

## Identifying Biomarkers and Drug Targets Using Systems Biology Approaches for Pancreatic Cancer

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### Editorial

Pancreatic Cancer (PC) is an incurable malignancy with an estimated 44,030 new cases and 37,660 deaths in 2012 [1]. The median survival is about 6 months and the five-year relative survival rate is less than 5% [1]. The reasons for this high mortality rate are partly due to symptoms at the early stages of this disease and lack of effective treatment by chemotherapeutic drugs [1]. Therefore, identification of newer diagnostic biomarkers and discovery of novel therapeutics for druggable targets are required to achieve better treatment outcome in PC patients.

In recent years, systems biology approaches have been applied to screen and identify the diagnostic biomarkers and druggable targets for prevention and treatment of PC [2,3]. It is well known that systems biology used mathematic, physics, computer science, engineering, and biological sciences to explore the biological behavior, which leads to better understand the complexities of biological functions [4]. In general, several systems biology approaches including cDNA microarray, mass spectrometry-based proteomics, tissue microarray, and microRNA microarray have often been employed in PC [5].

DNA microarray method used DNA microarray platforms to gain gene expression signatures. The cDNA and oligonucleotide-based microarrays are often used [6]. It is worth mentioning that DNA microarray has two disadvantages: the cost is relatively high and the data require further validation [7]. To discover new biomarkers and drug targets for PC, Han et al. [8] used cDNA microarray analysis to determine the differences in gene expression profiles between pancreatic cancer cell lines and normal pancreatic cells. This study identified multiple genes including urokinase-type plasminogen activator receptor, serine/threonine kinase 15, thioredoxin reductase, and CDC28 protein kinase 2 that could serve as potential clinical biomarkers and novel therapeutic targets [8]. Tan et al. [9] used cDNA microarray method and compared the gene expression pattern of PC with that of adjacent normal tissues. They found that 166 genes were up-regulated and 135 genes were down-regulated, suggesting that cDNA microarray could provide invaluable information regarding the discovery of innovative therapeutic targets for PC [9]. Similarly, Yu et al. [10] studied the gene expression profiles of PC by means of cDNA microarray consisting of 18,000 genes. Among these genes, 455 genes were found to be altered in PC. Moreover, studies have suggested that MBD1, EDG1 and gene hypermethylation mechanism might also play a crucial role in the progression of PC [10].

Notably, Nakamura et al. [11] analyzed gene expression profiles of PC using a cDNA microarray representing 23,040 genes. This group identified 260 genes that were up regulated and 346 genes that were down-regulated in PC. Among these genes, 76 genes were related to

lymph node metastasis, 30 genes were involved in the recurrence, and 168 genes were associated with liver metastasis [11]. Consistent with this study, Missiaglia et al. [12] used cDNA microarray and identified multiple candidate markers for pancreatic tumorigenesis and metastasis including insulin-like growth factor binding protein 3 and 4, S100P, S100A4, prostate stem cell antigen, lipocalin 2, claudins 3 and 4, trefoil factors 1 and 2, and Forkhead box J1. Furthermore, another study using cDNA microarray implicated that a set of 105 genes could be actively involved in the pathogenesis of PC [13]. Recently, Xu et al. [14] identified 278 up-regulated and 59 down-regulated genes upon Gli1 expression in PC cells, suggesting that SHH-Gli1 signals promote EMT by mediating a complex signaling network.

Strikingly, cDNA microarray method has also been employed to identify the gene expressions in drug resistant PC cells for providing molecular targets to overcome drug resistance. Nakai et al. [15] revealed that 30 genes including TNFSF6 were involved in gemcitabine sensitivity in PC. Additionally, cDNA microarray was also used to screen the invasion-metastasis-related factors in PC [16]. This study identified 46 up-regulated genes and 95 down-regulated genes, indicating that a highly organized regulation by many genes exists in tumor invasion and metastasis in PC [16]. Interestingly, cDNA microarray was recently used to explore long noncoding intronic RNAs expression profile in PC [17]. This work found that multiple intronic lncRNAs such as PPP3CB, MAP3K14 and DAPK1 were found to be correlated with tumor metastasis in PC [17]. Taken together, cDNA microarray could be a powerful method to provide useful information for the development of new therapeutic and diagnostic targets.

Tissue microarray, which often uses Immuno Histo Chemistry (IHC), has been broadly employed to investigate the biomarkers in various tumor tissue specimens [18]. Multiple proteins have been considered as the potential diagnostic and treatment biomarkers in PC. For example, using tissue microarray, osteopontin was found as a possible diagnostic marker in PC patients [19]. Gray et al. [20] identified Plk1 (polo-like kinase 1) as a potential therapeutic target

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in PC by a tissue microarray approach. Wang et al. [21] revealed that PDX1 (pancreatic duodenal homeobox 1) is an early potential diagnostic biomarker and could be a drug target for PC. Moreover, CEACAM6 expression was found to be more prevalent in high-grade PC and could be correlated with metastasis and survival using the tissue microarray approach [22]. Altogether, using a high-throughput tissue microarray could be a valuable approach to identify biomarkers for designing rational strategies for treatment of PC.

Tissue microarray method has also been used to explore the underlying mechanisms of EMT (Epithelial to Mesenchymal Transition) progression. One study used a tissue microarray approach and found that WISP-2/CCN5 silencing plays a critical role in promoting EMT process in PC [23]. To determine the role of EMT in PC, immunohistochemical stains for EMT markers such as Twist, Slug, and N-cadherin were performed using a tissue microarray containing 68 PC and 38 samples of normal pancreas or chronic pancreatitis tissues [24]. Interestingly, none of these markers could be used for prediction of patient outcome. However, loss of membrane localization and aberrant nuclear E-cadherin expression was found to be associated with invasion in PC [24]. Moreover, aberrant expression of both beta-catenin and E-cadherin was found to be correlated with lymph node spread and liver metastases in PC [25].

Recently, scientists constructed tissue microarray approach to determine the role of cancer stem cells in the development and progression of PC. It has been reported that the expression of the stem cell markers Oct4 and Nanog is associated with early stages of pancreatic carcinogenesis [26]. Consistent with this, ALDH1 (aldehyde dehydrogenase 1), a pancreatic cancer stem cell marker, has been considered as a prognostic marker in a PC tissue microarray [27]. Moreover, Zhu et al. [28] identified multiple glycoprotein markers including cytokeratin 8/CK8, integrin  $\beta$ 1/CD29, ICAM1/CD54, ribophorin 2/RPN2 and aminopeptidase N/CD13 for PC stem-like cells by tissue microarray and nano-LC-MS/MS methods. Taken together, tissue microarray is a powerful tool for identifying the function of cancer stem cell in pancreatic tumorigenesis.

It has been known that microRNAs (miRNAs) are involved in various cellular processes including cell growth, apoptosis, migration, invasion, angiogenesis, and metastasis in PC [29]. Therefore, defining miRNA expression profile is important to explore the insight into pancreatic carcinogenesis. To this end, miRNA microarray has been employed to measure short non-coding RNAs in PC. We investigated the different expression profile of miRNAs in the plasma between PC patients and healthy volunteers [30]. We observed that 54 miRNAs were up-regulated and 37 miRNAs were down-regulated. For example, miR-21 was increased, whereas miR-146a and let-7 families were decreased [30]. In line with this report, using miRNA microarray, 20 miRNAs were found to be associated with overall survival in PC [31]. Moreover, multiple miRNAs were correlated with lymph node metastasis and high tumor grade. Specifically, up-regulation of miR-21 and down-regulation of miR-34a and miR-30d were associated with poor overall survival [31]. Similarly, a high-throughput miRNAs microarray unravels the prognostic role of miR-211 in PC [32].

Although multiple studies suggest that miRNA microarray could be useful to explore the mechanisms of PC development, this method is not a very powerful tool for this purpose [33]. One study compared miRNA expression using miRNA microarray, TaqMan low density array (TLDA), and single tube quantitative RT-PCR (qRT-PCR) [33]. TLDA identified 19 miRNAs with up-regulation and 35 miRNAs with down-regulation in high metastatic PC cells compared with low

metastatic PC cells [33]. However, miRNA microarray only identified 27 up-regulated miRNAs. Moreover, all altered miRNAs identified by TLDA were validated by qRT-PCR, whereas miRNA microarray detected only 25% of qRT-PCR validated miRNAs, suggesting that miRNA microarray is not the best approach to determine miRNA expression profile in PC tumorigenesis [33].

Interestingly, scientists also employed other systems biology approaches such as CpG island microarray to analyze the methylation profile of CpG islands in PC [34]. Using this CpG island microarray, dozens of aberrantly methylated genes have been identified, indicating that identification of hypermethylated and silenced genes could have diagnostic, prognostic, and therapeutic applications in PC [34]. In support of this concept, another group used methylation CpG island amplification and agilent CpG island microarray analysis to detect the CpG island methylation profiles in PC. This group found that 245 genes were hypermethylated in PC, suggesting that PC could have extensive aberrant CpG island hypermethylation [35]. Recently, Messina was developed as a novel analysis tool to identify biologically important molecule aberrations in PC [36]. The advantage is that Messina can identify genes that only sometimes show aberrant expression in PC [36]. We believe that more systems biology approaches will be discovered to determine the biomarker and drug targets in PC in the near future.

It is important to note that systems biology approaches have some limitations. First, the good quantity and quality of mRNA, cDNA, miRNA and protein are highly required for microarray analysis. Second, the large amount of data obtained from microarray is required to validate and clarify which are the most critical genes for tumorigenesis. Third, since DNA and mRNA levels are not exactly paralleled by protein level due to post-translational modification, many conclusions from DNA microarray need to be validated at a protein level. Therefore, it is better to combine different assays to overcome these pitfalls. To this end, gene expression profile from DNA microarray need to be confirmed by RT-PCR and tissue microarray. Altogether, although systems biology approaches have some limitations, they have been considered as potentially useful tools to detect the prediction of treatment outcome, discover biomarker, and drug targets in PC.

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#### Conflict of Interest

The authors declare that they have no conflict of interest.

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