811. [CrossRef] [PubMed]

34. Manel, N.; Unutmaz, D.; Littman, D.R. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. *Nat. Immunol.* 2008, 9, 641–649. [CrossRef] [PubMed]

35. Regateiro, F.S.; Howie, D.; Nolan, K.F.; Ageroigannis, E.I.; Greaves, D.R.; Cobbold, S.P.; Waldmann, H. Generation of anti-inflammatory adenosine by leukocytes is regulated by TGF-beta. *Eur. J. Immunol.* 2011, 41, 2955–2965. [CrossRef] [PubMed]
36. He, S.; Fei, M.; Wu, Y.; Zheng, D.; Wan, D.; Wang, L.; Li, D. Distribution and clinical significance of Th17 cells in the tumor microenvironment and peripheral blood of pancreatic cancer patients. *Int. J. Mol. Sci.* **2011**, *12*, 7424–7437. [CrossRef] [PubMed]

37. McAllister, F.; Bailey, J.M.; Alsina, J.; Nirschl, C.J.; Sharma, R.; Fan, H.; Rattigan, Y.; Roesser, J.C.; Lankapalli, R.H.; Zhang, H.; et al. Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. *Cancer Cell* **2014**, *25*, 621–637. [CrossRef] [PubMed]

38. Wu, H.M.; Hwang-Verslues, W.W.; Lee, W.H.; Huang, C.K.; Wei, P.C.; Chen, C.L.; Shew, J.Y.; Lee, E.Y.; Jeng, Y.M.; Tien, Y.W.; et al. Targeting IL-17B-IL-17RB signaling with an anti-IL-17RB antibody blocks pancreatic cancer metastasis by silencing multiple chemokines. *J. Exp. Med.* **2015**, *212*, 333–349. [CrossRef] [PubMed]

39. Loncle, C.; Bonjoch, L.; Folch-Puy, E.; Lopez-Millan, M.B.; Lac, S.; Molejon, M.I.; Chuluyan, E.; Cordelier, P.; Dubus, P.; Lomberk, G.; et al. IL17 Functions through the Novel REG3beta-JAK2-STAT3 Inflammatory Pathway to Promote the Transition from Chronic Pancreatitis to Pancreatic Cancer. *Cancer Res.* **2015**, *75*, 4852–4862. [CrossRef] [PubMed]

40. Kurahara, H.; Shinchi, H.; Mataka, Y.; Maemura, K.; Noma, H.; Kubo, F.; Sakoda, M.; Ueno, S.; Natsugoe, S.; Takao, S. Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. *J. Surg. Res.* **2011**, *167*, e211–e219. [CrossRef] [PubMed]

41. Meng, F.; Li, C.; Li, W.; Gao, Z.; Guo, K.; Song, S. Interaction between pancreatic cancer cells and tumor-associated macrophages promotes the invasion of pancreatic cancer cells and the differentiation and migration of macrophages. *IUBMB Life* **2014**, *66*, 835–846. [CrossRef] [PubMed]

42. Monti, P.; Leone, B.E.; Zerbi, A.; Balzano, G.; Cainerca, S.; Sordi, V.; Pontillo, M.; Mercalli, A.; Di Carlo, V.; Alleva, P.; et al. Tumor-derived MUC1 mucins interact with differentiating monocytes and induce IL-10highIL-12low regulatory dendritic cell. *J. Immunol.* **2004**, *172*, 7341–7349. [CrossRef] [PubMed]

43. Karnevi, E.; Andersson, R.; Rosendahl, A.H. Tumour-educated macrophages display a mixed polarisation and enhance pancreatic cancer cell invasion. *Immunol. Cell Biol.* **2014**, *92*, 543–552. [CrossRef] [PubMed]

44. Helm, O.; Held-Feindt, J.; Grage-Griebenow, E.; Reiling, N.; Ungefroren, H.; Vogel, I.; Kruger, U.; Becker, T.; Ebsen, M.; Rocken, C.; et al. Tumor-associated macrophages exhibit pro- and anti-inflammatory properties by which they impact on pancreatic tumorigenesis. *Int. J. Cancer* **2014**, *135*, 843–861. [CrossRef] [PubMed]

45. Xu, S.; Chheda, C.; Ouhaddi, Y.; Benhaddou, H.; Bourhim, M.; Grippo, P.J.; Principe, D.R.; Mascarinas, E.; DeCant, B.; Tsukamoto, H.; et al. Characterization of Mouse Models of Early Pancreatic Lesions Induced by Alcohol and Chronic Pancreatitis. *Pancreas* **2015**, *44*, 882–887. [CrossRef] [PubMed]

46. Sainz, B., Jr.; Alcala, S.; Garcia, E.; Sanchez-Ripoll, Y.; Azevedo, M.M.; Cioffi, M.; Tatari, M.; Miranda-Lorenzo, I.; Hidalgo, M.; Gomez-Lopez, G.; et al. Microenvironmental hCAP-18/LL-37 promotes pancreatic ductal adenocarcinoma by activating its cancer stem cell compartment. *Gut* **2015**, *64*, 1921–1935. [CrossRef] [PubMed]

47. Gironella, M.; Calvo, C.; Fernandez-del Castillo, C.; Yajnik, V.; et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* **2003**, *425*, 851–856. [CrossRef] [PubMed]

48. Michl, P.; Gress, T.M. Improving drug delivery to pancreatic cancer: Breaching the stromal fortress by targeting hyaluronic acid. *Gut* **2012**, *61*, 1377–1379. [CrossRef] [PubMed]

49. Provenzano, P.P.; Cuevas, C.; Chang, A.E.; Goel, V.K.; Von Hoff, D.D.; Hingorani, S.R. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* **2012**, *21*, 418–429. [CrossRef] [PubMed]

50. Hingorani, S.R.; Harris, W.P.; Beck, J.T.; Berdov, B.A.; Wagner, S.A.; Pshevlotsky, E.M.; Tjulandin, S.A.; Gladkov, O.A.; Holcombe, R.F.; Korn, R.; et al. Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. *Clin. Cancer Res.* **2016**, *22*, 2848–2854. [CrossRef] [PubMed]

51. Thayer, S.P.; di Magliano, M.P.; Heiser, P.W.; Nielsen, C.M.; Roberts, D.J.; Lauwers, G.Y.; Qi, Y.P.; Gysin, S.; Fernandez-del Castillo, C.; Yajnik, V.; et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* **2003**, *425*, 851–856. [CrossRef] [PubMed]

52. Tian, H.; Callahan, C.A.; DuPree, K.J.; Darbonne, W.C.; Ahn, C.P.; Scales, S.J.; de Sauvage, F.J. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 4254–4259. [CrossRef] [PubMed]
Cancers 2017, 9, 93

53. Walter, K.; Omura, N.; Hong, S.M.; Griffith, M.; Vincent, A.; Borges, M.; Goggins, M. Overexpression of smoothened activates the sonic hedgehog signaling pathway in pancreatic cancer-associated fibroblasts. *Clin. Cancer Res.* 2010, 16, 1781–1789. [CrossRef] [PubMed]

54. Li, X.; Ma, Q.; Duan, W.; Liu, H.; Xu, H.; Wu, E. Paracrine sonic hedgehog signaling derived from tumor epithelial cells: A key regulator in the pancreatic tumor microenvironment. *Crit. Rev. Eukaryot. Gene Expr.* 2012, 22, 97–108. [CrossRef] [PubMed]

55. Mathew, E.; Zhang, Y.; Holtz, A.M.; Kane, K.T.; Song, J.Y.; Allen, B.L.; Pasca di Magliano, M. Dosage-dependent regulation of pancreatic cancer growth and angiogenesis by hedgehog signaling. *Cell Rep.* 2014, 9, 484–494. [CrossRef] [PubMed]

56. Heldin, C.H.; Miyazono, K.; ten Dijke, P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997, 390, 465–471. [CrossRef] [PubMed]

57. Yang, L.; Pang, Y.; Moses, H.L. TGF-beta and immune cells: An important regulatory axis in the tumor microenvironment and progression. *Trends Immunol.* 2010, 31, 220–227. [CrossRef] [PubMed]

58. Friess, H.; Yamanaka, Y.; Buchler, M.; Ebert, M.; Beger, H.G.; Gold, L.I.; Korc, M. Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. *Gastroenterology* 1993, 105, 1846–1856. [CrossRef]

59. Jaschinski, F.; Rothhammer, T.; Jachimczak, P.; Seitz, C.; Schneider, A.; Schlingensiepen, K.H. The antisense oligonucleotide trabedersen (AP 12009) for the targeted inhibition of TGF-beta2. *Curr. Pharm. Biotechnol.* 2011, 12, 2203–2213. [CrossRef] [PubMed]

60. Schnurr, M.; Duewell, P. Breaking tumor-induced immunosuppression with 5′-triphosphate siRNA silencing TGFbeta and activating RIG-I. *Oncoimmunology* 2013, 2, e24170. [CrossRef] [PubMed]

61. Tahara, H.; Sato, K.; Yamazaki, Y.; Ohyama, T.; Horiguchi, N.; Hashizume, H.; Kakizaki, S.; Takagi, H.; Ozaki, I.; Arai, H.; et al. Transforming growth factor-alpha activates pancreatic stellate cells and may be involved in matrix metalloproteinase-1 upregulation. *Lab. Invest.* 2013, 93, 720–732. [CrossRef] [PubMed]

62. Ostapoff, K.T.; Cenik, B.K.; Wang, M.; Ye, R.; Xu, X.; Nugent, D.; Hagopian, M.M.; Topalovski, M.; Rivera, L.B.; Carroll, K.D.; et al. Neutralizing murine TGFbetaR2 promotes a differentiated tumor cell phenotype and inhibits pancreatic cancer metastasis. *Cancer Res.* 2014, 74, 4996–5007. [CrossRef] [PubMed]

63. He, J.; Sun, X.; Qian, K.Q.; Liu, X.; Wang, Z.; Chen, Y. Protection of cerulein-induced fibrosis by pancreas-specific expression of Smad7. *Biochim. Biophys. Acta* 2009, 1792, 56–60. [CrossRef] [PubMed]

64. Creighton, C.J.; Gibbons, D.L.; Kurie, J.M. The role of epithelial-mesenchymal transition programming in invasion and metastasis: A clinical perspective. *Cancer Manag. Res.* 2013, 5, 187–195. [CrossRef] [PubMed]

65. Castellanos, J.A.; Merchant, N.B.; Nagathihalli, N.S. Emerging targets in pancreatic cancer: Epithelial-mesenchymal transition and cancer stem cells. *Onco Targets Ther.* 2013, 6, 1261–1267. [PubMed]

66. Sotomayor, E.M.; Borrello, I.; Tubb, E.; Rattis, F.M.; Bien, H.; Lu, Z.; Fein, S.; Schoenberger, S.; Levitsky, H.I. Conversion of tumor-specific CD4+ T-cell tolerance to T-cell priming through in vivo ligation of CD40. *Cancer Res.* 2012, 72, 720–732. [CrossRef] [PubMed]

67. Elgueta, R.; Benson, M.J.; de Vries, V.C.; Wasiuk, A.; Guo, Y.; Noelle, R.J. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immuno. Rev.* 2009, 229, 152–172. [CrossRef] [PubMed]

68. Beatty, G.L.; Torigian, D.A.; Chiorean, E.G.; Saboury, B.; Brothers, A.; Alavi, A.; Troxel, A.B.; Sun, W.; Teitelbaum, U.R.; Vonderheide, R.H.; et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* 2013, 19, 6286–6295. [CrossRef] [PubMed]

69. Lohr, M.; Kloppel, G.; Maisonneuve, P.; Lowenfels, A.B.; Luttges, J. Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis. *Neoplasia* 2005, 7, 17–23. [CrossRef] [PubMed]

70. Collins, M.A.; Bednar, F.; Zhang, Y.; Brisset, J.C.; Galban, S.; Galban, C.J.; Rakshit, S.; Flannagan, K.S.; Adsay, N.V.; Pasca di Magliano, M. Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *J. Clin. Invest.* 2012, 122, 639–653. [CrossRef] [PubMed]

71. Castellano, E.; Downward, J. Role of RAS in the regulation of PI 3-kinase. *Curr. Top. Microbiol. Immunol.* 2010, 346, 143–169. [PubMed]
72. Lesina, M.; Kurkowski, M.U.; Ludes, K.; Rose-John, S.; Treiber, M.; Kloppel, G.; Yoshimura, A.; Reindl, W.; Sipos, B.; Akira, S.; et al. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell* 2011, 19, 456–469. [CrossRef] [PubMed]

73. Yauch, R.L.; Gould, S.E.; Scales, S.J.; Tang, T.; Tian, H.; Ahn, C.P.; Marshall, D.; Fu, L.; Januario, T.; Kallop, D.; et al. A paracrine requirement for hedgehog signalling in cancer. *Nature* 2008, 455, 406–410. [CrossRef] [PubMed]

74. Mills, L.D.; Zhang, Y.; Marler, R.J.; Herreros-Villanueva, M.; Zhang, L.; Almada, L.L.; Couch, F.; Wetmore, C.; Pasca di Magliano, M.; Fernandez-Zapico, M.E. Loss of the transcription factor GLI1 identifies a signaling network in the tumor microenvironment mediating KRAS oncogene-induced transformation. *J. Biol. Chem.* 2013, 288, 11786–11794. [CrossRef] [PubMed]

75. Botta, G.P.; Reginato, M.J.; Reichert, M.; Rustgi, A.K.; Lelkes, P.I. Constitutive K-RasG12D activation of ERK2 specifically regulates 3D invasion of human pancreatic cancer cells via MMP-1. *Mol. Cancer Res.* 2012, 10, 183–196. [CrossRef] [PubMed]

76. Ying, H.; Kimmelman, A.C.; Lyssiotis, C.A.; Hua, S.; Chu, G.C.; Fletcher-Sananikone, E.; Locasale, J.W.; Son, J.; Zhang, H.; Coloff, J.L.; et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 2012, 149, 656–670. [CrossRef] [PubMed]

77. Wise, D.R.; Thompson, C.B. Glutamine addiction: A new therapeutic target in cancer. *Trends Biochem. Sci.* 2010, 35, 427–433. [CrossRef] [PubMed]

78. Ali, S.; Suresh, R.; Banerjee, S.; Bao, B.; Xu, Z.; Wilson, J.; Philip, P.A.; Apte, M.; Sarkar, F.H. Contribution of microRNAs in understanding the pancreatic tumor microenvironment involving cancer associated stellate and fibroblast cells. *Am. J. Cancer Res.* 2015, 5, 1251–1264. [CrossRef] [PubMed]

79. Pang, W.; Su, J.; Wang, Y.; Feng, H.; Dai, X.; Yuan, Y.; Chen, X.; Yao, W. Pancreatic cancer-secreted miR-155 implicates in the conversion from normal fibroblasts to cancer-associated fibroblasts. *Cancer Sci.* 2015, 106, 1362–1369. [CrossRef] [PubMed]

80. Kwon, J.J.; Nabinger, S.C.; Vega, Z.; Sahu, S.S.; Alluri, R.K.; Abdul-Sater, Z.; Yu, Z.; Gore, J.; Nalepa, G.; Saxena, R.; et al. Pathophysiologic role of microRNA-29 in pancreatic cancer stroma. *Sci. Rep.* 2015, 5, 11450. [CrossRef] [PubMed]

81. Valadi, H.; Ekstrom, K.; Bossios, A.; Sjostrand, M.; Lee, J.J.; Lotvall, J.O. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* 2007, 9, 654–659. [CrossRef] [PubMed]

82. Fabbri, M.; Paone, A.; Calore, F.; Galli, R.; Gaudio, E.; Santhanam, R.; Lovat, F.; Fadda, P.; Mao, C.; Nuovo, G.J.; et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proc. Natl. Acad. Sci. USA* 2012, 109, E2110–E2116. [CrossRef] [PubMed]

83. Jaffee, E.M.; Hruban, R.H.; Biedrzycki, B.; Laheru, D.; Schepers, K.; Sauter, P.R.; Goemann, M.; Coleman, J.; Grochow, L.; Donehower, R.C.; et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: A phase I trial of safety and immune activation. *J. Clin. Oncol.* 2001, 19, 145–156. [PubMed]

84. Lutz, E.; Yeo, C.J.; Lillemoe, K.D.; Biedrzycki, B.; Kobrin, B.; Herman, J.; Sugar, E.; Piantadosi, S.; Cameron, J.L.; Solt, S.; et al. A lethally irradiated allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: A Phase II trial of safety, efficacy, and immune activation. *Ann. Surg.* 2011, 253, 328–335. [PubMed]

85. Le, D.T.; Lutz, E.; Uram, J.N.; Sugar, E.A.; Onners, B.; Solt, S.; Zheng, L.; Diaz, L.A., Jr.; Donehower, R.C.; Jaffee, E.M.; et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. *J. Immunother.* 2013, 36, 382–389. [PubMed]

86. Lutz, E.R.; Wu, A.A.; Bigelow, E.; Sharma, R.; Mo, G.; Soares, K.; Solt, S.; Dorman, A.; Wamwea, A.; Yager, A.; et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. *Cancer Immunol. Res.* 2014, 2, 616–631. [CrossRef] [PubMed]

87. Le, D.T.; Wang-Gillam, A.; Picozzi, V.; Greten, T.F.; Crocenzi, T.; Springett, G.; Morse, M.; Zeh, H.; Cohen, D.; Fine, R.L.; et al. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J. Clin. Oncol.* 2015, 33, 1325–1333. [CrossRef] [PubMed]
88. Hassan, R.; Thomas, A.; Alewine, C.; Le, D.T.; Jaffee, E.M.; Pastan, I. Mesothelin Immunotherapy for Cancer: Ready for Prime Time? *J. Clin. Oncol.* **2016**, *34*, 4171–4179. [CrossRef] [PubMed]

89. Gong, Y.F.; Zhou, Q.B.; Liao, Y.D.; Mai, C.; Chen, T.J.; Tang, Y.Q.; Chen, R.F. Optimized construction of MUC1-VNTRn DNA vaccine and its anti-pancreatic cancer efficacy. *Oncol. Lett.* **2017**, *13*, 2198–2206. [CrossRef] [PubMed]

© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).