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An Argument to Examine Exosomal Survivin Splice Variant Expression and Patient Survival in Pancreatic Cancer

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Abstract

Important to pancreatic cancer (PCa) diagnosis and management is to determine an optimal combination of clinical indicators or biomarkers that could detect tumors early with high specificity/sensitivity and with limited invasiveness. In spite of the availability of a plethora of gene products considered as promising PCa biomarkers, it is recognized that their combined use with the available clinical information is still insufficient for early diagnosis and for guiding individualized therapeutic interventions and predicting outcomes [1]. Their main limitation is that they require invasive procedures such as biopsies. However, there is growing interest in using proteomics approaches to identify tumor-derived serum microvesicles called exosomes and their content, as serological biomarkers [2-8]. This interest stems from the notion that these blood components are considered "sensors" of molecular events associated with tumorigenesis [5,9,10]. One such target that may prove useful in early detection from PCa patient serum or plasma is the newly recognized exosomal protein Survivin [11] and its alternative splice variants. Validation that serum exosomes contain a specific panel of survivin splice variants may provide not only a cancer-specific marker of cancer but a means to identify disease in a non-invasive manner.

Introduction

PCa is the United State's fourth leading cause of cancer death in men and women [12]. In 2017 there will be 53,670 new cases of PCa diagnosed in the US with an estimated 43,090 deaths [12]. Adenocarcinomas make up 85% of all PCa with a range of 10 cases per 100,000 people worldwide [13]. It is the 8th leading cause of death from cancer in men and the 9th in women worldwide [13]. PCa is rarely diagnosed in persons younger than 40 years of age and has a median age at diagnosis of 71. More than 90% of patients diagnosed with PCa will die of it and 70% of these will die from metastasis, 30% with bulky primary tumors [14].

PCa risk factors include genetic syndromes, inheritance, family history, tobacco consumption and diets high in fat [15]. Some of the biological features associated with PCa are a high rate of activating mutations in KRAS (90%), distinct types of precursor lesions, local invasion and distant metastasis. Pancreatic adenocarcinoma is characterized with extensive stromal reaction leading to hypoxia and like most cancers PCa reprograms cellular metabolism and evades tumor immunity [1-18]. Common PCa symptoms include abdominal pain, weight loss, asthenia and anorexia [19]. Non-symptomatic jaundice is also a common manifestation of tumors in the head of pancreas and about 50% of PCa patients have diabetes [20]. To determine initial stage and treatment, abdominal CT is used to detect the arteries and veins involved [21].

The nature of the pancreatic tumor microenvironment makes it one of the most drug resisting cancers due to a dense stroma consisting of proliferating myofibroblasts (pancreatic stellate cells), type I collagen, hyaluronic acid, inflammatory cells, macrophages, mast cells, lymphocytes, and plasma cells. Additionally, the factors produced in the stroma such as connective-tissue growth factors promote survival of tumor cells [22]. The treatment of PCa depends on vessel involvement and metastasis. Surgery is the only potentially curative therapy with high success rate in resectable PCa stage 1 and 2. Only 15-20% patients are candidates for curative surgical resection [14]. Due to poor outcomes associated with surgery, adjuvant therapy is also used. Chemotherapy-using Gemcitabine or Fluorouracil improves survival. Multi-agent chemotherapy regimens such as the combination of fluorouracil, irinotecan, oxaliplatin, leucovorin (FOLFIRINOX) and gemcitabine

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plus albumin bound paclitaxel particles (nab-paclitaxel) have been shown to be more effective compared to single drug therapies [23]. Although FOLFIRINOX has been proven to provide better outcomes than single drug therapies, its ability to induce a resectable conversion was only 20% of all locally advanced PCa patients who were treated [23]. Additionally, toxicities were observed in patients who received this regimen. These results suggest that there is still need for further experimental therapy investigation for PCa. At present, numerous efforts are being made to improve treatment strategies for metastatic PCa, such as the search for new antimetabolite drugs, as well as using combinations of therapeutic agents.

Inhibitors of Apoptosis and Their Potential as Therapeutic Targets

Cancer is a disease that has acquired a number of molecular, biochemical and cellular changes which is common in most, even all types of cancer. These changes affect normal cellular physiology, are essential for malignant growth. The changes include independence from growth signals, loss of sensitivity to antigrowth signals, resistance to apoptosis, unlimited ability to replicate, angiogenesis maintenance, and invasion of tissue and metastasis [24]. Out of these acquired capabilities of cancer cells, we are most interested in the resistance to apoptosis. The inhibitor of apoptosis (IAP) family of proteins is of special interest, which includes cIAP1, cIAP2, XIAP, Livin (ML-IAP), NAIP, Bruce (apollon), ILP-2 and Survivin [25-27].

IAPs are characterized by an ~70 amino acid baculovirus IAP repeat (BIR) domain and a RING domain in the C-terminus of each family member [25,28]. IAPs are known to be endogenous caspase inhibitors [29]. Activated caspase-3, -7 and -9 are inhibited by cIAP1, cIAP2 and XIAP by directly binding to the caspases using their BIR domains [27,30-32]. Survivin is the smallest IAP family member and is the only IAP that has only one BIR domain and no RING domain, making Survivin structurally unique among the rest of the family [33]. Another unique feature of Survivin is its multifunctional role in various cellular activities, which includes the regulation of mitosis, protection from cell death, and adaptation to stressful environments [34,35]. Survivin is found to be localized in the cytoplasm, mitochondria and nucleus, with its subcellular location determining its function [36,37]. It has been shown that Survivin's role in the regulation of mitosis is carried out by a nuclear Survivin pool [38]. Alternatively, mitochondrial Survivin is able to suppress cell death in tumor cell lines and plays a part in tumorigenesis in immunocompromised mice [39].

IAPs are found over expressed in PCa compared to non-malignant pancreatic ductal cells or pancreatic tissues [40,41]. A number of approaches have been developed in order to target their expression and function in PCa IAP. To date, most have targeted XIAP as it is the most broadly expressed IAP and is the most consistent as well as potent [42]. These approaches include antisense oligonucleotides and RNA interference (RNAi) [26], second mitochondrial activator of caspases (Smac) peptides, and small molecule XIAP inhibitors [43,44]. Synthetic small molecule inhibitors such as Embelin from the Japanese Ardisia herb inhibits Survivin and XIAP resulting in increased apoptosis [45].

Survivin in Cancer & Treatment

Survivin expression is normally seen during the embryonic and fetal developmental stages, but is either low in expression or absent in tissues that are terminally differentiated. Survivin has also been shown to be present in highly proliferative adult cells, such as thymocytes, CD34+ bone-marrow-derived stem cells, T cells, vascular endothelial cells and gastrointestinal tract mucosa. Expression levels of Survivin in these cells are significantly lower compared to tumor cells, where there is a striking overexpression of this IAP in virtually every cancer type. High levels of Survivin expression in cancer cells have been associated with dismal prognosis, disease progression, metastatic dissemination, therapy resistance and overall dismal disease outcome [35,46,47]. Survivin levels were shown to promote radio-resistance in colorectal cancer cell lines and siRNA to survivin promoted increased levels of apoptosis with activation of caspase 3 and 7 in response to radiotherapy [48].

This group also investigated the effect of survivin levels on the risk of local relapse in rectal cancer patents. A 6% to 26% increased risk difference was observed in low levels of survivin compared to high levels [48]. Another study has shown that both Survivin mRNA and protein levels were higher in Cisplatin-treated gastric cancer cells compared to untreated cells [49]. Both these studies give indication that Survivin plays an essential role in chemotherapy and radiotherapy resistance, increasing the ability of cancer cells to evade apoptosis, thus providing cytoprotection to malignant cells [50].

To date, Survivin is one of the most tumor specific transcriptomes [35], and in addition to its presence in both solid tumor and hematopoietic malignancy, this IAP makes an exciting target for anti-cancer treatment. There have been many efforts in recent years to develop novel anti-cancer therapeutics targeting Survivin to both inhibit tumor growth as well as increase tumor cells' sensitivity to conventional chemotherapeutic agents [47,51]. Thus far, there are numerous strategies to target Survivin from mRNA to protein levels. Small molecule inhibitor YM155 acts by inhibiting transcription of Survivin mRNA, while anti-sense oligonucleotides, hammerhead ribozymes and siRNA are designed to degrade Survivin mRNA and/or inhibit protein translation. Strategies to inhibit Survivin at the protein level include small molecule antagonist sheperdin, which prevents Hsp90/Survivin interaction, as well as expression of two Survivin dominant negative mutants Cys84Ala and T34A into tumor cells introduced by plasmid or viral vectors [47,51].

In recent years, many studies have been accomplished to determine whether downregulation of Survivin could reverse chemotherapy and radiotherapy resistance in cancer cells. Several groups have shown that inhibition of Survivin expression by shRNA, RNAi, as well as emodin, a natural compound, re-sensitizes a variety of cancer cells, including squamous cell carcinoma of the tongue [50], osteosarcoma [52], breast cancer [53], and PCa [54,55] to cisplatin, adriamycin, and gemcitabine. All the Survivin based therapies mentioned previously have shown to be successful in decreasing Survivin expression levels, inhibiting further growth of malignant cells and increasing sensitivity to chemo- and radiotherapies.

Survivin Splice Variants

Alternative splicing, or differential splicing, is a regulated process during gene expression that results in a single gene coding for multiple proteins [56,57]. Inherited and acquired changes in pre-mRNA splicing have been documented to play a significant role in human disease development and many cancer-associated genes are regulated by alternative splicing [57]. Loss of fidelity, variation of the splicing process, and controlled switching to specific splicing alternatives may occur during tumor progression and could play a major role in

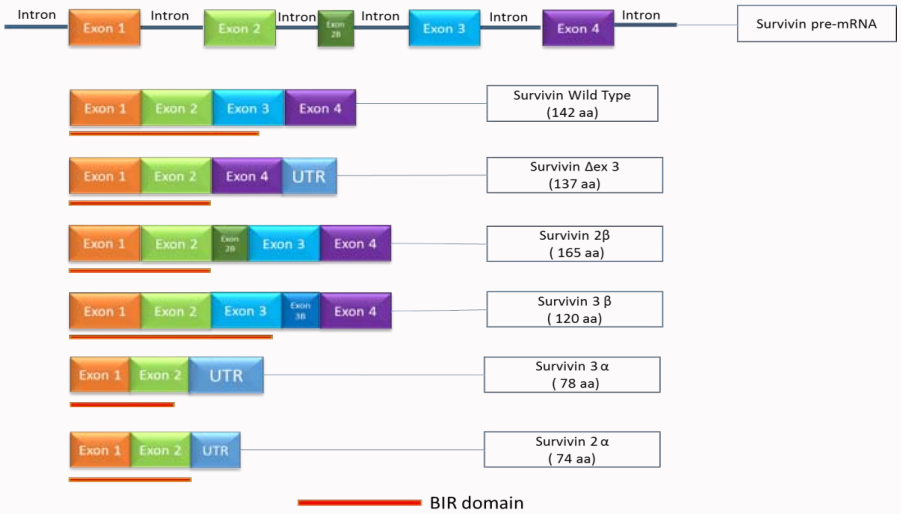

Figure 1: Survivin splice variants. **A** Survivin pre-mRNA has 5 exons (1, 2, 2B, 3, and 4) and 6 introns; **B** Survivin mRNA possesses exons 1, 2,3, and 4; **C** Survivin delta Ex3 (Δex 3) shows a loss of exon 3 and a frame shift with an extension of the reading frame into ORF of 3' UTR; **D** Survivin 2β has an additional exon 2β inserted between exons 2 and 3; **E** Survivin 3β has all regular 4 exons plus an additional exon 3β derived from intron 3 (165 bp) and flanked by exon 3 and 4; **F** Survivin 3α retains the intronic sequence of survivin-2β and an additional 32 nucleotides from the intron 2 as a cryptic exon; and **G** Survivin 2α has exons 1 and 2 plus 197 (195 are non-coding) nucleotides of the 3' end of intron 2 leading to an early stop just before exon 2 [82].

carcinogenesis. Splice variants that are found in tumors have clear diagnostic value and may provide potential drug targets [56,57]. Through alternative splicing, survivin pre-mRNA generates five splice variants including what is called wild type survivin itself along with survivin 2α survivin 2β, survivin 3α, survivin 3β and survivin delta Ex3 [56] (Figure 1). Survivin splice variants have been characterized in many cancers such as oral cancers [58], thyroid malignancies [59,60], breast cancer [61-66], pituitary tumors [67], acute myeloid leukemia, [68] pediatric acute precursor B lymphoblastic leukemia [69], colorectal cancer [70], laryngeal carcinoma [71], renal cell carcinoma [72], gastric cancer [73], uterine cervical carcinoma [74], astrocytoma [75], bladder cancer [76] and PCa [41]. Tabulating the presence and abundance of these six splice variants given their conflicting anti and pro-apoptotic characteristics (Table 1) could prove useful in determining and selecting either one or more to be able to predict the presence and aggressive nature of the disease and one day this code may be used to identify specific cancers.

Survivin 2α

Caldas et al. [77] first described survivin 2α, in 2005, in different malignant cell lines and primary tumors. Functional assays showed that Survivin 2α attenuates the anti-apoptotic activity of Survivin. It was also shown that Survivin 2α directly interacts and colocalizes subcellularly with Survivin during different stages of mitosis. Of potential interest is survivin 2α's high expression in the non-neoplastic surgical margin with concomitant low expression in the malignant thyroid nodule tissue [60]. Furthermore, findings in breast cancer showed survivin 2α being the dominant variant of survivin expressed in breast cancer [78]. These findings may supply further evidence of Survivin 2α's opposition to wild type Survivin's role but further confirmatory work must be accomplished and Survivin 2α's expression in other tumor types, including PCa, must be studied.

Survivin 2β

Survivin 2β results from the introduction of a 69-bp portion of intron 2 forming a new exon 2β [79]. The Survivin 2β protein has a molecular weight of 18.5 kDa, possessing a truncated BIR domain,

Table 1: Known apoptotic status of the six survivin splice variants.

Survivin Splice Variant	Apoptotic Phenotype
Survivin WT	Antiapoptotic
Survivin 2a	Proapoptotic
Survivin 2B	Proapoptotic
Survivin 3a	Believed to be Antiapoptotic
Survivin 3B	Antiapoptotic
Survivin ΔEx3	Conflicting between Anti- & Proapoptotic

and is thus believed to have pro-apoptotic functions with no evidence for a role in cell cycle regulation [80]. It has been shown, along with Survivin delta Ex3 to dimerize *in vitro* with its wild type homologue. However, it is unable to rescue siRNA-mediated effects of survivin depletion [81]. To date, little has been accomplished in dissecting the role of Survivin 2β in cancer progression or control and thus further evaluation of this splice variant must continue.

Survivin 3α

Survivin 3α contains 78 amino acids made up of 73 amino acids from the canonical Survivin protein and 5 additional amino acids MRELC [82]. Survivin 3α was found in breast cancer tumors and not in marginal tissues [78] and is thus currently considered important or indicative of the onset and progression of breast cancer [82]. There is therefore a need for more exploration into the role of this splice variant.

Survivin 3β

Vegran et al. [83] showed that Survivin 3β induced cancer cell resistance to natural killer cell cytotoxicity with a reduced cell viability in the Survivin 3β siRNA treated cells. In addition, Survivin 3β inhibited the apoptotic effect of FASL treatment suggesting that Survivin 3β may play a role in cancer resistance to therapy. This property might be attributed to its complete BIR domain, as truncating the BIR domain prohibited its antiapoptotic affects. Survivin 3β's antiapoptotic abilities was shown to result from its ability to engage procaspase-6 [83]. Additionally, siRNA directed to this splice variant

sensitized tumor cells to 5-FU [83]. Like patients expressing Survivin wild type, patients with high level of Survivin 3β expression had a shorter overall survival [66].

Survivin DeltaEx3

Survivin delta Ex3 lacks exon 3 causing a frameshift to alter the C-terminal end of the resulting protein of 137 amino acids instead of 142 amino acids of the wild type Survivin [79]. Having a disrupted BIR domain and the presence of a nuclear localization signal suggests that Survivin delta Ex3 prefers to reside in the nucleus [80]. It has been shown expressed in at least 13 different types of cancer but unlike its wild type isoform Survivin delta Ex3 has been described as having conflicting anti- and pro-apoptotic abilities [82,84]. In addition, when dimerized with Survivin it supports angiogenesis [85]. All in all, this Survivin isoform will continue to draw attention and may serve as a diagnostic tool in cancer evaluation and therapeutic monitoring.

Survivin Splice Variants have not Adequately been Investigated in PCa

Several studies have looked at the role that survivin plays in PCa biology. Glienke et al evaluated the role of increasing concentration of curcumin, a turmeric root derivative that has been considered as a potential PCa therapeutic agent [86]. In their results, they observed a correlation between survivin levels and increasing concentration in curcumin treated PCa cells [87]. This suggests that Survivin might serve as a target for PCa treatment. Survivin was also shown to play a role in radiation sensitivity and cell proliferation in PCa. In this study, Survivin transduction in MiaPaca2, the radiosensitive PCa cell line, resulted in an increased proliferation and less radio-sensitivity compared to vector transduced cells. Additionally, Panc1 cells, the radio-resistant cell line had enhanced radio-sensitivity when transduced with dominant negative Survivin gene using RT-PCR [88]. A study that evaluated the Survivin levels in correlation to chemotherapy found that the non-responder group showed high Survivin expression. However, this was evaluated in only 14 patients and thus no statistical significance [89]. Serum from PCa patients expressed higher levels of Survivin compared to serum from healthy controls. Additionally, serum Survivin levels were significantly associated with tissue Survivin expression. A positive correlation was also observed for serum Survivin with clinicopathological factors including TNM staging, lymphatic invasion, perineural invasion, venous invasion, cell differentiation and recurrence [90]. This study also found a correlation between Survivin expression in PCa and survival with high expression of Survivin with short survival [91]. Although Survivin has been sufficiently studied in PCa, the role of Survivin splice variants is yet to be studied in PCa.

Existence of Secreted Membrane Vesicles in Cancers

In the past few decades, extracellular vesicles have gained the attention of many scientists due to their presence in virtually all bodily fluids. According to their cellular biogenesis, there are three types of extracellular vesicles: microvesicles, apoptotic bodies and exosomes [92,93]. Among these three, exosomes- with a size ranging from 30-150 nM in diameter are the most studied. These vesicles have been shown to contain nucleic acids, miRNA, proteins and lipids [94,95]. All cells such as B- and T- lymphocytes, dendritic cells, neurons, intestinal epithelia cells as well as tumor cells release exosomes [96-98]. In particular, human and mouse tumor cells have been shown to secrete tumor cell-derived exosomes (TEX), constitutively into the extracellular space [99]. The morphology, density and certain membrane markers expressed, such as LAMP1, MHC class I, HSP70 and HSP80, on the released TEX are similar to the dendritic cell-derived exosomes (DEX) [100]. Despite similarities to DEX, there are differences in the molecular profiles and biological roles of TEXs, both of which give an indication of the cell of origin [101]. The specific protein content found on and within TEX not only reflects their origin, but in addition, establishes their functional role [102]. TEX secreted from neoplastic cells express diverse tumor antigens, which signify the type of tumor cells from where TEXs were released [103]. Elevated Glypican homologues have been associated with unfavorable prognosis in pancreatic ductal adenocarcinoma [104-106] and recently, the discovery of Glypican-1 in PCa exosomes has produced optimism for early diagnosis possibilities. *In vitro*, it has been shown that TEX released from breast carcinoma cells contain HER2, while carcinoembryonic antigen (CEA) was found in the exosomes secreted from colon carcinoma cells, and proteins MelanA/Mart-1 and gp100 that are expressed in melanoma cells are found on the released TEX [100,107]. This phenomenon is also evident *in vivo*, where plasma from cancer patients contain membrane vesicles that are characterized by the expression of tumor antigens which reflect the tumor of origin [108,109]. In PCa, TEX have been reported to facilitate the formation of pro-metastatic microenvironment in primary cancer tissue through the stimulation of angiogenesis [110,111], facilitate epithelial to mesenchymal transition [112], enhance tumor cell invasiveness [113], promote vascular destruction and invasion [114], regulate energy metabolism by regulating glucose uptake [115], and transport oncogenic abilities between different cell types [116]. TEX within the tumor microenvironment are aided by exosomes secreted from other cellular components of the primary PCa tissue [116].

When immunocompetent and nude mice were pre-treated with murine mammary TEX, an accelerated growth of the tumor was observed [117]. This observation led to various studies to try to elucidate the role of secreted membrane vesicles in cancer. TEX can be described as "multi-purpose carriers" which have important roles in the communication, protection, as well as the exchange of genetic information with neighboring cells [118]. The production and secretion of TEX is important for the tumor. They serve a protective function, have a supportive role in the survival and growth of the tumor cells, are involved in the promotion of host tissue invasion and subsequent metastasis, and facilitate evasion from the immune response [119,120]. Acting in a paracrine fashion, the diverse function of TEX is speculated to be due to the various bioactive molecules found within and on the vesicles having a strong influence on the surrounding environment [103,108,109,21].

The promotion of angiogenesis is due in part to the upregulation of Vascular Endothelial Growth Factor (VEGF) [122] and release of matrix metalloproteinases (MMPs) in neighboring, even distant endothelial cells, which are brought by TEX containing tetraspanin family members [124], epithelial growth factor receptor (EGFR) [124], platelet-derived tissue factor (TF) [125] or developmental endothelial locus-1 protein [108]. TEX has also been implicated in the further growth of tumor by the exchange of genetic material. Functional mRNA was detected within exosomes released from glioblastoma cells. Neighboring microvascular endothelial cells that take up the exosomes and translate the mRNA become liable for further tumor growth leading to the stimulation of angiogenesis [122]. In addition, tissue invasion and stromal remodeling can be facilitated by proteases

and MMP transport and release via exosomes [126,127]. These exosomes also provide a protective role to the cancer cells, which can be manifested in different ways. Survivin, a member of the inhibitor of apoptosis (IAP) protein family, was found to be released from tumor cells via exosomes [11]. The protective role of TEX can be attained by the accumulation and packaging of chemotherapeutic drugs or its metabolites into the vesicles, thus decreasing cellular levels of the drug, a factor leading to drug resistance [128,129]. This phenomenon has been observed in various cancer cells. Cisplatin enhanced the shedding of the vesicle from melanoma cells [130], while doxorubicin was found in the exosomes released from ovarian carcinoma cells [129]. Despite the beneficial roles of TEX for the tumor cells and the tumor microenvironment, TEX can be a useful tool for detecting the malignant condition. Serum levels of exosomes taken from cancer patients are significantly increased. These vesicles taken from serum [131], as well as from malignant tumor fluids, such as ascites fluids [132], pleural effusions [100] and urine [4] positively correlate with the tumor progression. Unfortunately, the process whereby exosomes are purified is quite arduous, making it not only difficult to isolate, but also difficult to replicate findings sample to sample [133]. It is also difficult, using commercial isolation techniques to use these now purified exosomes as treatment modalities [134].

Constitutive and Inducible Vesicle Secretion in Cancer and Cancer Therapy

In the tumor microenvironment, various changes are taking place, which could affect the release of vesicles, such as exosomes. Environmental stress such as chemo- and radio-therapy, can modulate TEX release and the biome they contain, inducing the tissues to adapt to changes taking place in the microenvironment [135]. Tumor cells that have undergone radiation or chemotherapy treatment have been shown to increase the release of TEX [136,137]. Interestingly, when treated with chemotherapeutic agents, there is a significantly enhanced membrane vesicle secretion in chemoresistant cells compared to chemosensitive cells. This activity may be a factor leading to drug resistance [128,129]. TSAP6 is an important cellular component as it regulates the secretion of protein via the non-classical pathway or the ER/Golgi-independent protein secretion pathway needed for the enhanced release of exosomes [136,138,139]. Normally, the secretion of exosomes in various cell types happens at a low rate. However, when p53 is activated, endosomal compartment activities are activated. Simultaneously, there is an increased expression of TSAP6, inducing the release of exosomes at a higher rate [140]. It is suggested that following p53 activation, exosomal release may act as a 'detoxifier' to expel unwanted chemotherapeutic agents [128-130,139]. Communication to the microenvironment is the other proposed role of TSAP6 and exosomal release after p53 activation, which may act as a warning signal to the neighboring cells, the immune system, and the extracellular matrix, that there are abnormal intracellular events happening [139,140].

TEX can be used as an important biomarker for the disease, which will give information not only on the disease progression, but also on the tumor type. As previously mentioned, TEX express specific tumor antigens, reflecting the protein content of the originating tumor, which gives an indication of the tumor type. The content of these vesicles can also be useful as markers for the aggressiveness of the disease.

Exosomal Survivin

Survivin is found localized in various subcellular locations. Depending on its function, this IAP is shown to be in the cytoplasm, mitochondria and nucleus [37,141]. Recently, our lab has discovered that Survivin exists in the extracellular space packaged in exosomes [142]. In addition we have shown that the extracellular pool of Survivin has the ability to cause neighboring cancer cells to become resistant to therapy, rapidly proliferate and acquire an increased potential to become invasive [142], providing a protective role to the neighboring tumor cells [93]. The ability of extracellular Survivin to cause these effects in the surrounding cancer cells is no surprise as an overexpression of this IAP is seen in virtually every human cancer type [35]. TEX as biomarkers can be also used as tools to detect malignant conditions. Serum taken from cancer patients had an increased level of TEX [126,131], which had a positive correlation with the progression of the tumor [93]. In addition to serum, TEXs were shown to be isolated from malignant tumor fluids, such as urine [4], ascites fluids [143] and pleural effusions [100]. We have recently shown that exosomal Survivin may be a useful tool for early detection and diagnosis or even monitoring prostate cancer progression. Newly diagnosed and advanced prostate cancer patients with high or low-grade cancer had significantly higher levels of exosomal Survivin compared to control subjects or patients with pre-inflammatory BPH [144,145].

Survivin Splice Variants in Exosomes

Our lab has for the first time identified some of survivin splice variants in exosomes isolated from breast cancer patients' sera. These survivin splice variants may prove useful as potential diagnostic biomarkers and/or prognostic markers. In our study, survivin 2β protein expression levels varied the most among breast cancer stages [63] with little changes in the other variants investigated. It will be important to continue to evaluate these splice variants in PCa exosomes in order to more fully control and identify pancreatic cancers at a time when treatment might be affective.

Conclusion

Most efforts on the identification of candidate PCa biomarkers, and on analyzing differences in PCa biology between therapeutic sensitive and resistant patients, have emphasized the analysis of differential gene expression in tumor tissues, methylation patterns, or single nucleotide polymorphisms (SNPs) [146-149]. While these efforts are necessary and provide important clues for understanding biological mechanisms associated with PCa treatment disparities, it is also imperative to develop innovative, non-invasive approaches that analyze indirectly and early in the disease process, the molecular profile of pancreatic tumors. Small membrane-bound vesicles called exosomes constitute the latest mode of intercellular information transfer or communication [150,151]. This exchange of molecular information is facilitated by their unique composition, which is enriched with enzymes, structural proteins, adhesion molecules, lipid rafts, microRNAs (miRNAs), RNAs and double stranded DNA [150,152]. Importantly, cancer cells have been shown to secrete more exosomes than do their normal counterparts indicating that exosomes can be used as diagnostic markers and their active secretion has functional implications. In addition, genes involved in inflammation and autoimmune responses are differentially upregulated in PCa patients compared to controls [153-156]. This implies that differences in antitumor immune responses may exist between these disease groups in pancreatic tumors. We have recently shown Survivin's exosomal presence and the possibility that Survivin-containing exosomes, once secreted into the tumor microenvironment have

not only the ability to modulate the immune system's response the tumor [157] but that a number of Survivin's splice variants are also exosomally localized [63]. We propose that by better understanding the role these TEX and the Survivin splice variants they contain play in modulating the pancreatic tumor microenvironment the better early diagnosis for this most horrible of cancers may one day become.

Author Contribution

Conception and design: (NRW). Wrote the first draft of the manuscript: (JK). Jointly developed the structure and arguments for the paper: (JK, NRW). Contributed to the writing of the manuscript: (JK, NRW). Made critical revisions and approved final version: (JK, NRW). Responsible for all aspects of this research: (NRW). All authors reviewed and approved the final manuscript: (JK, NRW).

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