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Systems and Network-Centric Understanding of Pancreatic Ductal Adenocarcinoma Signalling

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease that is intractable to currently available treatment modalities (Vincent et al. 2011). Failure of standard chemo-, radio- and neoadjuvant single pathway targeted therapies indicate that before newer treatment regimens are designed, one has to re-visit the basic understanding of the origins and complexity of PDAC. As such, PDAC is now appreciated to have not only a highly heterogeneous pathology but is also a disease characterized by dysregulation of multiple pathways governing fundamental cell processes (Kim and Simeone 2011). Such complexity has been suggested to be governed by molecular networks that execute metabolic or cytoskeletal processes, or their regulation by complex signal transduction originating from diverse genetic mutations (Figure 1). A major challenge, therefore, is to understand how to develop actionable modulation of this multivariate dysregulation, with respect to both how it arises from diverse genetic mutations and to how it may be ameliorated by prospective treatments in PDAC. Lack of understanding in both these areas is certainly a major underlying reason for failure of most of the available and clinically used drugs (Stathis and Moore 2010). The pharmaceutical industry handpicked drugs have been generally based on their specificity towards a particular protein and the subsequent targeted pathway (K-Ras, PI3K, MEK, EGFR, p53 etc) without considering the effect of modulating secondary and interacting pathways (Almhanna and Philip 2011; Philip 2011). However, as results from integrated network modeling and systems biology studies indicate, targeting one protein is not straightforward as each protein in a cellular system works in a complex interacting network comprised of a myriad interconnected pathways (Wist et al. 2009a). Silencing one protein/pathway can have multiple effects on different secondary pathways leading to secondary effects. For example, activation of salvage pathways (commonly observed in PDAC) can result in diminished drug response or in some cases acquired resistance. Therefore, in order to decode this complexity and to understand both the PDAC disease and identify drug targets, it requires a departure from a protein-centric to a more advanced network-centric view. This chapter deals with recent advancements on deciphering PDAC disease networks and drug response networks based on integrated systems and network biology-driven science. It is believed that such integrated and holistic approach will help in not only delineating the mechanism of resistance of this complex disease, it will also aid in the future design of targeted drug combinations that will improve the dismal cure rate.

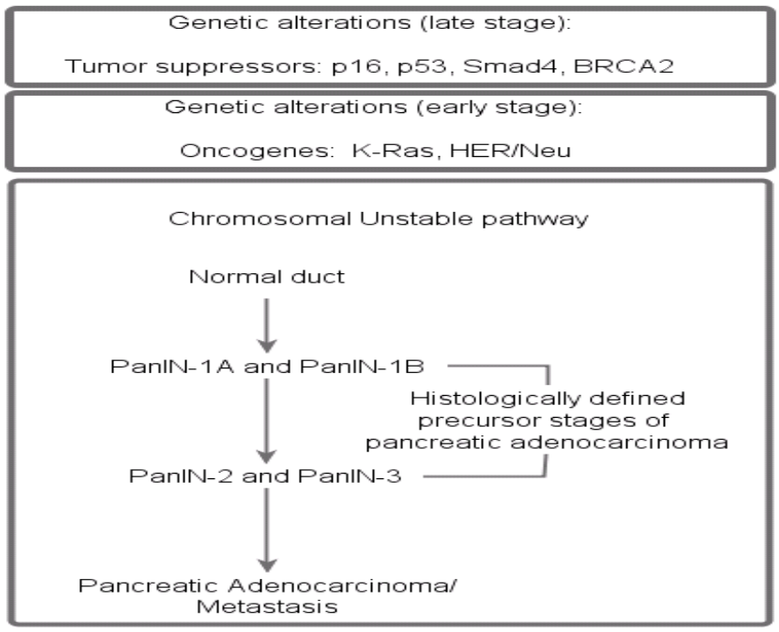

Fig. 1. Genetic alterations in PDAC are categorized into early state (oncogenes, K-Ras, Her2/Neu); Late Stage (tumor suppressors, p16, Smad4, BRCA2) and chromosomal instability pathways that accelerate progression from PanIN-1A lesions to metastatic PDAC.

2. Complex PDAC genetic network

PDAC is highly complex malignancy with myriad set of de-regulated mechanisms involved and affecting the tissue at different stages of the disease. Detailed molecular mechanisms of initiation, development and progression of PDAC have been thoroughly studied since the basic principles of the disease were revealed in the 1970s (Pour et al. 2003; Morosco et al. 1981; Morosco and Goeringer 1980). The most acceptable model is the classical one that describes morphological as well as molecular transformation from precursor lesions into invasive carcinoma (Hruban et al. 2000a; Hruban et al. 2000b). While the standard nomenclature and diagnostic criteria for classification of PDAC has primarily been based on grades of pancreatic intraepithelial neoplasia (PanIN) (Hruban et al. 2001), cumulatively it has been accepted that PDAC is a genetically and epigenetically complex disease that arises through a combination of events. It is increasingly being accepted that these complexities cannot be fully understood by traditional molecular biology techniques and integrated approaches may play pivotal role in the better understanding of PDAC as are discussed below.

2.1 Interaction of oncogenes and tumor suppressor genes in PDAC

PDAC origin and progression is broadly classified to be result of three major events (a) early stage genetic alterations in the proto-oncogenes mainly K-ras and Her-2/Neu; (b) late stage alterations in tumor suppressor genes such as p53, p16, Smad4 and BRCA2 and (c) chromosomal instability/precursor lesion in the normal duct (i.e. formation of PanIN-1a and PanIN-1B to Pan-IN-2 and Pan-IN3 (summarized in Figure 1).

These early and late genetic alterations have fundamental roles affecting key guardians of cellular signaling, which induces instability of entire molecular systems such as cell growth, division, apoptosis and migration. Mutation in proto-oncogenes gives rise to oncogenes that are often present in PDAC. These mutations cause the protein products of oncogenes to be permanently activated, resulting in uncontrolled cell proliferation. Oncogenic mutations exhibit a dominant characteristic and deficiency of one allele (i.e. heterozygous mutation) is sufficient for a lethal outcome. There are several key proto-oncogenes involved in PDAC, including KRAS, Her2/Neu, CTNNB1 (β-catenin), PIK3CA or AKT1. The most common oncogenic mutation types are point mutations, deletions, gene amplifications, and gene re-arrangements.

On the other hand, tumor suppressor genes code for proteins that act against cell proliferation. As a result of late event genetic alterations, their normal function may be reduced or even completely eliminated. Mutations in tumor suppressor genes have recessive characteristics and hence, the cell looses its function only when both alleles are affected. Commonly, described as a double hit model, one allele is initially mutated while the other is subsequently mutated or lost completely (Serra et al. 1997). In addition, there are numerous epigenetic controls of tumor suppressors that involve deactivation by hypermethylation (Herman et al. 1996). In PDAC, the frequently affected tumor suppressors include the guardian regulator TP53 (Barton et al. 1991), APC (Horii et al. 1992); SMAD4 (Bartsch et al. 1999) and TP16 (Caldas et al. 1994).

2.1.1 Complex de-regulatory signaling mechanisms in PDAC

Intense research over the last three decades have revealed that PDAC has a highly intricate web of de-regulatory signaling. In pancreatic duct cells, molecular biologist have identified some of the core signaling pathways that are aberrantly expressed that consequently leads to development of PDAC. Major cell surface receptor de-regulatory mechanisms include the c-MET/HGF (hepatocyte growth factor) signaling pathway which is a key factor in early progression of PDAC. This pathway is responsible for invasive growth of PDAC through activation of key oncogenes, angiogenesis and scattering (cell dissociation and metastasis). c-MET is a proto-oncogene that encodes an HGF receptor that has a primary function in embryonic development and wound healing (Chmielowiec et al. 2007). Even though c-MET mRNA is present at very small amounts in normal human exocrine pancreas, it is upregulated in a majority of PDAC. Interestingly overexpression of c-MET has been observed in regenerative tissue affected by acute pancreatitis (Otte et al. 2000), and has been linked to early events in PDAC carcinogenesis. HGF is a primary ligand of c-MET. Upon c-MET/HGF interaction, several different signaling pathways are activated, including the Ras, phosphoinositide 3-kinase (PI3K), JAK signal transducer and activator of transcription (STAT) and β-catenin (Wnt) pathways.

The second major cell surface signaling found altered in PDAC is the Ras/Raf/MAPK pathway. The Ras/Raf/mitogen-activated protein kinase (MAPK) pathway is one of the most elaborately studied signaling pathways in PDAC and other cancers (Molina and Adjei 2006). The role of Ras/Raf/MAPK signaling is critical for many carcinogeneic processes, including cell growth, division, cell differentiation, invasion and migration, wound healing repair, and angiogenic processes. The central regulator of this multivariate signal transduction from extracellular to intracellular environment is the Ras protein, which is

localized at the inner side of the cellular membrane. Under normal physiological conditions, the hydrophobic Ras protein is in its inactive GDP-bound form. In the event of an extracellular signal coming through growth factor receptors, their is removal of GDP from Ras protein and its subsequent activation upon binding to GTP. Activated Ras complex triggers kinase activity of Raf kinase, which ultimately results in activation of an MAPK. MAPK kinase (MAPKK) in turn is an important regulator of DNA transcription and mRNA translation. Mutations that affect any of the Ras/Raf/MAPK members produce an increase in tumorigenicity through hyper-activation of DNA machinery and mRNA translation. Besides Raf and MAPK, there are other downstream effectors of Ras protein, including PI3K, thus providing crosstalk between multiple pathways.

Aside from Ras pathway, the PTEN/PI3K/AKT signaling axis is found altered in PDAC. This pathway is fundamentally based on regulated activation of AKT through its localization at the cell membrane (Carnero et al. 2008). PI3K and PTEN phosphatases are two important protein families involved in the membrane localization of AKT. PI3K phosphorylates certain membrane-bound lipids known as phosphoinositides producing three different phosphatidylinositol 3-phosphate (PIP), phosphatidylinositol (3,4)-bisphosphate (PIP_2), and phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). The phosphorylated forms, PIP_3 and, to a lesser extent, PIP_2, attract important protein kinases to the cell membrane. The most prominent is AKT, a family of serine/threonine protein kinases that trigger a number of key cellular processes, including glucose metabolism, cell proliferation, and apoptosis, transcription, and cell migration (Maitra and Hruban 2005). AKT activity is strongly dependent on its proper localization on the cell membrane. The positioning of AKT at the membrane is achieved through its strong binding to PIP_3. In pancreatic carcinogenesis, AKT1 acts as an oncogene that upholds cell survival by overcoming cell cycle arrest, blocking apoptosis, and promoting angiogenesis. PTEN is a phosphatase that acts in opposition to PI3K. It has tumor suppression ability by converting PIP_3 back to PIP_2 and to PIP, hence disrupting membrane localization and reducing activity of AKT. In most cancers, expression levels of PI3Ks and AKT are high, while PTEN is often deactivated by mutation, or deleted completely. Through its key role in pancreatic carcinogenesis, PI3K/AKT/PTEN signaling is an important target for anticancer therapy.

The JAK/STAT signaling pathway also has an important role in regulation of DNA transcription by inducing chemical signals from cytokine receptors into the cell nucleus. The signal is phosphorylation dependent prompting activation and dimerization in a family of STAT proteins. Activated STAT dimers initiate DNA transcription inside the nucleus. It is known that inhibition of JAK/STAT signaling induces apoptosis in various human cancers, and is therefore, a primary focus for potential new drug candidates (Buettner et al. 2002). A recent study has reported reduced growth of pancreatic cancer cells *in vitro* when exposed to benzyl isothiocyanate, through suppression of STAT3 signaling and subsequent induction of apoptosis. This is suggested as a possible explanation of the anti-carcinogenic effect of cruciferous vegetables (such as broccoli, cauliflower, cabbage or horseradish) that are rich in isothiocyanates.

TGF-β is a ligand that binds to type II cytokine receptor dimer, which then interacts and activates type I cytokine receptor dimer, triggering phosphorylation of receptor-regulated SMADs (R-SMADs), mainly SMAD2 and SMAD3. In the phosphorylated form, the R-

SMADs form a complex with SMAD4, which localizes it in the nucleus and where it interacts with other factors to stimulate transcription of genes that are important for cell cycle arrest and migration. SMAD4 is therefore a key mediator for TGF-β signals. Due to its frequent absence in proliferating PDAC tissue, it is also known as DPC or "deleted in pancreatic cancer" (Schutte et al. 1995). Relatively high frequency of SMAD4 mutations and loss of heterozygosity at the DPC4 locus (18q21.1) strongly suggest that the protein is a primary tumor suppressor involved in PDAC carcinogenesis process. However, it should be noted that reinstating SMAD4 expression results in tumor growth suppression only *in vivo* and not *in vitro*. It has also been found that a SMAD4-independent pathways may be responsible for tumorigenic effect of TGF-β signaling (Levy and Hill 2005).

Wnt signaling is crucial to formation and maintenance of pancreas (Dessimoz and Grapin-Botton 2006; Dessimoz et al. 2005). During PDAC development, hyper-activation of Wnt triggers transcription of a number of genes that have a direct impact on cell proliferation, differentiation and migration (Cano et al. 2008; Rulifson et al. 2007). Activation of Wnt signaling is through interaction of a family of membrane-bound receptors known as Frizzleds with Wnt ligands. Once activated, the downstream signals may proceed through independent pathways. In a canonical pathway, signal transduction is mediated through stabilization and translocation of β-catenin from the cytosol into the nucleus followed by its interaction with T-cell factor that in turn activates transcription of target genes. The localization of high expression levels of β-catenin in the nucleus has been experimentally confirmed in various high grade PanIN lesions, as well as in advanced PDAC (Al-Aynati et al. 2004). In non-canonical, β-catenin-independent pathways, other signaling mediators are involved, that block the β-catenin assisted transcription. The nuclear localization of β-catenin and high expression levels of WNT5a, a gene involved in non-canonical Wnt pathways, suggests involvement of both pathways in PDAC progression.

The cell cycle control genes have profound importance in PDAC and CDKN2A is one of key factors in its negative control. The CDKN2A has two promoters and alternative splicing sites that give rise to two alternative protein products: cyclin-dependent kinase inhibitor p16INK4a and p53-activator p14ARF. Although both proteins are active in negative control of the cell cycle, only the function of p16INK4a is frequently lost in PDAC due to point mutations, deletions or hypermethylation . p16INK4a protein (also known as p16) inhibits key elements of cell cycle progression at the G1 checkpoint. p16 inactivation is an early event in pancreatic carcinogenesis, and low levels of p16 expression are associated with larger tumors, risk of early metastases and poor survival. The network interactions of de-regulatory signaling pathways in PDAC are depicted in Figure 2.

In summary, the above comprehensive set of studies accumulated over the years clearly show that PDAC is a highly complex disease. Traditional molecular biology focuses on studying these alterations in a single protein-centric manner honing on individual pathways. There are unanswered questions regarding the interaction between these de-regulatory signaling mechanisms that may be related to the cause of such dismal outcomes in PDAC. This is indeed the case as pharmaceutical companies handpick drugs to target individual protein and not multiple pathways. Even if a drug blocks one signaling molecule in the tumor, another salvage pathway becomes activated leading to diminished efficacy of the drugs. Therefore, we are of the view that an integrated holistic approach is needed to to

first understand the interactions between individual pathways that will aid in the design of single or combination regimens for the elimination of PDAC.

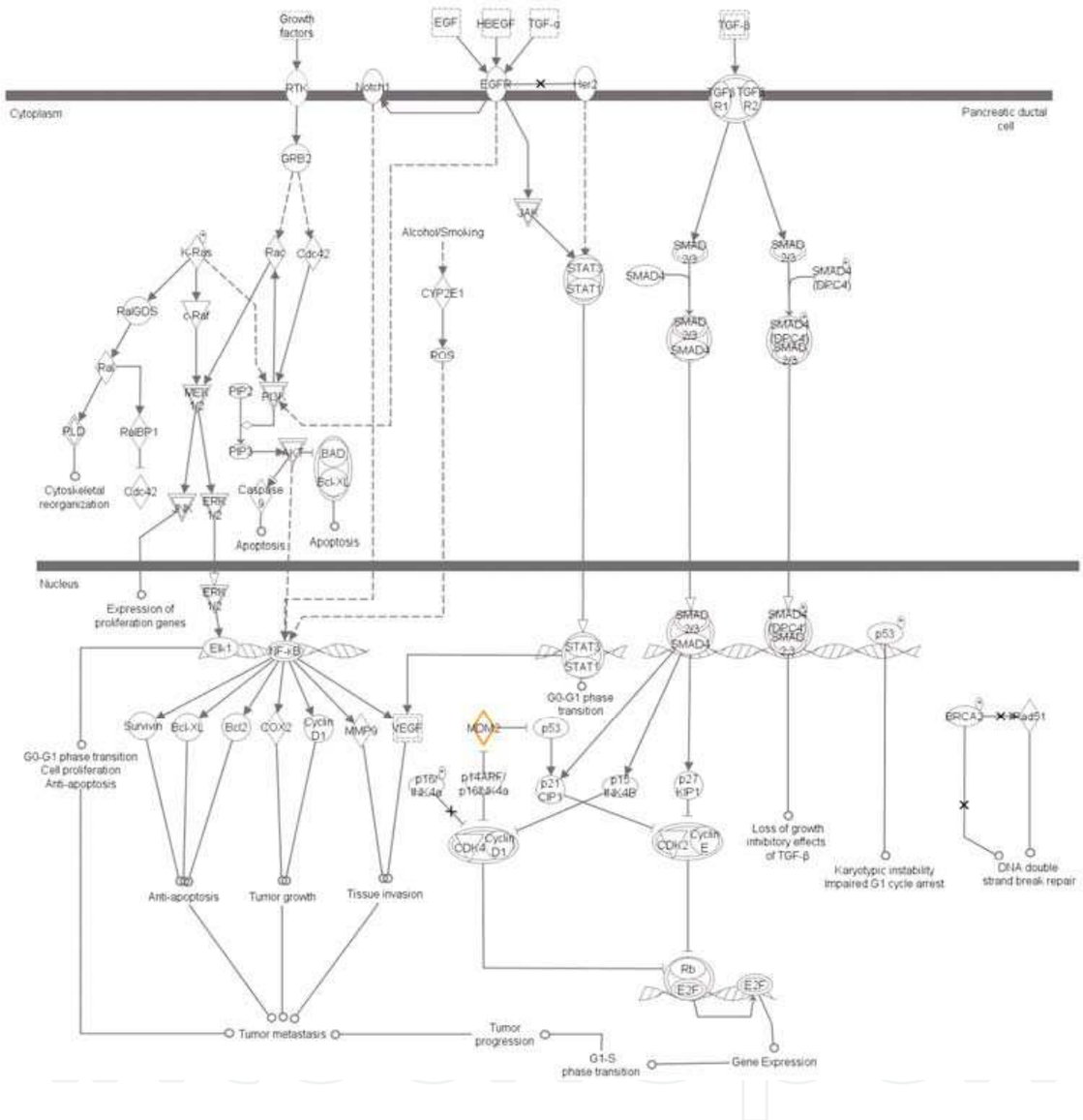

Fig. 2. Complex de-regulatory network of PDAC obtained from Ingenuity Pathway Analysis Database.

3. Systems biology and its use in understanding the complexity of PDAC

Applicability of systems biology is slowly being realized in the clinic (Faratian et al. 2009). Currently, combining information on patient history with high throughput bioinformatics such as genotyping, transcriptomics and comparative genomic hybridization, sequencing, and proteomics, followed by molecular network analysis, one can predict biomarkers and

targets and that would ultimately benefit in designing personalized medicine (Figure 3 depicting integration of multiple high-throughput technologies for better approach and treatment to a disease).

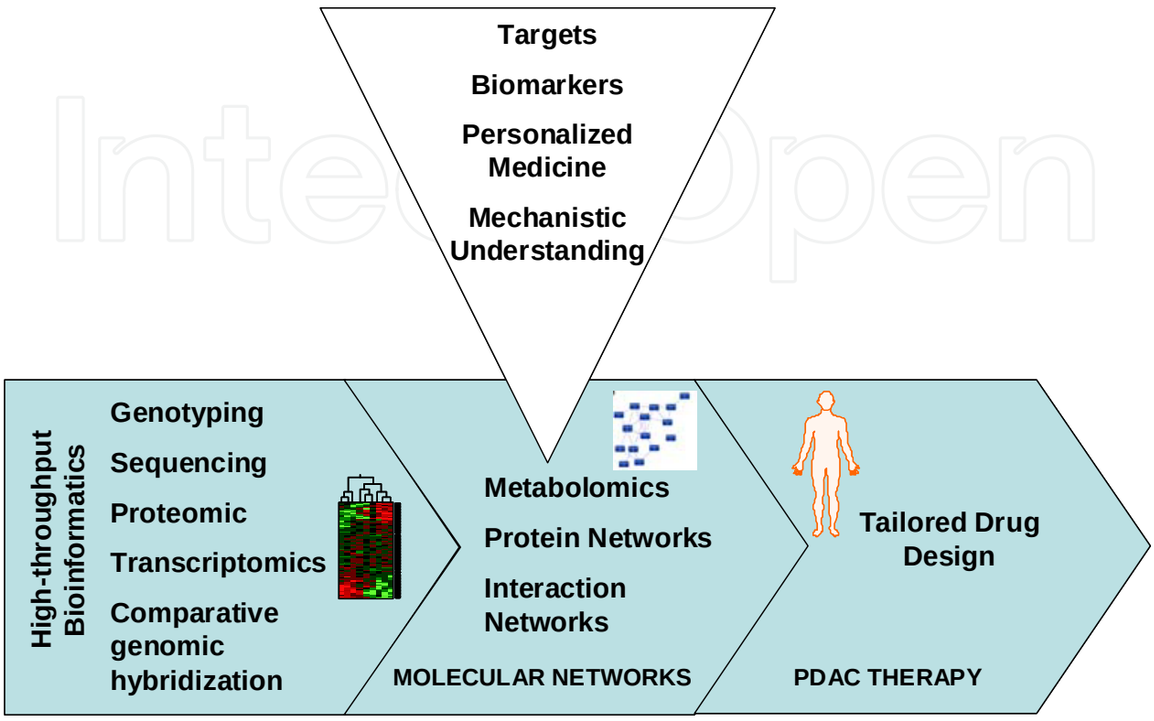

Fig. 3. Systems Biology is a potent tool for designing personalized medicine, predicting biomarkers and targets and mechanistic understanding of complex diseases.

This type of association study can be applied to both affected and healthy cohorts, or in relation to particular phenotypes, such as disease susceptibility (for example, diabetes) (Saxena et al. 2007), or to study individual responses to drugs. As a result, genetic variations have been identified through comprehensive re-sequencing studies of cancer-related mutations in colon and breast tumors, leading to the identification of around 80 DNA alterations in a typical cancer (Wood et al. 2007). This technology has been applied to understand PDAC genetics, pathway interactions and in identifying PDAC stem cells and are discussed below.

3.1 Systems understanding of PDAC expression datasets

As a proof of concept, the first study on the use of proteomic profiling was published by Lohr and group and they showed how integrated technologies could be utilized in obtaining PDAC biomarkers (Lohr et al. 2006). In this study, it was postulated that this type of proteomic approach was extremely necessary in the rationale for the design of drugs for this deadly malignancy. Later, a number of investigations have demonstrated that indeed this technology can be applied to unwind the complex web of interacting pathways in PDAC. For example, in an elegant study, Chelala and colleagues provided pancreatic expression database that was a generic model for organization, integration and mining of

complex pancreatic cancer datasets (Chelala et al. 2007). The database holds 32 datasets comprising 7636 gene expression measurements extracted from 20 different published gene or protein expression studies from various PDAC types, pancreatic precursor lesions (PanINs) and chronic pancreatitis. The pancreatic data are stored in a data management system based on the BioMart technology alongside the human genome gene and protein annotations, sequence, homologue, SNP and antibody data. Interrogation of the database can be achieved through both a web-based query interface and through web services using combined criteria from pancreatic (disease stages, regulation, differential expression, expression, platform technology, publication) and/or public data (antibodies, genomic region, gene-related accessions, ontology, expression patterns, multi-species comparisons, protein data, SNPs). This database enables connections between otherwise disparate data sources and allows relatively simple navigation between all data types and annotations. The database structure and content provides a powerful and high-speed data-mining tool for cancer research. It can be used for target discovery i.e. of biomarkers from body fluids, identification and analysis of genes associated with the progression of cancer, cross-platform meta-analysis, SNP selection for pancreatic cancer association studies, cancer gene promoter analysis as well as mining cancer ontology information. The data model is generic and can be easily extended and applied to other types of cancer and is available online with no restrictions for the scientific community at http://www.pancreasexpression.org/. Building on this database, the same group has updated their PDAC expression studies combining newly discovered and emerging molecules in 2011 (Cutts et al. 2011). These studies were not possible through traditional molecular biology approach which has its own limitations. In addition to the 32 datasets discovery, the group has added newer, more sophisticated query types that serve as a prototype for possible questions of interest that might be addressed towards greater understanding of PDAC (Chelala et al. 2009).

3.1.1 Integrated systems biology in identification of PDAC biomarkers

Comprehensive progress has been made on the use of systems biology in identification of biomarkers for PDAC. In a recent study, PDAC cell line related conditioned media and pancreatic juice were both mined for identification of putative diagnostic leads (Makawita et al. 2011). The proteome of the condition media were identified using strong cation exchange chromatography, followed by LC-MS/MS on an LTQ-Orbitrap mass spectrometer from six pancreatic cancer cell lines (BxPc3, MIA-PaCa2, PANC1, CAPAN1, CFPAC1 and SU.86.86), one normal human pancreatic ductal epithelial cell line, HPDE, and two pools of six pancreatic juice samples from ductal adenocarcinoma patients. These studies identified 1261 and 2171 proteins with two or more peptides, in each of the cell lines, while an average of 521 proteins were identified in the pancreatic juice pools. In total, 3,479 non-redundant proteins were identified with high confidence, of which ~40% were extracellular or cell membrane-bound based on genome ontology classifications. Three strategies were employed for identification of candidate biomarkers (1) examination of differential protein expression between the cancer and normal cell lines using label-free protein quantification, (2) integrative analysis, focusing on the overlap of proteins between the multiple biological fluids, and (3) tissue specificity analysis through mining of publically available databases. However, further validation of these proteins is warranted, as is the investigation of the remaining group of candidate biomarkers in PDAC. In another study on PDAC, secreted

serum biomarker identification the profiling pancreatic cancer-secreted proteome using 15N amino acids and serum-free media was performed (Xiao et al. 2010). In this study the effect of oxythiamine chloride on PDAC cell secreteome was studied. The authors further improved on the existing biomarker discovery technology (i.e. coupling of proteomics and in vitro labeling of proteins in cells (SILAC) to enhance the efficacy of biomarker discovery. The authors concluded that labeling protein with ^{15}N amino acids in conjunction with depleted serum allows the identification of actively secreted proteins from pancreatic cancer cells, and the rate of production of a secreted protein may be used as an independent biomarker of the presence of tumor.

3.1.2 Integrated analysis of pathways collectively targeted by co-expressed microRNAs in PDAC

Apart from investigations on signaling pathway de-regulation, multiple recent studies have found aberrant expression profiles of small non-coding RNAs (microRNAs) in PDAC. While several target genes have been experimentally identified for some microRNAs in various tumors, the global pattern of cellular functions and pathways affected by co-expressed microRNAs in PDAC remained elusive. Here too systems biology has found application in identification through computational approach and global analysis of the major biological processes and signaling pathways that are most likely to be affected collectively by co-expressed microRNAs in cancer cells. In a recent study, using five datasets of aberrantly expressed microRNAs in pancreatic and other cancers (breast cancer, colon cancer, lung cancer and lymphoma) and combinatorial target prediction algorithm miRgate and a two-step data reduction procedure Gene Ontology categories were determined (Gusev 2008; Gusev et al. 2007). These studies demonstrated biological functions, disease categories, toxicological categories and signaling pathways that are: targeted by multiple microRNAs; statistically significantly enriched with target genes; and known to be affected in PDAC. The analysis of predicted miRNA targets suggests that co-expressed miRNAs collectively provide systemic compensatory response to the abnormal phenotypic changes in cancer cells by targeting a broad range of functional categories and signaling pathways known to be affected in PDAC. The analysis revealed that E2F1 is a predicted microRNA target as well as caspase3 that were also validated experimentally as a target of multiple miRNAs in PDAC. Such a systems biology based approach provides new avenues for biological interpretation of miRNA profiling data and generation of experimentally testable hypotheses regarding collective regulatory functions of miRNA in PDAC for the design of effective therapies.

3.1.3 Proteomic profiling in identification of PDAC stems cells

PDAC tumors are heterogenous in nature and harbor many different types of cells. In recent years it has been realized that PDAC and other tumors carry a sub-population of cells with stem cell characteristics that are resistant to chemotherapeutic treatment modalities. However, this concept is still controversial since these cells have yet to be comprehensively identified and characterized. PDAC stem cells (CSCs) are such a group of cells that only constitute 0.2-0.8% of the total tumor cells but have been found to be the origin of carcinogenesis and metastasis. However, the extremely low availability of pancreatic tissue

CSCs (around 10 000 cells per xenograft tumor or patient sample) has limited the utilization of currently available molecular biology techniques. Global proteome profiling of pancreatic CSCs from xenograft tumors in mice using integrated systems biology is a promising way to unveil the molecular machinery underlying the signaling pathways in these CSCs. Using a capillary scale shotgun technique by coupling offline capillary isoelectric focusing (cIEF) with nano reversed phase liquid chromatography (RPLC) followed by spectral counting peptide quantification, Lubman and group investigated the proteomic profile of PDAC stems cells (Dai et al. 2010). In comparison with a non-tumorigenic tumor cell sample, among 1159 distinct proteins identified with FDR and less than 0.2%, 169 differentially expressed proteins are identified after multiple testing corrections where 24% of the proteins are up-regulated in the CSCs group. Ingenuity Pathway analysis of these differential expression signatures further indicated that a significant involvement of signaling pathways related to cell proliferation, inflammation, and metastasis were indentified. This was the first study to represents the proteome profiling study on PDAC stem cells from xenografted tumors in mice.

4. Systems biology can aid understanding of the drug mechanism of action in PDAC

Although partially successful in PDAC, new adjuvant targeted therapies (k-ras, EGFR, VEGF, src etc) have been met with more failure than success. The major reason for the low response is related to incomplete understanding and validation of the specific molecular targets at the gene level. The complexities of genetic and epigenetic changes in PDAC, coupled with redundancies and cross-talk in signaling pathways may explain the failure of single-pathway targeted therapies. This can be envisioned from the fact that of the 25,000 genes representing the human genome, about 1,800 are involved in the etiology of numerous diseases including cancer (Wist et al. 2009b). Currently available FDA approved drugs (~ 1200 in the market) have been designed to target approximately 400 genes (**Drugome)**. However, targeting this drugome by individually analyzing each gene is an impossible task because the functional product of each gene or (Proteome) is under multiple control, including splice variants and post translational modifications, giving rise to >40,000 functionally distinct proteins. In addition, such studies, thus far have been hindered by lack of suitable rapid technology. Therefore, novel and high-throughput data acquisition technologies coupled with integrated systems network modeling are urgently required to identify target genes in a tumor-specific manner. Such technologies are crucial for identifying and understanding the mechanisms of potential target candidates in complex diseases like PDAC.

4.1 Systems pharmacology view of drug action

Most of the known targeted drugs currently being used in the clinic were initially designed to affect a single gene. Unfortunately, contrary to the original idea, even the most specific drugs eventually target more than one gene (in most cases, >10 secondary targets). The use of systems pharmacology categorizes these off-targets into two types i) off-targets (resulting in side effects [often toxic]) and ii) secondary targets resulting in partial synergy] (Figure 4) (Berger and Iyengar 2009). These secondary targets exist within a complex network which

can mediate the response to the drugs leading to both therapeutic and adverse effects. Understanding these beneficial secondary targets specially observed in potent synergistic combinations will provide fundamental information for the design of the most potent drug combination for individualized/personalized treatments.

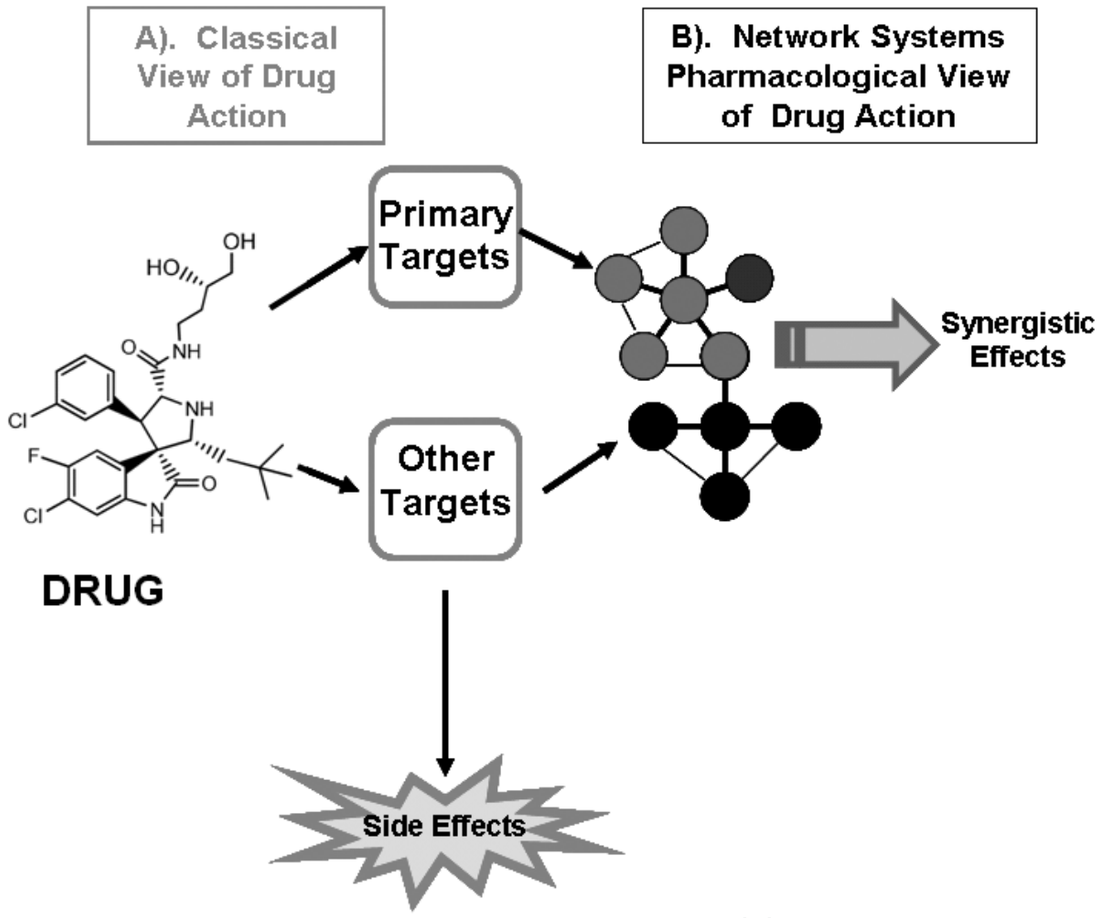

Fig. 4. Traditional vs Network view of drug mechanism of action. Network view differs in understanding the mechanism of action of drugs. Classic view pools all secondary effects as off targets that are considered to cause side effects and toxicity. Network pharmacology categorizes secondary targets into off targets and interacting secondary targets which can mediate the response to the drugs to both the therapeutic or adverse effects. Adopted from Azmi et al., 2011b

Such an understanding requires mechanistic studies in the laboratory to be coupled with robust, state of the art computational tools to obtain irrevocably strong proof for the integration of pathways involved in the observed synergy. One such approach involves the use of network modeling which provides mathematically and statistically robust information regarding the involvement of effector genes in the efficacy or synergy between two drugs. These network models can also predict key secondary targets of such interaction, thus, also providing information on novel previously unrecognized targets and pathways which could be useful for future therapeutic interventions in the treatment of different cancers where, at present, information is gravely lacking, such as in PDAC.

4.1.1 Validation of the systems approach for predicting potent drug combination in PDAC

Our laboratory has been working on a specific small molecule inhibitor of MDM2 (MI-219) and indentifying, in greater detail, its mechanism of action in PDAC (Azmi et al. 2010b). MI-219 is currently in Phase I clinical trial (Brown et al. 2009). Our initial studies were restricted to evaluating its efficacy against wt-p53 tumors. However, we have recently found that MDM2 inhibitor, when combined with chemotherapy such as oxaliplatin, synergistically enhanced apoptosis in wt-p53 cancers and most importantly, 50% of tumor bearing mice treated with this combination remained tumor free without recurrence for 120 days (Azmi et al. 2010a). We used this model to validate a systems approach in predicting potent drug combinations in PDAC and to obtain critical information into understanding the mechanism for this synergy. Therefore, our study included integrated microarray gene expression profiling (IGEMP) and pathway network modeling (PNM) (Azmi et al. 2011a). The systems analysis data for MI-219-oxaliplatin combination treated wt-p53 capan-2 cells revealed that indeed synergy is at the gene level. Principle component analysis showed that one can differentiate the gene signatures between single treatment versus combination. The emergence of certain unique synergy-related genes indicated their potential as key players supporting the overall response of MI-219-oxaliplatin in positively regulating the p53 re-activation (Azmi et al. 2010c; Azmi et al. 2011b). Presented with this vast amount of information regarding the mechanism involved in the response to MI-219-oxaliplatin synergy, we believe it validates the applicability of this technology for use in identifying the relevant pathways involved in both cure and resistance. Ultimately, results of these studies will significantly aid in the design of clinically successful drug combinations for PDAC, which will benefit the overall survival of patients.

4.1.2 Systems identification of biomarker of response with implications for PDAC therapy

Our intended goal in using IGEMP and PNM analysis was to demonstrate the synergy between MI-219-oxaliplatin at the gene level and to demonstrate the local network of p53 and crucial neighboring network that augment p53 re-activation mediated events. Systems network modeling, although a powerful technological tool has not yet been fully explored for use in PDAC (the most genetically complex cancer). We had previously identified several genes responsible for cross-talk within the local network of p53 which included NF-

kB, cadherin anti-tumor module, the tumor suppressor EGR1 and MDM2 negative regulator CREBBP. Our more in-depth analysis using these integrated approach, revealed the prominent role of HNF4A (hepatocyte nuclear factor 4 alpha) that modulates a totally distinct yet p53-linked set of proteins driving apoptosis (Azmi et al. 2010c). The identification of HNF4A as a key player was certainly revealing since it has not been well defined in PDAC cells used in this study (Capan-2 (wt-p53)). However, a search of the literature indicated that this gene is highly expresses in pancreatic tumors compared to their normal counterpart. HNF4A is known to interact with the p53 positive regulator CREBBP (Yoshida et al. 1997) and thus, confirmed its role in augmenting apoptotic effects in this synergic combination. Therefore, not only does systems biology provide information on the networks involved in drug efficacy, it can also provide information on biomarkers of therapeutic response that can be utilized for evaluation of drug response during actual clinical trials in PDAC patients.

5. Conclusion

PDAC is a complex disease that arises from a complex set of genetic mutations and pathway alterations. Traditional sciences have not been very successful in clearly delineating the interaction between these multiple pathways and this could be the primary reason for the observed failure of chemo- and targeted therapies. All of these genetic alterations can now be "re-discovered" using next-generation integrated systems technology. As described above, integrated sciences have revealed that these signaling pathways cross talk with one another and can regulate cell growth, proliferation, survival, angiogenesis and metastasis in PDAC. In addition, these high-throughput technologies can achieve many different goals such as cataloging the driver mutations, exploring functional role of cancer genes, proteins and interaction networks, identifying microRNAs, understanding protein–DNA interactions, and comprehensive analyses of transcriptomes and interactomes. Furthermore, these technologies can be utilized to identify, understand and differentiate sub population of CSCs in PDAC heterogeneous tumor mass. Systems biology has the power to catalog complex events leading to origin, progression, recurrence and resistance of PDAC and can greatly assist in understanding how cancer genomes operate as part of the whole biological system. Now, high-quality clinical treatment and outcomes (death or survival) data from biobanks, and extensive genetics and genomics data for some PDAC and other tumors, including breast, colorectal, and lung are available. How all these clinical and genetics data could be integrated into reverse engineering-based network modeling to approach the extremely complex genotype–phenotype map of different tumors is currently being explored. These studies will pave way for the discovery of new molecular innovations, both predictive markers and therapies, towards personalized treatment of PDAC. Therefore systems biology can aid in the overall understanding of PDAC.

6. Acknowledgment

We thank Dr. Frances W.J. Beck for critically evaluating this manuscript. All members of Dr. Fazlul H. Sarkar's team who could not be added in this chapter are acknowledged.

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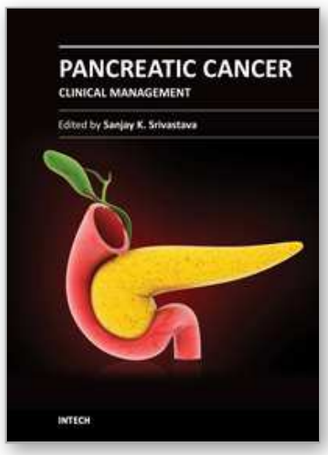

Pancreatic Cancer - Clinical Management
Edited by Prof. Sanjay Srivastava

ISBN 978-953-51-0394-3
Hard cover, 312 pages
Publisher InTech
Published online 28, March, 2012
Published in print edition March, 2012

This book covers pancreatic cancer risk factors, treatment and clinical procedures. It provides an outline of pancreatic cancer genetic risk factors, biomarkers and systems biology for the better understanding of disease. As pancreatic cancer suffers from lack of early diagnosis or prognosis markers, this book encompasses stem cell and genetic makers to identify the disease in early stages. The book uncovers the rationale and effectiveness of monotherapy and combination therapy in combating the devastating disease. As immunotherapy is emerging as an attractive approach to cease pancreatic cancer progression, the present book covers various aspects of immunotherapy including innate, adaptive, active, passive and bacterial approaches. Management of anesthesia during surgery and pain after surgery has been discussed. Book also takes the reader through the role of endoscopy and fine needle guided biopsies in diagnosing and observing the disease progression.

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