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RESEARCH ARTICLE

THE UNSUCCESSFUL HUNT FOR PANCREATIC CANCER BIOMARKERS – TIME TO SEARCH DEEPER IN THE PROTEOME

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ABSTRACT

Proteomics has been successful in the identification of reliable diagnostic and prognostic biomarkers for a wide variety of malignancies. In addition, therapeutic biomarkers have allowed for the identification of radio- or chemo-resistant phenotypes and the selection of sub-type-specific therapies. However, the hunt for biomarkers of any kind related to pancreatic cancer has so far turned up unfruitful. The few high-potential candidates which have been found so far, still do not show the desired sensitivity and specificity. In this light, it is hence better to rely on a combination of proteins in the form of a biomarker panel, which attempts to cover the entire spectrum of the disease state. The observed heterogeneity and the presence of cancer stem cells in different pancreatic cancers are important issues that need to be tackled efficiently in the search for significant biomarkers. With the next-generation of mass spectrometers combining quadrupole, orbitrap, and ion trap mass analysis systems it is now time to go beyond the search for biomarkers at a whole protein level and start looking at post-translational modifications (PTMs) such a phosphorylation, ubiquitination, acetylation and methylation for subtle changes which are known to have a dramatic effect on protein activation, function or half-life. This makes PTMs a key focus for future investigations in this area of research.

INTRODUCTION

Most proteomic studies analyse protein expression profiles through a collection of methods referred to as 'expression proteomics' (Blackstock and Weir, 1999), in an attempt to isolate and identify biomarkers, for subsequently confirmation by immunohistochemistry or western blotting. Cancer biomarkers give quantifiable information of aberrant cellular processes and thus are not only useful to understand what is happening at a molecular level but also as a direct clinical tool. At a clinical level, diagnostic biomarkers are used to classify a tumourhistopathologically, prognostic markers provide information about the potential malignancy of a tumour, and finally predictive biomarkers direct clinicians to more effective treatment regimens based on therapeutic sensitivity. So far the available biomarkers for pancreatic cancer are scarce and unreliable. The standard pancreatic cancer biomarker is Carbohydrate Antigen 19-9 (CA19-9), which has been used for decades together with Carcinoembryonic antigen (*CEA*) and Kras mutations to diagnose and classify pancreatic cancers (Satake *et al.*, 1994; Steinberg, 1990; Posner and Mayer, 1994). A detailed review of CA19-9 use for diagnosis, prognosis as well as in monitoring has recently been published (Duffy *et al.*, 2010). However, CA19-9 is not expressed in individuals with a Lewis negative (Le a- and Le b-) genotype (Takasaki *et al.*, 1988) and not sensitive enough for early or small-diameter pancreatic cancers (Steinberg, 1990) or poorly differentiated pancreatic cancers compared to moderately or well-differentiated tumours (Steinberg, 1990).

Apart from that, several diseases including chronic and acute pancreatitis, liver cirrhosis, hepatitis, cholangitis, obstructive jaundice, as well as various gastrointestinal cancers (bile duct cancer, gastric cancer, colorectal cancer, esophageal cancer and hepatocellular carcinoma) may present elevated CA19-9 levels (Gullo, 1994; Duffy, 1998; Lamerz, 1999; Steinberg, 1990; Goonnetilleke and Siriwardena, 2007; Albert *et al.*, 1988).

Combining various biomarkers into a panel

The isolation and identification of pancreatic cancer biomarkers is difficult due to the lack of specific symptoms which results in late diagnosis. This also implies that studies on pancreatic cancer are generally carried out on terminal stage tumour samples, by which time a tumour has undergone several compound changes at a DNA, protein and post-

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translational level. Additionally, each tumour harbours a number of sub-populations presenting different molecular aberrations (and hence clinical phenotypes), which can potentially mask and nullify changes in individual protein expression profiles. Recently, Harsha *et al.* (2009) reviewed the literature describing changes observed in pancreatic cancers at a transcriptional and translational level, which might have biomarker potential. In total, 2,516 genes were included of which, 1,868 genes were over-expressed at the mRNA level, 207 genes were over-expressed at a protein level, and most interestingly 441 genes over-expressed at both levels. Of the over-expressed genes identified, 162 genes were for secreted proteins and 166 genes were for membrane-bound proteins (at both RNA and protein levels), which give ample choice for easily accessible biomarkers. The downside of all this was that only 200 genes (less than 8%) were reported to be over-expressed in four or more pancreatic cancer studies.

In the light of all this, there is a drive towards improved detection including the availability of a screening tool for asymptomatic individuals.With the extent of putative biomarkerinformation available resulting from changes undergone by the various forms of pancreatic cancer, the aim should be to develop a panel of reliable biomarkers, for the combined assessment of various biochemical pathways. This should result in an increased sensitivity and specificity in comparison to using any single biomarker (Makawita *et al.*, 2011). The use of such a panel of biomarkers should improve the chances of detecting and treating pancreatic cancer at an earlier stage. When it comes to choosing the type of proteins to be included in such a screening panel for routine clinical use, the preference is for proteins available in serum, or other body fluids such as urine, as these can be easily collected and assayed essentially since tissue specimens are difficult to obtain, requiring invasive procedures and biopsies. Proteins in serum may increase as a result of release by the tumour or decrease as a result of protein degradation processes within the tumour (Wulfkuhle *et al.*, 2003). Membrane-bound proteins are often found in serum as they are shed by the tumour and are attractive candidates for inclusion in a screening panel because they represent the majority of drug targets use to treat cancer and can direct towards an effective therapy in addition to being useful for imaging of tumours.

Understanding the level of heterogeneity

In order to better assess the viability of such biomarkers, it is useful to look at the precursor lesions of pancreatic cancer including pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) (Hruban *et al.*, 2001; Hruban *et al.*, 2004; Maitra *et al.*, 2005), although the frequency and speed of this progression is not as yet known. Thus potential pancreatic cancer biomarkers also over-expressed in such precursor lesions (particularly PanIN-3) are possibly the most promising diagnostic markers. Approximately 1,100 genes were found to be over-expressed in Pan INs and IPMNs, most of which were also being expressed in invasive pancreatic ductal adenocarcinomas (PDACs) (Buchholz *et al.*, 2005b). To initially screen the number of pancreatic cancer biomarkers reported, it is important to disregard those proteins that are common to pancreatitis, especially considering that 40% of proteins reported to be differentially expressed in pancreatic cancer, undergo a similardysregulation in chronic pancreatitis (Chen *et al.*, 2007a; Chen *et al.*, 2007b). However, there is as yet no clear agreement in the literature as to the list of such proteins with many contradictory reports. Furthermore, it was shown using biological network analysis,that the transcription factor c-MYC is prominent in both pancreatic cancer and chronic pancreatitis(Chen *et al.*, 2007a). This does not mean that there is no significance to such common elements, as patients with chronic pancreatitis have a 2-fold increased risk of pancreatic cancer. There are underlying mechanisms common to both disease which can be unravelled using the observed commonalities at both a gene and protein level. Specialised techniques have been applied to better isolate sub-populations of interest within a tumour and further increase sample specificity. Laser Capture Microscopy (LCM) has proved to be excellent at separating sub-populations from heterozygous samples (Emmert-Buck *et al.*, 1996) in sufficient amounts for downstream analysis such as antibody arrays (Knezevic *et al.*, 2001) or 2D-electrophoresis (Wulfkuhle *et al.*, 2002, Craven *et al.*, 2002), which thanks to post-labelling techniques can be carried out with as few as 10,000 cells (Zang *et al.*, 2004).LCM has been successfully applied to the analysis of pancreatic cancer proteomics (Shekouh *et al.*, 2003).

Pancreatic cancer stem cells (CSCs)

The study ofpancreatic cancer stem cells (CSCs) are as yet another special sub-class of cells and are considered of extremely high prognostic value. Pancreatic CSCs are of particular interest because they dysregulate cell proliferation and are resistant to apoptosis as well as being associated with inflammation and metastasis (Dai *et al.*, 2010), however they constitute just 0.2-0.8% of the total tumour cells. One such isolated sub-population of $CD44^{+}CD24^{+}ESA^{+}$ pancreatic tumour cells was shown to possess CSC properties including self-renewal and differentiation (Li *et al.*, 2007). The advantage of looking at proteins impacting the tumorigenic ability of CSCs is that these are linked to a restricted number of pathways, with mitochondrial dysfunction being central, as a consequence of its role in apoptosis and tumor genesis (Newmeyer and Ferguson-Miller, 2003; Lu *et al.*, 2009; Bapat, 2007), together with inflammation, which has been shown to possibly accelerate the process of mutagenesis and mutation accumulation (Lee *et al.*, 2008; Guerra *et al.*, 2007; Dai *et al.*, 2010).

Comparing expression profiles of pancreatic tumour CSCs with their non-CSC counterparts lead to the isolation of two interesting proteins, inter-alpha try sin inhibitor H3 (ITIH3) and mitochondrial apoptosis-inducing factor (AIFM1) (Dai *et al.*, 2010). ITIH3 (a downstream target genes of Sonic Hedgehog (Shh)) has been linked to inflammatory response in local tissue (Zhou and Kimata, 2008) and over-expression of ITIH3 corroborates up-regulated Shhm RNA expression in pancreatic CSCs (Li *et al.*, 2007), important for the self-renewal and apoptosis-inhibition functions of CSCs (Kato *et al.*, 2001). Similarly, AIFM1 inactivation is known to make embryonic stem cells resistant to cell death (Joza *et al.*, 2002). Other proteins reported to be dysregulated in CSCs include NF-κβ, c-MET and CXCL5(Donahue and Hines, 2009; Wente *et al.*, 2006; Hansel *et al.*, 2004; Birnie *et al.*, 2009; Alvero *et al.*, 2009; Dai *et al.*, 2010).

Promoising protein candidates

Despite the need for such an elimination process to be carried out with care and attention based on sound and reproducible protein data, there are a small number of very promising candidates which deserve further mention and particular attention.

Fibrinogen and plasminogen

Fibrinogen β and γ chains and fibrinogen precursors were reported to be higher in pancreatic cancer serum and juice than normal controls (Bloomston *et al.*, 2006; Chen *et al.*, 2005a; Charlton *et al.*, 1999). Immuno-histochemistry has shown that fibrinogen exists throughout the tumour stroma but the pancreatic islets lack fibrinogen in both chronic pancreatitis and pancreatic cancer, with the highest expression being in the cytoplasm of pancreatic cancer cells (Wojtukiewicz *et al.*, 2001; Bloomston *et al.*, 2006).Thus tumour cells are surrounded by fibrin (Wojtukiewicz *et al.*, 2001) and the adjacent stromal fibroblasts have been shown to promote pancreatic cancer progression (Hwang *et al.*, 2008). This up-regulation of fibrinogen in pancreatitis juice may be due to its role as a major acute response protein involved in the inflammation of pancreas (Chen *et al.*, 2007b).

The role of fibrinogen and fibrinogen degradation products in carcinogenesis has been suggested for various tumor types (Gerner *et al.*, 2001; Palumbo *et al.*, 2005; Palumbo *et al.*, 2002; Bloomston *et al.*, 2006). This is thought to occur through deposition of fibrin or fibrinogen which induces fibrinolytic activity, mainly via plasmin, which leads to the degradation of the extracellular matrix aggravating proliferation, invasion and metastasis (Gerner *et al.*, 2001; Pollanen *et al.*, 1991; Hatzfeld *et al.*, 1982; Wojtukiewicz *et al.*, 2001). Although fibrinogen has been associated with various malignancies, there are known ties between pancreatic cancer and fibrinogen storage disease (Radhi and Lukie, 1998) or migratory thrombophlebitis (Trousseau, 1865). Plasminogen has been shown to be concomitantly differentially expressed in pancreatic cancer (Bloomston *et al.*, 2006) with a down-regulation of plasminogen reported from the secretome of pancreatic cancer (Grønborg *et al.*, 2006). In addition to its role in inflammation, plasminogen activation is known to be a key factor in invasion and metastasis (Andreasen *et al.*, 2000; Schmitt *et al.*, 1997). In fact the generation of plasmin at the surface of pancreatic cancer cells is considered a key event in invasion and metastasis (Andreasen *et al.*, 2000; Schmitt *et al.*, 1997).

S100 protein family

The S100 gene family consists of over 20 members distinguished in part by the calcium binding EF-hand motif (Heizmann *et al.*, 2002). A number of S100 family genes including S100A2, S100A4, S100A6, S100A8, S100A11, and S100P have been shown to be over-expressed at an mRNA (Friess *et al.*, 2003; Han *et al.*, 2002; Logsdon *et al.*, 2003) and/or protein level (Shekouh *et al.*, 2003; Shen *et al.*, 2004) in pancreatic cancer. Such an up-regulation has been observed at different stages of various human cancers suggesting that S100 family proteins might play a role in carcinogenesis (Emberley *et al.*, 2004). In disagreement, S100A2 has been found to be down-regulated in the secretome of pancreatic cancer (Grønborg *et al.*, 2006). Although S100 protein dysregulation is not unique to pancreatic cancer (Luo *et al.*, 2004; El-Rifai *et al.*, 2002; Zucchini *et al.*, 2001; Ott *et al.*, 2003) they are an important consideration because of their role in cell survival, apoptosis and drug resistance (Sommer *et al.*, 2003) particularly S100A4 is a suitable pancreatic cancer biomarkeras it is thought to mediate drug resistance through the BCL2/adenovirus E1B 19 kd-interacting protein 3 (BNIP3) gene regulation, a hypoxia-induced pro-apoptotic gene (Mahon *et al.*, 2007; Erkan *et al.*, 2005).

Annexins

The annexins are a very large family of proteins which share in common the 'annexin repeat' and the ability to bind negatively-charged phospholipids in a calcium-dependent manner (Gerke and Moss, 2002). Members of this protein family that have been reported to be dysregulated in pancreatic cancer include annexin 1, annexin A2 and annexin A8 (Bai *et al.*,2004; Han *et al.*, 2002; Nedjadi *et al.*, 2009; Takano *et al.*, 2008; Vishwanatha *et al.*, 1993; Karanjawala *et al.*, 2008) with the most promising being annexin A2, even though over-expression of annexin A2 has been observed in othercancer types (Zimmermann *et al.*, 2004; Sharma *et al.*, 2006; Syed *et al.*, 2007; Gillette *et al.*, 2004; Huang *et al.*, 2008; Duncan *et al.*, 2008). An immunohistochemical study has shown no or mild annexin A2 expression in acinar cells, ductal cells, and islet cells of normal pancreas, while 93% (118/127) of pancreatic ductal adenocarcinoma samples showed strong expression (Chen *et al.*, 2005b). Over-expression of annexin A2 at an mRNA (Kumble *et al.*, 1992; Vishwanatha *et al.*, 1993) and protein level (Crnogorac-Jurcevic *et al.*, 2005; Chen *et al.*, 2005a; Lu *et al.*, 2004; Buchholz *et al.*, 2005a; Sitek *et al.*, 2005) and is a suitale biomarker candidate as it is not over-expressed in chronic pancreatitis (Chen *et al.*, 2007a) has been extensively reported in pancreatic cancer. Annexin A2 and A4 could also be used to distinguish pancreatic cancer from pancreatitis as these proteins are only over-expressed in the former (Shen *et al.*, 2004; Chen *et al.*, 2007a). Additionally, annexin A3 and annex in 5 have been found to be down-regulated in pancreatic cancer secretome (Grønborg *et al.*, 2006) making them easily accessible for analysis.

IGFBP2

In pancreatic cancer Insulin-like Growth Factor-binding Protein 2 (IGFBP-2) was found to be over-expressed at an mRNA level (Nakamura *et al*., 2004) and at a protein level in pancreatic juice (Chen *et al.*, 2006), while in chronic pancreatitis the protein levels of IGFBP-2 are similar to those in normal pancreas (Chen *et al.*, 2007a).IGFBP-2 has however been shown to be over-expressed in the serum and cerebrospinal fluid of patients with ovarian cancer, malignant solid tumors, acute leukemiaand hepatocellular carcinoma (Flyvbjerg *et al.*, 1997; Muller *et al.*, 1994; Ranke *et al.*, 2003).

AGR2

Anterior gradient homolog 2 (AGR2) was first isolated in *Xenopus laevis* in which it plays a role in ectodermal patterning (Aberger *et al.*, 1998), however the function of

human AGR2 is as yet largely unknown. It is known to be up-regulated invarious human cancers including pancreatic cancer and possibly serum (Liu *et al.*, 2005; Park *et al.*, 2011; Pohler *et al.*, 2004; Zhang *et al.*, 2005; Barraclough *et al.*, 2009; Fritzsche *et al.*, 2007; Zhang *et al.*, 2007; Chen *et al.*, 2010; Makawita *et al.*, 2011) AGR2 has been shown to play a role in invasion, metastasis and poor prognosis (Smirnov *et al.*, 2005; Barraclough *et al.*, 2009; Ramachandran *et al.*, 2008; Zhang *et al.*, 2010). AGR2 is not normally expressed in the pancreas but its expression has been confirmed in all sporadic and familial samples from the earliest PanINs to late stage PDACs as well as circulating tumour cells and metastases (Sitek *et al.*, 2009; Dumartin *et al.*, 2011).

In one study, expression of AGR2 led to its localisation to the endoplasmic reticulum (ER) and the external surface of tumour cells and resulted in the up-regulation of three chaperone proteins (PDI/P4HB, CALU and RCN1) as well as the deregulation of several proteins within the ubiquitin-proteasome degradation pathway (HIP2, PSMB2, PSMA3, PSMC3, and PSMB4) (Dumartin *et al.*, 2011). In another, AGR2 expression resulted in over-expression of the lysosomal proteases cathepsin B and cathepsin D, as reported in pancreatic cancer by various authors (Shen *et al.*, 2004; Tumminello *et al.*, 1996; Iacobuzio-Donahue *et al.*, 2003), including up-regulation of cathepsin B and cathepsin D in the secretome of pancreatic cancer (Grønborg *et al.*, 2006). Cathepsins are known to play a role in the dissemination of cancer cells (Rao, 2003; Joyce *et al.*, 2004; Tzanakakis *et al.*, 2003). Cathepsin D up-regulation has also been observed in pancreatitis (Chen *et al.*, 2007a). When on the other hand, AGR2 was silenced in the pancreatic cancer cell line MPanc-96, these cells showed a decrease in metastatic ability (Ramachandran *et al.*, 2008). Additionally, it was found that the level of AGR2 expression has a directly proportional relationship to the invasiveness of pancreatic cancer cells (Dumartin *et al.*, 2011).

mTOR-related proteins

Ras proteins are small GTPases essential in signaling pathways that control the transduction of growth and differentiation signals, regulating various important cellular operations, from the receptor tyrosine kinases to the cell nucleus, where gene transcription is initiated (Khosravi-Far and Der, 1994). It has been known for a long time that most pancreatic cancer harbour specific activating KRAS mutations, which occur early on in pancreatic cancer progression, indicating it as a key factor in this initial phase (Hruban *et al.*, 1993) however Ras inhibitors failed in pancreatic cancer phase III trials (Van Cutsem *et al.*, 2004). The Mammalian target of rapamycin (mTOR) pathway is a downstream target of Ras. The mTOR pathway is known to regulate both the translation initiation and the inactivation of 4E-binding protein (4E-BP1) and both mTOR and P70-S6 Kinase 1 were found to be activated in all of the pancreatic cancer cell lines tested (Ito *et al.*, 2006). When phosphoinositol 3-kinase (PI3K) is phosphorylated it stimulates the catalytic activity of Akt, which leads to the phosphorylation of a variety of proteins that affect processes such as cell growth, cell cycle entry and cell survival having many implications for cancinogenesis (Vara *et al.*, 2004). In a preclinical study it has been shown that pancreatic cancer xenografts can be induced to apoptose in a dose-dependent manner by administering PI3K inhibitors such as wortmannin and LY294002 (Ng *et al.*, 2001). PI3K inhibitors have also been shown to increase apoptosis induced by gemcitabine directly proportional to concentration (Ng *et al.*, 2000) although another study disagrees (Arlt *et al.*, 2003). The reduction in phosphorylated Akt levels correlates directly to the increase in gemcitabine-induced apoptosis, suggesting that the mTOR pathway is one of the major pathways by which pancreatic cancer becomes resistant to apoptosis caused by chemotherapy or molecular targeting agents (Ng *et al.*, 2000).

Another important target of Akt is c-MYC and in fact a number of Ras-dependent phosphorylation pathways have been shown to regulate Myc protein stability (Sears *et al.*, 2000). The regulatory protein c-MYC interacts with five differentially expressed proteins observed in a pancreatic cancer study, namely HBB protein, integrinβ1, NDRG1 protein, thioredoxin, and tropomyosin 2. Apart from this, the same study identified four other transcription factors important in the network which were c-FOS, c-JUN, NF-κB1, and p53 (Chen *et al.*, 2007a). PI3K-mediated signaling can be terminated by phosphatase and tensin homologue deleted from chromosome 10 (PTEN), a tumour suppressor gene, which acts as a lipid phosphatase that regulates major signal transduction pathways and is particularly important in embryonic development, cell migration and apoptosis. In solid tumors where PTEN is mutated there is activation of the PI3K/Akt pathway, resulting in resistance to apoptosis. In pancreatic cancer however PTEN is not mutated, but in over 60% of cases it is functionally suppressed due to loss of expression (Maitra and Hruban, 2005).

The potential of post-translational modifications (PTMs)

The above data reflects the importance of screening for, analysing and understanding PTMs (particularly phosphorylation) to provide a clearer picture of the activation state of kinase pathways, particularly those involved in metastasis and apoptosis. Such an argument can be extended to other protein properties related to their function, which affect the working order of a cell in both normal and disease states. While most of the routine lab based protein analysis of tumour samples is done by Western Blotting with antibodies that recognise an unknown epitope within the protein, the more detailed and sensitive proteomic work is done by mass spectrometry. First generation mass spectrometers, despite being sensitive enough to identify proteins from nanogram quantities of peptides isolated using two-dimensional polyacrylamide gel electrophoresis (2D PAGE) methods, could not be used to determine the presence and position of PTMs.

Next-generation mass spectrometers have greatly improved the resolution of protein analysis by combining quadrupole, orbitrap, and ion trap mass analysis systems. This has now enabled scientists to sequence proteins and identify the position (on which amino acid residue) and degree (e.g. mono, di- or tri-methylation) of PTMs based on the shift in mass over charge (m/z) resulting from the adition of such small chemical groups as a phosphate or a methyl group. In terms of the variety of PTMs, the list is quite extensive, but among those that seem to show most potential as biomarkers in relateion to

cancer are phosphorylation, ubiquitination, acetylation and methylation. Each one fulfils a different biological role and as such provides information as to unique processes of dysregulation. Phosphorylation (the addition of a phosphate group to serine, threonine or tyrosine residues) is synonymous with protein activation and as mentioned above aberrant phosphorylation can be one of the hallmarks of a sub-set of pancreatic cancers with implications in cell growth, survival, apoptosis, or response to extracellular stimuli. On the other hand, ubiquitination (the addition of a chain of ubiquitin monomers onto a lysine residue)is mostly linked to protein stability and half-life as it generally leads to proteosomal degradation, while acetylation (the addition of an acetyl group to lysine residues) is generally linked with activation, mainly through the alteration of DNA-binding properties. The importance of methylation (the addition of a methyl group to a lysine or arginine) in protein function and tumourigenesis unfortunately is still not well characterised and understood. However, many ubiquitination, acetylation and methylation sites overlap, which may suggest a competition between the three modifications and an inbalance in which could lead to catastrophic downstream effects. Thusnow is the time to start considering the role of PTMs in pancreatic cancer as a viable option for biomarker searches in various stages of the disease and looking for processes which share common dysregulation at a post-translational level. The PTM data collected from such studies would most definitely have similar importance in other malignancies, as a number of common processes take place during cancer initiation and progression.

Conclusion

The volume of data available for pancreatic cancer is vast but the region of overlap between the results is disappointingly small. This is mainly due to the fact that pancreatic cancer displays no symptoms until the very late stages of malignancy, most of which are not specific. By that time, the tumour has undergone a number of changes and formed various sub-populations and possibly even metastases, making the identification of biomarkers difficult to say the least. When looking at the total levels of a protein in cells, it unfortunately groups together all the different variants present. Individual peptides may be presenting PTMs which fulfill a variety of important functions (activation, change conformation, mark for degradation) but this information is not being collected when using traditional proteomic methods. Thus it is now time to start going beyond and looking for answers at a deeper protein level. This is achievable with the current technology and will definitely improve our understanding of malignant transformation, protein biology and possibly provide us with better biomarkers and direct us towards better therapeutics for pancreatic cancer.

REFERENCES

Aberger, F., Weidinger, G., Grunz, H. and Richter, K. 1998 Anterior specification of embryonic ectoderm: the role of the Xenopus cement gland-specific gene XAG-2. *Mech. Dev.,* 72**,** 115–130

Albert, M.B., Steinberg, W.M. and Henry, J.P. 1988. Elevated serum levels of tumor marker CA 19-9 in acute cholangitis. *Dig Dis Sci*., 33: 1223–1225.

Alvero, A.B., Fu, C. R., Montagna, H.H., Schwartz, M., Rutherford, P.E., Silasi, T., Steffensen, D.A., Waldstrom, K.D., Visintin, M., Mor, I. and Molecular, G. 2009. Phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemo resistance. Cell *Cycle.,* 8(1):158–66.

Andreasen, P.A., Egelund, R. and Petersen, H.H. 2000. The plasminogen activation system in tumor growth, invasion, and metastasis. *Cell Mol. Life. Sci.,* 57:25–40.

Arlt, A., Gehrz, A. and Muerkoster, S., Vorndamm, J., Kruse, M.L., Folsch, U.R., and Schafer, H. 2003. Role of NF-nB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine induced cell death. Oncogene 22:3243–51.

Bai, X. F., Ni, X. G., Zhao, P., Liu, S. M., Wang, H. X., Guo, B., Zhou, L. P., Liu, F., Zhang, J. S., Wang, K., Xie, Y. Q., Shao, Y. F. and Zhao, X. H. 2004. Over expression of annexin 1 in pancreatic cancer and its clinical significance. *World J. Gastroenterol*., 10, 1466-1470

Bapat, S. 2007. Evolution of cancer stem cells. *Semin Cancer Bio*., 17(3):204–13.

Barraclough, D. L., Platt-Higgins, A., de Silva Rudland, S., Barraclough, R., Winstanley, J., West, C. R., and Rudland, P. S. 2009. The metastasis- associated anterior gradient 2 protein is correlated with poor survival of breast cancer patients. *Am. J. Pathol.,* 175**,** 1848–1857

Birnie, R. B. S., Roome, C., Dussupt, V., Droop, A., Lang, S.H., Berry, P.A., Hyde, C.F., Lewis, J.L., Stower, M.J., Maitland, N.J. and Collins, A.T. 2008. Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. *Genome Biol.,* 9(5):R83.

Blackstock, W. P. and Weir, M. P. 1999. Proteomics: quantitative and physical mapping of cellular proteins. *Trends Biotechnol, 17*, 121-127.

Bloomston, M., Zhou, J.X., Rosemurgy, A.S., Frankel, W., Muro-Cacho, C.A. and Yeatman, T.J. 2006. Fibrinogenγ; Over expression in Pancreatic Cancer Identified by Large-scale Proteomic Analysis of Serum Samples.

Buchholz, M., Braun, M., Heidenblut, A., Kestler, H.A., Kloppel, G., Schmiegal, W., Hahn, S.A., Luttges, J., Gress, T.M. 2005a. Transcriptome analysis of micro dissected pancreatic intraepithelial neoplastic lesions. Oncogene 24: 6626–6636.

Buchholz, M., Kestler, H. A., Bauer, A., Bock, W., Rau, B., Leder, G., Kratzer, W., Bommer, M., Scarpa, A., Schilling, M. K., Adler, G., Hoheisel, J. D. and Gress, T. M. 2005b. Specialized DNA arrays for the differentiation of pancreatic tumors. *Clin. Cancer Res.,* 11, 8048–8054

Charlton, L.A., Sayed, M., Clark-Lewis, I., Aebersold, R., Pelech, S.L. 1999. Characterization of an activated ribosomal S6 kinase variant from maturing sea star oocytes: association with phosphatase 2A and substrate specificity. *J. Cell Biochem.,* 75:310–326.

Chen, R., Brentnall, T.A., Pan, S., Cooke, K., Moyes, K.W., Lane, Z., Crispin, D.A., Goodlett, D.R., Aebersold, R. and Bronner, M.P. 2007a. Quantitative Proteomics Analysis Reveals That Proteins Differentially Expressed in Chronic Pancreatitis Are Also Frequently Involved in Pancreatic Cancer. Molecular and Cellular Proteomics 6:1331–1342, 2007.

Chen, R., Pan, S., Brentnall, T.A. and Aebersold, R. 2005a. Proteomic Profiling of Pancreatic Cancer for Biomarker

Discovery. Molecular and Cellular Proteomics 4:523–533, 2005.

Chen, R., Pan, S., Cooke, K., White Moyes, K., Bronner, M.P., Goodlett, D.R., Aebersold, R. and Brentnall, T.A. 2007b. Comparison of Pancreas Juice Proteins from Cancer Versus Pancreatitis Using Quantitative Proteomic Analysis. *Pancreas*., 2007 ; 34(1): 70–79.

Chen, R., Pan, S., Duan, X., Nelson, B. H., Sahota, R. A., de Rham, S., Kozarek, R. A., McIntosh, M., and Brentnall, T. A. 2010. Elevated level of anterior gradient-2 in pancreatic juice from patients with premalignant pancreatic neoplasia. *Mol. Cancer*, 9, 149

Chen, R., Pan, S., Yi, E. C., Donohoe, S., Bronner, M. P., Potter, J. D., Goodlett, D. R., Aebersold, R. and Brentnall, T. A. 2006. Quantitative proteomic profiling of pancreatic cancer juice. *Proteomics*, 6**,** 3871–3879

Chen, R., Yi, E. C., Donohoe, D., Pan, S., Eng, J., Crispin, D. A., Lane, Z., Goodlett, D. A., Bronner, M. P., Aebersold, R. and Brentnall, T. A. 2005b. Pancreatic cancer proteome: the proteins that underlie invasion, metastasis, and immunologic escape. *Gastroenterology*, 129**,** 1187–1197

Craven, R. A., Totty, N., Harnden, P., Selby, P. J. and Banks, R. E. 2002. Laser capture micro dissection and two-dimensional polyacrylamide gel electrophoresis: evaluation of tissue preparation and sample limitations. *Am. J. Pathol.*, *160*, 815-822.

Crnogorac-Jurcevic, T., Gangeswaran, R., Bhakta, V., Capurso, G., Lattimore, S., Akada, M., Sunamura, M., Prime, W., Campbell, F., Brentnall, T. A., Costello, E., Neoptolemos, J. and Lemoine, N. R. 2005 Proteomic analysis of chronic pancreatitis and pancreatic adenocarcinoma. *Gastroenterology*, 129**,** 1454–1463

Dai, L., Li, C., Shedden, K.A., Lee, C.J., Li, C., Quoc, H.V., Simeone, D.M. and Lubman, D.M. 2010. Quantitative Proteomic Profiling Studies of Pancreatic Cancer Stem Cells. *J. Proteome. Res*., 2010 July 2; 9(7): 3394–3402

Donahue, T.R., Hines, O.J. 2009. CXCR 2 and RET single nucleotide polymorphisms in pancreatic cancer. *World J. Surg.*, 33(4):710–5.

Duffy, M.J. C.A. 1998. 19-9 as a marker for gastrointestinal cancers: a review. *Ann. Clin. Biochem*., 35: 364–370.

DuffyM. J., Sturgeon, C., Lamerz, R., Haglund, C., Holubec, V. L., Klapdor, R., Nicolini, A., Topolcan, O. and Heinemann, V. 2010. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. Annals of Oncology 21: 441–447, 2010

Dumartin, L., Whiteman, H.J., Weeks, M.E., Hariharan, D., Dmitrovic, B., Iacobuzio-Donahue, C.A., Brentnall, T.A., Bronner, M.P., Feakins, R.M., Timms, J.F., Brennan, C., Lemoine, N.R., and Crnogorac-Jurcevic, T., 2011. AGR2 Is a Novel Surface Antigen That Promotes the Dissemination of Pancreatic Cancer Cells through Regulation of Cathepsins B and D. *Cancer Res.*, 2011;71:7091-7102.

Duncan, R., Carpenter, B., Main, L.C., Telfer, C. and Murray, G.I. 2008. Characterisation and protein expression profiling of annexins in colorectal cancer. *Br. J. Cancer*. 2008; 98(2): 426–433.

El-Rifai, W., Moskaluk, C.A., Abdrabbo, M.K., Harper, J., Yoshida, C., Riggins, G.J., Frierson, H.F., Jr, Powell, S.M. 2002. Gastric cancers over express S100A calcium-binding proteins. *Cancer Res.*, 62:6823– 6.

Emberley, E.D., Murphy, L.C., Watson, P.H. 2004. S100 proteins and their influence on pro-survival pathways in cancer. *Biochem Cell Biol.*, 82:508 –15

Emmert-Buck, M.R.; Bonner, R.F.; Smith, P.D., Chuaqui, R.F., Zhuang, Z., Goldstein, S.R., Weiss, R.A., Liotta, L.A. 1996. Laser capture micro dissection. *Science, 274*, 998-1001.

Erkan, M., Kleeff, J., Esposito, I., Giese, T., Ketterer, K., Buchler, M.W., Giese, N.A., Friess, H. 2005. Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemo resistance and worsened prognosis. Oncogene, 24:4421–32.

Flyvbjerg, A., Mogensen, O., Mogensen, B., and Nielsen, O. S. 1997. Elevated serum insulin-like growth factor-binding protein 2 (IGFBP-2) and decreased IGFBP-3 in epithelial ovarian cancer: correlation with cancer antigen 125 and tumor-associated trypsin inhibitor. *J. Clin. Endocrinol. Metab.*, 82, 2308–2313

Friess, H., Ding, J., Kleeff, J., Fenkell, L., Rosinski, J.A., Guweidhi, A., Reidhaar-Olson, J.F., Korc, M., Hammer, J., Buchler, M.W. 2003. Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer. *Cell Mol Life Sci*., 60:1180 –99.

Fritzsche, F. R., Dahl, E., Dankof, A., Burkhardt, M., Pahl, S., Petersen, I., Dietel, M., and Kristiansen, G. 2007. Expression of AGR2 in non small cell lung cancer. *Histol. Histopathol,* 22, 703–708

Gerke, V. and Moss, S. 2002. Annexins: form structure to function. *Physiol. Rev*., 82 (2): 331–71

Gerner, C., Steinkellner, W., Holzmann, K. Gsur, A., Grimm, R., Ensinger, C., Obrist, P., Sauermann, G. 2001. Elevated plasma levels of crosslinked fibrinogen g-chain dimer indicate cancer-related fibrin deposition and fibrinolysis. Thromb Haemost 85:494–501.

Gillette, J. M., Chan, D. C., Nielsen-Preiss, S. M. 2004. Annexin 2 expression is reduced in human osteosarcoma metastases. *J. Cell Biochem*., 92:820–832.

Goonnetilleke, K. S. and Siriwardena, A. K. 2007. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. *Eur. J. SurgOncol.*, 33: 266–270.

Grønborg, M., Kristiansen, T.Z., Iwahori, A., Chang, R., Reddy, R., Sato, N., Molina, H., Jensen, O.N., Hruban, R.H., Goggins, M.G., Maitra, A. and Pandey, A. 2006. Biomarker Discovery from Pancreatic Cancer Secretome Using a Differential Proteomic Approach. *Molecular and Cellular Proteomics, 5:157–171, 2006.*

Guerra, C.S.A., Cañamero, M., Grippo, P.J., Verdaguer, L. Pérez-Gallego, L., Dubus, P., Sandgren, E. P., Barbacid, M. 2007. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell*. 11(3):291–302.

Gullo, L. 1994. CA 19-9: the Italian experience. Pancreas 9, 717 – 719.

Han, H., Bearss, D. J., Browne, L. W., Calaluce, R., Nagle, R. B. and Von Hoff, D. D. 2002. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res.*, 62, 2890–2896

Hansel, D.E., R. A. House, M. Ashfaq, R. Berg, K. Yeo, C. J and Maitra, A. 2004. Met proto-oncogene and insulin like growth factor binding protein 3 over expression correlates

with metastatic ability in well differentiated pancreatic endocrine neoplasms. Clin *Cancer Res.,* 10(18):6152–8.

Harsha, H.C., Kandasamy, K., Ranganathan, P., Rani, S., Ramabadran, S., Gollapudi, S., Balakrishnan, L., Dwivedi S.B., Telikicherla, D., Selvan, L.D.N., Goel, R, Mathivanan, S., Marimuthu, A., Kashyap, M., Vizza, R.F., Mayer, R.J., DeCaprio, J.A., Srivastava, S., Hanash, S.M., Hruban, R.H., Pandey, A. 2009. A Compendium of Potential Biomarkers of Pancreatic Cancer. PLoS Med 6(4): e1000046

Hatzfeld, J.A., Hatzfeld, A. and Maigne, J. 1982. Fibrinogen and its fragment D stimulate proliferation of human hemopoietic cells in vitro. *Proc. Natl. Acad. Sci.,* U S A 79:6280–4.

Heizmann, C. W., Fritz, G., Schafer, B. W. S. 2002. 100 proteins: structure, functions and pathology Front *Biosci*., 7:d1356–68.

Hruban, R.H., Adsay, N.V., Albores-Saavedra, J., Compton, C., Garrett, E.S., Goodman, S.N., Kern, S.E., Klimstra, D.S., Kloppel, G., Longnecker, D.S., Luttges, J., Offerhaus, G.J. 2001. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. *Am. J. SurgPathol.,* 25:579–86.

Hruban, R.H., Takaori, K., Klimstra, D.S., Adsay, N.V., Albores-Saavedra, J., Biankin, A.V., Biankin, S.A., Compton, C., Fukushima, N., Furukawa, T. 2004. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am. J. SurgPathol*., 28: 977–987.

Hruban, R.H., van Mansfeld, A.D., Offerhaus, G.J., van Weering, D.H., Allison, D.C., Goodman, S.N., Kensler, T.W., Bose, K.K., Cameron, J.L., Bos, J.L. 1993. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol*., 143:545–54.

Huang, Y., Jin, Y., Yan, C. H., Yu, Y., Bai, J., Chen, F., Zhao, Y. Z., Fu, S. B. 2008. Involvement of Annex in A2 in p53 induced apoptosis in lung cancer *Mol. Cell. Biochem.,* 309 1−2 11723

Hwang, R.F., Moore, T., Arumugam, T., Ramachandran, V., Amos, K.D., Rivera, A., Ji, B., Evans, D.B. and Logsdon, C.D. 2008. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res*., 68: 918–926.

Iacobuzio-Donahue, C. A., Maitra, A., Olsen, M., Lowe, A. W., Van Heek, N. T., Rosty, C., Walter, K., Sato, N., Parker, A., Ashfaq, R., Jaffee, E., Ryu, B., Jones, J., Eshleman, J. R., Yeo, C. J., Cameron, J. L., Kern, S. E., Hruban, R. H., Brown, P. O., and Goggins, M. 2003. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. *Am. J. Pathol.,* 162, 1151–1162

Ito, D., Fujimoto, K., Mori, T., Kami, K., Koizumi, M., Toyoda, E., Kawaguchi, Y., Doi, R. 2006. In vivo antitumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer. *Int. J. Cancer*, 118: 2337–2343.

Joyce, J.A., Baruch, A., Chehade, K., Meyer-Morse, N., Giraudo, E., Tsai, F.Y., Greenbaum, D.C., Hager, J.H., Bogyo, M., Hanahan, D. 2004. Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumor genesis. Cancer Cell 5:443–53.

Joza, N., Susin S.A., Daugas, E., Stanford, W.L., Cho, S.K., Li, C.Y.J., Sasaki, T., Elia, A.J., Cheng, M.H.Y., Ravagnan, L., Ferri, K.F., Zamzami, N., Wakeham, A., Hakem, R., Yoshida, H., Kong, Y.Y., Mak, T.W., Zúñiga-Pflücker, J.C., Kroemer, G., and Penninger, J.M. 2002. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature*. 410 (6828):549–54.

Karanjawala, Z.E., Illei, P. B., Ashfaq, R., Infante, J. R., Murphy, K. Pandey, A., Schulick, R., Winter, J., Sharma, R., Maitra, A., Goggins, M., Hruban, R.H. 2008. New markers of pancreatic cancer identified through differential gene expression analyses: Claudin 18 and annex in A8. *Am. J. Surg. Pathol.,* 32: 188–196.

Kato, M., S. N., Sugano, S., Hashimoto, K., Masuho, Y., Muramatsu, M., Kaibuchi, K., Nakafuku, M. 2001. Identification of sonic hedgehog-responsive genes using cDNA microarray. *Biochem Biophys Res Commun*. 289(2):472–8.

Khosravi-Far, R., Der, C.J. 1994. The Ras signal transduction pathway. Cancer Metastasis Rev., 13:67–89.

Knezevic, V., Leethanakul, C., Bichsel, V. E., Worth, J. M., Prabhu, V. V., Gutkind, J. S., Liotta, L. A., Munson, P. J., Petricoin, E. F., 3rd, Krizman, D. B. 2001. Proteomic profiling of the cancer microenvironment by antibody arrays. *Proteomics, 1*, 1271-1278.

Kumble, K. D., Hirota, M., Pour, P. M., and Vishwanatha, J. K. 1992. Enhanced levels of annexins in pancreatic carcinoma cells of Syrian hamsters and their intrapancreatic allografts. *Cancer Res.,* 52**,** 163–167

Lamerz, R. 1999. Role of tumor markers, cytogenetics. Ann Oncol 10 (Suppl 4): 145–149.

Lee, C.J. D. J., Simeone, D.M. 2008. Pancreatic cancer stem cells. *J. Clin Oncol*., 26(17):2806–12.

Li, C. H. D., Dalerba, P., Burant, C.F., Zhang, L., Adsay, V., Wicha, M., Clarke, M.F. and Simeone, D.M. 2007. Identification of Pancreatic Cancer Stem Cells. *Cancer Research.,* 67(3):1030–1037.

Liu, D., Rudland, P.S., Sibson, D.R., Platt-Higgins, A., Barraclough, R. 2005. Human homologue of cement gland protein, a novel metastasis inducer associated with breast carcinomas. *Cancer Res.,* 65:3796–805.

Logsdon, C. D., Simeone, D. M., Binkley, C., Arumugam, T., Greenson, J. K., Giordano, T. J., Misek, D. E. and Hanash, S. 2003. Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. *Cancer Res.* 63, 2649–2657

Lu, J. S. L., Bai, Y. 2009. Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis. *Cell Res.,* 19(7):802–15.

Lu, Z., Hu, L., Evers, S., Chen, J. and Shen, Y. 2004. Differential expression profiling of human pancreatic adenocarcinoma and healthy pancreatic tissue. *Proteomics*, 4, 3975–3988

Luo, A., Kong, J., Hu, G., Liew, C.C., Xiong, M., Wang, X., Ji, J., Wang, T., Zhi, H., Wu, M., Liu, Z. 2004. Discovery of Ca2+-relevant and differentiation-associated genes down-regulated in esophageal squamous cell carcinoma using cDNA microarray. *Oncogene*, 23:1291–9.

Mahon, P.C., Baril, P. and Bhakta, V. Chelala, C., Caulee, K., Harada, T., Lemoine, N.R. 2007. S100A4 contributes to the suppression of BNIP3 expression, chemo resistance, and

inhibition of apoptosis in pancreatic cancer. *Cancer Res.*, 67:6786–95.

Maitra A, Hruban, R.H. 2005. A new mouse model of pancreatic cancer: PTEN gets its Akt together. *Cancer Cell*, 8:171–2.

Maitra, A., Fukushima, N., Takaori, K., Hruban, R.H. 2005. Precursors to invasive pancreatic cancer. *Adv. Anat. Pathol.*, 12:81–91.

Makawita, S., Smith, C., Batruch, I., Zheng, Y., Rückert, F., Grützmann, R., Pilarsky, C., Gallinger, S., and Diamandis, E.P. 2011. Integrated Proteomic Profiling of Cell Line Conditioned Media and Pancreatic Juice for the Identification of Pancreatic Cancer Biomarkers. Molecular and Cellular Proteomics 10: 10.1074/mcp.M111.008599, 1–20, 2011.

Muller, H. L., Oh, Y., Lehrnbecher, T., Blum, W. F. and Rosenfeld, R. G. 1994. Insulin-like growth factor-binding protein-2 concentrations in cerebrospinal fluid and serum of children with malignant solid tumors or acute leukemia. *J. Clin. Endocrinol. Metab.*, 79, 428–434

Nakamura, T., Furukawa, Y., Nakagawa, H., Tsunoda, T., Ohigashi, H., Murata, K., Ishikawa, O., Ohgaki, K., Kashimura, N., Miyamoto, M., Hirano, S., Kondo, S., Katoh, H., Nakamura, Y., and Katagiri, T. 2004. Genome-wide cDNA microarray analysis of gene expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection. *Oncogene*, 23**,** 2385–2400

Nedjadi, T. N., Kitteringham, F., Campbell, R.E., Jenkins, B.K., Park, P., Navarro, F., Ashcroft, A., Tepikin, J.P. and Neoptolemos, E. 2009. CostelloS100A6 binds to annexin 2 in pancreatic cancer cells and promotes pancreatic cancer cell motility. *Br. J. Cancer*, 101, pp. 1145–1154

Newmeyer, D.D., F-M S. 2003. Mitochondria: releasing power for life and unleashing the machineries of death. Cell. 112(4):481–90.

Ng, S.S., Tsao, M.S., Nicklee, T., Hedley, D.W. 2001. Wortmannin inhibits pkb/akt phosphorylation and promotes gemcitabine antitumor activity in orthotopic human pancreatic cancer xenografts in immunodeficient mice. *Clin Cancer Res.*, 7:3269–75.

Ng, S.S.W., Tsao, M.S., Chow S, Hedley, D.W. 2000. Inhibition of phosphatidylinositide 3-kinase enhances gemcitabine-induced apoptosis in human pancreatic cancer cells. *Cancer Res.*, 60:5451–5.

Ott, H.W., Lindner, H., Sarg, B., Mueller-Holzner, E., Abendstein, B., Bergant, A., Fessler, S., Schwaerzler, P., Zeimet, A., Marth, C., Illmensee, K. 2003. Calgranulins in cystic fluid and serum from patients with ovarian carcinomas. *Cancer Res.*, 63:7507–14.

Palumbo, J.S., Potter, J.M., Kaplan, L.S., Talmage, K., Jackson, D.G., Degen, J.L. 2002. Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice. *Cancer Res.*, 62:6966–72.

Palumbo, J.S., Talmage, K.E. and Massari, J.V. La Jeunesse, C.M., Flick, M.J., Kombrinck, K.W., Jirousková, M., Degen, J.L. 2005. Platelets and fibrin (ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. Blood, 105:178–85.

Park, K., Chung, Y.J., So, H., Kim, K., Park, J., Oh, M., Jo, M., Choi, K., Lee, E.J., Choi, Y.L., Song, S.Y., Bae, D.S., Kim, B.G., Lee, J.H. 2011. AGR2, a mucinous ovarian cancer marker, promotes cell proliferation and migration. *Exp. Mol. Med.*, 43:91–100.

Pohler, E., Craig, A.L., Cotton, J., Lawrie, L., Dillon, J.F. and Ross, P. Kernohan, N., Hupp, T.R. 2004. The Barrett's antigen anterior gradient-2 silences the p53 transcriptional response to DNA damage. *Mol. Cell. Proteomics.*, 3:534–47.

Pollanen, J., Stephens, R.W., Vaheri, A. 1991. Directed plasminogen activation at the surface of normal and malignant cells. *Adv. Cancer Res*., 57:273–328.

Posner, M.R. and Mayer, R.J. 1994. The use of serological tumor markers in gastrointestinal malignancies. Hematol/ OncolClin North Am 8, 533 – 553.

Radhi, J.M., Lukie, B.E. 1998. Pancreatic cancer and fibrinogen storage disease. *J. Clin. Pathol.*, 51:865–7.

Ramachandran, V., Arumugam, T., Wang, H. and Logsdon, C. D. 2008. Anterior gradient 2 is expressed and secreted during the development of pancreatic cancer and promotes cancer cell survival. *Cancer Res.*, 68**,** 7811–7818

Ranke, M.B., Maier, K.P., Schweizer, R., Stadler, B., Schleicher, S., Elmlinger, M.W. and Flehmig, B. 2003. Pilot study of elevated levels of insulin-like growth factor-binding protein-2 as indicators of hepatocellular carcinoma. *Horm. Res.*, 60, 174–180

Rao, J.S. 2003. Molecular mechanisms of glioma invasiveness: the role of proteases. *Nat. Rev. Cancer*, 3:489–501.

Satake, K., Takeuchi, T., Homma, T. and Ozaki, H. 1994. CA19-9 as a screening and diagnostic tool in symptomatic patients: the Japanese experience. Pancreas 9, 703 – 706.

Schmitt, M., Harbeck, N., Thomssen, C., Wilhelm, O., Magdolen, V., Reuning, U., Ulm, K., Hofler, H., Janicke, F., Graeff, H. 1997. Clinical impact of the plasminogen activation system in tumor invasion and metastasis: prognostic relevance and target for therapy. Thromb Haemost 78:285–296.

Sears, R., Nuckolls, F., Haura, E., Taya, Y., Tamai, K., Nevins, J.R. 2000. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes. Dev.*, 14:2501–14.

Sharma, M.R., Koltowski, L., Ownbey, R.T., Tuszynski, G.P. and Sharma, M.C. 2006. Angiogenesis-associated protein annex in II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. *Exp. Mol. Pathol*., 81:146–156.

Shekouh, A. R., Thompson, C. C., Prime, W., Campbell, F., Hamlett, J., Herrington, C. S., Lemoine, N. R., Crnogorac-Jurcevic, T., Buechler, M. W., Friess, H., Neoptolemos, J. P., Pennington, S. R. and Costello, E. 2003. Application of laser capture micro dissection combined with two-dimensional electrophoresis for the discovery of differentially regulated proteins in pancreatic ductal adenocarcinoma. *Proteomics*, *3*, 1988-2001.

Shen, J., Person, M.D., Zhu, J., Abbruzzese, J.L. and Li, D. 2004. Protein expression profiles in pancreatic adenocarcinoma compared with normal pancreatic tissue and tissue affected by pancreatitis as detected by two-dimensional gel electrophoresis and mass spectrometry. *Cancer Res.*, 64, 9018–9026

Sitek, B., Luttges, J., Marcus, K., Kloppel, G., Schmiegel, W., Meyer, H.E., Hahn, S.A. and Stuhler, K. 2005. Application of fluorescence difference gel electrophoresis saturation labelling for the analysis of micro dissected precursor

lesions of pancreatic ductal adenocarcinoma. *Proteomics*, 5, 2665–2679

Sitek, B., Sipos, B., Alkatout, I., Poschmann, G., Stephan, C., Schulenborg, T., Marcus, K., Lüttges, J., Dittert, D.D., Baretton, G. 2009. Analysis of the pancreatic tumor progression by a quantitative proteomic approach and immunhistochemical validation. *J. Proteome Res.*, 8:1647–56.

Smirnov, D.A., Zweitzig, D.R., Foulk, B.W., Miller, M.C., Doyle, G.V., Pienta, K.J. Meropol, N.J., Weiner, L.M., Cohen, S.J., Moreno, J.G., Connelly, M.C., Terstappen, L.W., O'Hara, S.M. 2005. Global gene expression profiling of circulating tumor cells. *Cancer Res.*, 65:4993–97.

Sommer, A., Hoffmann, J., Lichtner, R.B., Schneider, M.R., and Parczyk, K. 2003. Studies on the development of resistance to the pure antiestrogen Faslodex in three human breast cancer cell lines. *J. Steroid Biochem. Mol. Biol*., 85:33– 47.

Steinberg, W. 1990. The clinical utility of the CA 19-9 tumor-associated antigen. *Am. J. Gastroenterol, 85*, 350 – 355.

Syed, S.P., Martin, A.M., Haupt, H.M., Arenas-Elliot, C.P., Brooks, J.J. 2007. Angiostatin receptor annexin II in vascular tumors including angiosarcoma. Hum Pathol. 38:508–513.

Takano, S., Togawa, A., Yoshitomi, H., Shida, T., Kimura, F., Shimizu, H., Yoshidome, H., Ohtsuka, M., Kato, A., Tomonaga, T., Nomura, F. and Miyazaki, M. 2008. Annex in II over expression predicts rapid recurrence after surgery in pancreatic cancer patients undergoing gemcitabine-adjuvant chemotherapy *Ann. Surg. Oncol.*, 15 (11) 3157–68

Takasaki, H., Uchida, E., Tempero, M.A., Burnett, D.A., Metzgar, R.S., Pour, P.M. 1988. Correlative study on expression of CA19-9 and DU-Pan-2 in tumor tissue and in serum of pancreatic cancer patients. *Cancer Res*., 48: 1435–1438

Trousseau, A. 1865. Phlegmasiaalbacolens. Clinique Medicale de l'Hotel-Dieu de Paris, the New Sydennam Society, London, 3:94.

Tumminello, F.M., Leto, G., Pizzolanti, G., Candiloro, V., Crescimanno, M., Crosta, L., Flandina, C., Montalto, G., Soresi, M., Carroccio, A., Bascone, F., Ruggeri, I., Ippolito, S., Gebbia, N. 1996. Cathepsin D, B and L circulating levels as prognostic markers of malignant progression. *Anticancer Res.*, 16: 2315–9.

Tzanakakis, G.N., Margioris, A.N., Tsatsakis, A.M., Vezeridis, M.P. 2003. The metastatic potential of human pancreatic cell lines in the liver of nude mice correlates well with cathepsin B activity. *Int. J. Gastrointest Cancer*, 34:27–38.

Van Cutsem, E., van de Velde, H., Karasek, P. Oettle H., Vervenne, W.L., Szawlowski, A., Schoffski, P., Post, S., Verslype, C., Neumann, H., Safran, H., Humblet, Y., Perez Ruixo, J., Ma, Y., Von Hoff, D. 2004. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J. Clin Oncol*, 22:1430–8.

Vishwanatha, J.K., Chiang, Y., Kumble, K.D., Hollingworth, M.A. and Pour, M.A. 1993. Enhanced expression of annexin II in human pancreatic carcinoma cells and primary pancreatic cancer. *Carcinogenesis*, 14: 2575–2579.

Wente, M.N., K. M., Burdick, M.D., Friess, H., Büchler, M.W., Ceyhan, G.O., Reber, H.A., Strieter, R.M., Hines, O.J. 2006. Blockade of the chemokine receptor CXCR2 inhibits pancreatic cancer cell-induced angiogenesis. *Cancer Lett.*, 241(2):221–7.

Wojtukiewicz, M.Z., Rucinska, M., Zacharski, L.R., Kozłowski, L., Zimnoch, L., Piotrowski, Z., Kudryk, B.J., Kisiel, W. 2001. Localization of blood coagulation factors in situ in pancreatic carcinoma. Thromb Haemost, 86:1416–1420.

Wulfkuhle, J. D., Sgroi, D. C., Krutzsch, H., McLean, K., McGarvey, K., Knowlton, M., Chen, S., Shu, H., Sahin, A., Kurek, R., Wallwiener, D., Merino, M. J., Petricoin, E. F., 3rd, Zhao, Y. and Steeg, P. S. 2002. Proteomics of human breast ductal carcinoma in situ. *Cancer Res*., *62*, 6740-6749.

Wulfkuhle, J.D., Liotta, L.A. and Petricoin, E.F. 2003. III Proteomic applications for the early detection of cancer. *Nat. Rev. Cancer*, 3, 267 – 275.

Zang, L., Toy, D. P., Hancock, W. S., Sgroi, D. C. and Karger, B. L. 2004. Proteomic analysis of ductal carcinoma of the breast using laser capture micro dissection, LC-MS, and 16O/18O isotopic labeling. *J. Proteome Res., 3*, 604-612.

Zhang, J.S., Gong, A., Cheville, J.C., Smith, D.I. and Young, C.Y. 2005. AGR2, an androgen-inducible secretory protein over expressed in prostate cancer. *Genes Chromosomes Cancer*, 43:249–59.

Zhang, Y., Ali, T. Z., Zhou, H., D'Souza, D. R., Lu, Y., Jaffe, J., Liu, Z., Passaniti, A., and Hamburger, A. W. 2010. ErbB3 binding protein 1 represses metastasis-promoting gene anterior gradient protein 2 in prostate cancer. *Cancer Res.* 70, 240–248

Zhang, Y., Forootan, S. S., Liu, D., Barraclough, R., Foster, C. S., Rudland, P. S. and Ke, Y. 2007. Increased expression of anterior gradient-2 is significantly associated with poor survival of prostate cancer patients. *Prostate Cancer Prostatic Dis.*, 10**,** 293–300

Zhuo, L. and Kimata, K. 2008. Structure and function of inter-alpha-trypsin inhibitor heavy chains. *Connect Tissue Res.*, 49(5):311–20.

Zimmermann, U., Woenckhaus, C., Pietschmann, S., Junker, H., Maile, S., Schultz, K., Protzel, C. and Giebel, J. 2004. Expression of annex in II in conventional renal cell carcinoma is correlated with Fuhrman grade and clinical outcome. *Virchows Arch.*, 445:368–374.

Zucchini, C., Biolchi, A., Strippoli, P., Solmi, R., Rosati, G., Del Governatore, M., Milano, E., Ugolini, G., Salfi, N., Farina, A., Caira, A., Zanotti, S., Carinci, P., Valvassori, L. 2001. Expression profile of epidermal differentiation complex genes in normal and anal cancer cells. *Int J. Oncol.*, 19:1133– 41.

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