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Original Article

Analysis of whole genomic expression profiles and screening of the key signaling pathways associated with pancreatic cancer

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Abstract: The tumorigenesis of pancreatic cancer is thought to be a complex process. Investigation of the molecular mechanism of pancreatic cancer and exploring the specific markers for early diagnosis and specific targets of therapy is a key point to prevent and treat pancreatic cancer effectively and to improve their prognosis. In this study, expression profiles experiment was performed using Agilent human whole genomic oligonucleotide microarrays with 41,000 genes. Differentially expressed genes related with pancreatic cancer were screened, and analyzed further by GO term analysis and KEGG Pathway analysis. Our results showed that there were 1276 differentially expressed genes associated with pancreatic cancer. 691 genes were up regulated and 585 were down regulated in pancreatic cancer group. The present study confirmed that the occurrence of pancreatic cancer was involved in multiple-gene interaction. In addition, our study found that pancreatic cancer was related to an activation of the mTOR signaling pathway and renal cell carcinoma pathway.

Keywords: Pancreatic cancer, microarray, gene expression profile, signaling pathway

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal cancers in the world with a 5-year survival rate of <5% [1], and the incidence rate showed an upward trend in the domestic and foreign. There are about 43,140 new cases are diagnosed with pancreatic cancer in the United States in 2010 and 36,800 of them will die of the disease [1], which is in fourth place in cancer mortality [2]. In addition, because of its metastatic potential and the cytotoxic chemotherapy drug tolerance, which usually results in ineffective non-surgical treatment, the vast majority of patients will die within one year. Although in recent years, the molecular pathogenesis of pancreatic cancer research has made great progress, the clinical diagnosis and treatment of pancreatic cancer is still intractable.

The tumorigenesis of pancreatic cancer is thought to be a complex process and involves multiple genetic alterations. Investigation of the molecular mechanism of pancreatic cancer and exploring the specific markers for early diagnosis and specific targets of therapy are key points to prevent and treat pancreatic cancer effectively and to improve their prognosis. Over the past several years, a number of cancer-related genes have been detected in human PDAC, most commonly affected genes in pancreatic cancer are K-ras, Smad4, p53 and p16 [3]. These genetic discoveries have given important insights into the molecular pathogenesis of PDAC and have stimulated efforts to develop diagnostic and therapeutic agents. However, considering the complexity of the genome, it is most likely that many of human genes still have not been indentified in pancreatic cancer. Moreover, it has been shown that aberrant activation of

signaling pathways, such as hedgehog [4], Wnt [5] and Notch signaling pathway [6] is frequently observed in pancreatic cancer. Defective, overactive or dominating signaling pathways can motivate tumor growth and survival, as well as progression of invasion and metastasis [7]. Consequently, exploring the key alterations of signaling pathways involving genesis and progression of pancreatic cancer may provide promising targets for rational molecular-targeted anticancer drug design. For example, the ras-signaling pathway has attracted considerable attention as a target for anticancer therapy because of its important role in carcinogenesis [8]. However, the signaling pathways which are implicated in pancreatic cancer are not well defined. Effective cancer treatment strategies are required to aim at the changes of genes and signaling pathways of individual characteristics.

Therefore, in order to extensively investigate molecular mechanism involved in pancreatic carcinogenesis, and lay the theoretical basis for early diagnosis and treatment of pancreatic cancer, gene expression profiles were compared in whole genome expression levels with high-throughput oligonucleotide microarray technology in this study. We hoped to screen additional genes and key signaling pathways which could guide future research on pancreatic cancer.

Materials and methods

Tissue samples

From January 2007 to March 2008 six cases of pancreatic cancer and paracancerous tissues were obtained by surgery and screened by pathology (Saved by the Shanghai national co-existence Biochip Research Center Tissue Bank). There is no difference in gender, age and underlying diseases: 3 patients were male and 3 were female, aged from 41 to 67 years and the mean age was 52 years. Biopsies were placed in liquid nitrogen for chip hybridization. The tissue samples were obtained for this study with patient informed consent, and Ethical approval for the study was obtained from the ethical committee of biobank center related hospitals.

Total RNA extraction and purification from tissue

Six cases of pancreatic cancer tissues and paracancerous tissues were remove from liquid nitrogen, adding 1 ml TRIzol (Invitrogen, Carlsbad, USA) per 50 ~ 100mg tissue. According to the instruction, tissue should be samshed completely with a homogenizer. Total RNA was extracted using method of phenol / chloroform, salt was washed away with 70% alcohol. After air-dried in 15 ~ 30℃, adding the appropriate amount of RNase-free water to dissolve RNA. With Nanodrop spectrophotometer (NanoDrop Inc, Wilmington, DE USA) and agarose gel electrophoresis, quantity, quality and purity of total RNA were evaluated the total RNAs from tissues were select and extract respectively.

Sample labeling, gene chip hybridization

Sample RNAs were labeled using indirect method. cDNA Synthesis from total RNA: 1μg of total RNA were used as sample RNA, then with T7-oligo (dT) Promotor primer, first strand and second strand of cDNA were synthesized. Following Agilent Low RNA Input Linear Amplification Kit (Agilent, Palo Alto, CA, USA), fluorescent cRNA was Synthesized and amplified. This is a process of in vitro transcription and incorporation of cyanine 3-CTP. We used Nanodrop ND-1000 (NanoDrop Inc, Wilmington, DE USA) to quantitate yielded cRNA. Hybridization mix was prepared following the manual of Agilent oligonucleotide microarray *in situ* hybridization plus kit. 750ng fluorescence labeled probe was fragmentated at 60℃. Hybridization mix and probes were added on Human Whole-Genome 60-mer oligonucleotide microarrays (44K Agilent Human Genome, Agilent Technologies, Palo Alto, CA, USA). Hybridization oven was set to 60℃, hybirdization rotator was set to rotate at 10rpm, hybridization was 17 hours. When hybridization was finished, Microarray washing was performed according to the procedure of Agilent Gene Expression Wash Buffer Kit. Microarray was scanned by Agilent Scanner and image was extracted by Feature Extraction Software to acquire the original data.

Microarray scanning and data analysis

Microarray image scanning and quantitative analysis were acquired using Feature Extraction which is the corresponding system integrated with Agilent's Feature Extraction 7.1 image analysis software (Agilent Technologies, Palo

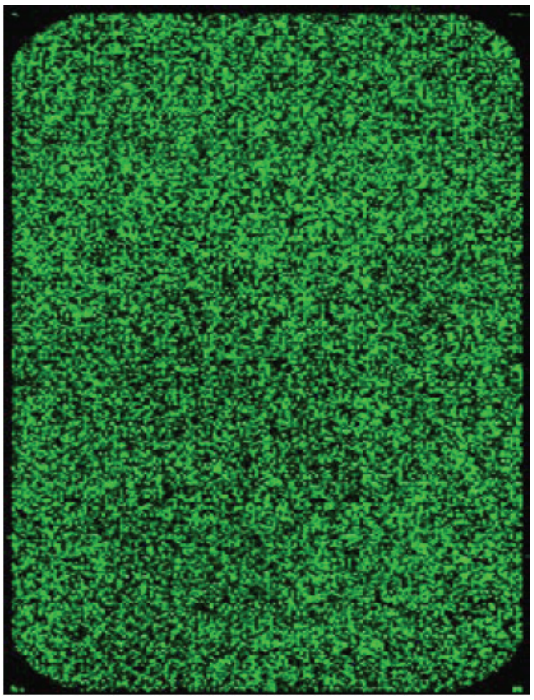

Figure 1. Gene chip hybridization fluorescence signal graph.

Alto, CA, USA), image quantification and standardization of data processing. After standardization of the original signals the low expression probes were to be filtered, stringent selection were performed, all samples' "gIsFound" should be equal to 1. Screening of differential genes: To screen the differential probes of between pancreatic cancer tissue samples and paracancerous tissues samples, *P* value of each probe was calculated by T test. According to the criterion of $P < 0.01$, probes were selected, then switched from the corresponding GeneBank Accession number to Entrez IDs.

Criterion for Significant difference of GO functional classification was $P < 0.05$, and significant gene functional classification was sort in accordance with the *P* value from low to high. In GO functional classification, the gene with the highest score which takes into account the two indicators *Enrichment* and *Count of Genes* possess, the most biology significance.

KEGG's Pathway Analysis: From a practical point of view of gene function annotation, KEGG Pathway database is the most widely used and comparatively more comprehensive database of annotation information. The pathway classification criterion for Significance is the EASE Score *P*-value < 0.05. Significant Pathway classification sorts the P-values from low to high sort, and analyses the relative ratios based on the actual differential genes in all Pathway classification to obtain the Enrichment of Pathway Categories. The higher the Enrichment value of differential genes it is, the greater the proportion accounted for the Pathway classification and the higher participation of the contribution of differential expression it will be. Enrichment and the Count of Genes, these two indicators with the highest score in Pathway classification have the most biological significance.

Results

Microarray hybridization and data analysis

Microarray experiment was qualified according with quality standards. The experimental system was stable, fluorescent signal intensity was strong and homogenous (**Figure 1**). In the 12 chips 27,574 probes had clearly signals, representing 67.3% of 41 000 probe. The differentially expressed genes associated with pancreatic cancer were 1276, in which 691 were up regulated and 585 were down regulated. We used GO (Gene Ontology) to analyze these differentially expressed genes by three domains. In biological process, we found the *P* values of 22 functional description nodes were less than 0.01, which were mainly related to the regulation of cellular or cellular metabolic process, nucleic acid metabolic process and transcription, chromatin modification, intracellular signaling cascade, post-translational protein modification, G-protein signaling and so on (**Table 1**). In cellular component, 3 of functional description nodes were found less than 0.01, which were mainly located in the nucleus, the cell and local (**Table 2**). In molecular function , the P values of 12 functional description nodes were less than 0.01, such as DNA, RNA or protein binding, zincion binding, transcription regulation and activation activity, etc. (**Table 3**).

Screening of the signaling pathways related to pancreatic cancer

KEGG Pathway analysis: The two highest scores of indicators of Enrichment and Count are hsa04150: Mammalian target of rapamycin (mTOR) signaling pathway, and hsa05211:

Table 1. GO (Gene Ontology) analysis of pancreatic cancer with different biological processes related to gene

Category	Term	Count of genes	Percent of count of genes	Fold enrichment	*P*-value
GOTERM_BP_ALL	Programmed cell	478	61.20%	1.072124483	2.30E-05
GOTERM_BP_ALL	Programmed cell regulation	190	24.33%	1.249948604	2.05E-04
GOTERM_BP_ALL	Biological regulation	219	28.04%	1.216286577	2.43E-04
GOTERM_BP_ALL	Metabolic processes of biological macromolecules	220	28.17%	1.193807126	7.38E-04
GOTERM_BP_ALL	Biological process regulation	198	25.35%	1.210338306	8.48E-04
GOTERM_BP_ALL	Chromatin modification	16	2.05%	2.315694256	0.004008869
GOTERM_BP_ALL	Intracellular signaling cascade	71	9.09%	1.380651434	0.004459034
GOTERM_BP_ALL	DNA dependent transcriptional regulation	107	13.70%	1.279904306	0.004671531
GOTERM_BP_ALL	Gene regulation	119	15.24%	1.25428195	0.0050482
GOTERM_BP_ALL	DNA dependent transcriptional regulation	108	13.83%	1.263296414	0.00640369
GOTERM_BP_ALL	Protein modification process	82	10.50%	1.32231405	0.006550157
GOTERM_BP_ALL	Cellular metabolic process	313	40.08%	1.101155466	0.006596904
GOTERM_BP_ALL	Post-transcriptional protein modification	71	9.09%	1.357066061	0.0066885
GOTERM_BP_ALL	RNA synthesis	108	13.83%	1.26177437	0.006918278
GOTERM_BP_ALL	G-protein signaling	11	1.41%	2.735042735	0.006968409
GOTERM_BP_ALL	Transcriptional regulation	112	14.34%	1.254111554	0.006969324
GOTERM_BP_ALL	Cellular metabolic process regulation	122	15.62%	1.23532576	0.007422386
GOTERM_BP_ALL	Basic metabolic process	313	40.08%	1.098233572	0.007941417
GOTERM_BP_ALL	Transcription	115	14.72%	1.241356047	0.008236733
GOTERM_BP_ALL	Polymer metabolism	276	35.34%	1.112678895	0.008454144
GOTERM_BP_ALL	Modification of biological macromolecules	84	10.76%	1.301884051	0.008663423
GOTERM_BP_ALL	Regulate nucleic acid bases, nucleotides, nucleic acid metabolism	113	14.47%	1.2367467	0.009761074

Table 2. GO (Gene Ontology) analysis of pancreatic cancer associated with the cellular location of differentially expressed genes

Category	Term	Count of genes	Percent of count of genes	Fold enrichment	*P*-value
GOTERM_CC_ALL	Nucleus	196	25.10%	1.196364067	0.001908819
GOTERM_CC_ALL	Cells	399	51.09%	1.077291739	0.005076384
GOTERM_CC_ALL	Local cell	375	48.02%	1.077702214	0.009388117

Table 3. GO (Gene Ontology) analysis of pancreatic cancer associated with molecular function of differentially expressed genes

Category	Term	Count of genes	Percent of count of genes	Fold enrichment	*P*-value
GOTERM_MF_ALL	Combine	498	63.76%	1.089757696	1.70E-05
GOTERM_MF_ALL	RNA polymerase II transcription factor activity	23	2.94%	2.628040404	6.97E-05
GOTERM_MF_ALL	Activity of RNA polymerase II transcription media	5	0.64%	8.926767677	0.001921619
GOTERM_MF_ALL	Total RNA polymerase II transcription factor activity	7	0.90%	5.127169127	0.002169247
GOTERM_MF_ALL	Protein binding	287	36.75%	1.136764203	0.00241749
GOTERM_MF_ALL	Zinc ion binding	109	13.96%	1.297356902	0.003004454
GOTERM_MF_ALL	Transcription activity	74	9.48%	1.378004293	0.003936181
GOTERM_MF_ALL	Transcriptional activation	22	2.82%	1.933675214	0.005204198
GOTERM_MF_ALL	DNA binding	109	13.96%	1.270880231	0.00568968
GOTERM_MF_ALL	Chelate metal ions transfer	126	16.13%	1.237714143	0.006488172
GOTERM_MF_ALL	Transcriptional co-activator activity	15	1.92%	2.2671156	0.006696523
GOTERM_MF_ALL	Nucleic acid binding	154	19.72%	1.192171033	0.009547129

Table 4. Pancreatic cancer KEGG pathway analysis result

KEGG pathway ID and name	Count of genes	Percent of count of genes	NCBI entrez gene ID of genes	Fold enrichment	*P*-value
hsa04150:mTOR signaling pathway	9	1.15%	9706, 8503, 5170, 1978, 5228, 7423, 25989, 253260, 23533,	4.5622519	6.23E-04
hsa05211:Renal cell carcinoma	9	1.15%	7043, 2034, 8503, 54583, 5228, 2113, 7423, 1387, 23533	3.4727589	0.003787618

Renal cell carcinoma pathway (**Table 4**). In mTOR Pathway, the 9 key genes of ULK2, PIK3R3, PDPK1, EIF4EBP1, PGF, VEGFB, ULK3, RICTOR and PIK3R5 had significant difference ($P < 0.05$, **Table 5**). In renal carcinoma pathway, the 9 key genes of TGFB3, EPAS1, PIK3R3, EGLN1, PGF, ETS1, VEGFB, CREBBP and PIK3R5 had significant difference ($P < 0.05$, **Table 6**).

Discussion

The DNA microarray gene expression profiles have been successful in the large-scale analysis of gene expression differences, particularly in cancer investigations. Recently, numerous studies have reported gene expression profiles of various cancers [9-14], including pancreatic cancer [15-18]. For example, Nakamura *et al* [19] examined gene-expression profiles of 18 pancreatic cancers. The analysis identified 260 commonly up-regulated genes and 346 down-regulated genes in pancreatic cancer. In present study, through comparison of gene expression profile of pancreatic cancer and paracancerous tissue, differentially expressed genes were screened and analyzed further by GO term analysis. Our results showed that there were 1276 differentially expressed genes associated with pancreatic cancer. 691 genes were up regulated and 585 were down regulated in pancreatic cancer group. Furthermore, our study found that pancreatic cancer was related to an activation of the mTOR signaling pathway and renal cell carcinoma pathway.

By GO analysis, genes of significant difference involved in biological process mainly related to regulation of cellular or cellular metabolic process, nucleic acid metabolic process and tran-

Table 5. Key gene list of mTOR signaling pathway related to pancreatic cancer

Entrez gene ID	Gene symbol	Cancer/paracancerous tissue ratio (2)	*P*-value	Description
9706	ULK2	-0.25170218	0.048056072	Homo sapiens unc-51-like kinase 2 (C. elegans) (ULK2), mRNA [NM_014683]
8503	PIK3R3	-1.88099339	0.033034565	Homo sapiens Phosphatidylinositol 3-kinase regulatory subunit gamma 3 (p55, gamma) (PIK3R3) , mRNA [NM_003629]
5170	PDPK1	0.39752027	0.033717114	Homo sapiens 3-phosphoinositide dependent protein kinase-1(PDPK1), mRNA [NM_002613]
1978	EIF4EBP1	-0.28287385	0.040891956	Homo sapiens eukaryotic translation initiation factor 4E bindingprotein 1 (EIF4EBP1), mRNA [NM_004095]
5228	PGF	-0.29988699	0.012464356	Homo sapiens placental growth factorascular endothelial growth factor-related protein (PGF), mRNA [NM_002632]
7423	VEGFB	-0.31859571	0.044830214	Homo sapiens Vascular endothelial growth factor B (VEGFB), mRNA [NM_003377]
25989	ULK3	0.254669903	0.001672132	Human mRNA; cDNA DKFZp434C131 (from clone DKFZp434C131). [AL117482]
253260	RICTOR	0.649677387	0.009706675	Homo sapiens rapamycin-insensitive companion of mTOR (RICTOR), mRNA [NM_152756]
23533	PIK3R5	-0.73838379	0.030512859	Homo sapiens Phosphoinositide 3-kinase regulatory subunit 5, p101, p101 (PIK3R5), mRNA [NM_014308]

Table 6. Key gene list of renal cell carcinoma pathway related to pancreatic cancer

Entrez gene ID	Gene symbol	Cancer/paracancerous tissues ratio (2)	*P*-value	Description
7043	TGFB3	-1.05930128	0.00163691	Homo sapiens transforming growth fact β3 (TGFB3), mRNA [NM_003239]
2034	EPAS1	0.92358726	0.034126314	Human Endothelial PAS Protein1(EPAS1), mRNA [NM_001430]
8503	PIK3R3	-1.88099339	0.033034565	Homo sapiens Phosphatidylinositol 3-kinase regulatory subunit gamma (p55, gamma) (PIK3R3) , mRNA [NM_003629]
54583	EGLN1	0.217749398	0.01410161	Homo sapiens EGLN1, mRNA [NM_022051]
5228	PGF	-0.29988699	0.012464356	Homo sapiens placental growth factorascular endothelial growth factor-related protein(PGF), mRNA [NM_002632]
2113	ETS1	0.670387076	0.049698783	Homo sapiens v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1), mRNA [NM_005238]
7423	VEGFB	-0.31859571	0.044830214	Homo sapiens Vascular endothelial growth factor B (VEGFB), mRNA [NM_003377]
1387	CREBBP	0.410273283	0.004572845	Homo sapiens CREB binding protein (Rubinstein–Taybi syndrome) (CREBBP), mRNA [NM_004380]
23533	PIK3R5	-0.73838379	0.030512859	Homo sapiens Phosphoinositide 3-kinase regulatory subunit 5, p101 (PIK3R5), mRNA [NM_014308]

scription, chromatin modification, intracellular signaling cascade, post-translational protein modification, G-protein signaling and so on. Genes of significant difference involved in cellular location mainly located at nucleus and intracellular part. Genes of significant difference involved in molecular function mainly related to DNA, nucleic acid or protein binding, zinc ion binding, transcription regulator and activator activity, etc.

Cellular programming is essential to normal cell growth, proliferation and apoptosis, and this "procedure" is not a single process, but rather a component process. We found 478 significant abnormal genes involved in cellular programming associated with pancreatic cancer. Regulating the cellular programming supposed to inhibit tumor growth. Recently, Mouratidis *et al* [20] studied cell killing effect on pancreatic cancer of two new PDE4 is (Phosphodiesterase-4 inhibitors) inhibitor compounds CC-8075 and CC-8062 .The results showed significant antiproliferation effect, which may be effected by intervening the activation of p38MAPK signal transduction pathway. In addition, cytokines such as TNF trigger intracellular signal transduction cascade and induce apoptosis, mediated through the activation of appropriate cell membrane receptors [21]. If the intracellular signal transduction cascade is affected, it will undoubtedly lead to abnormal apoptosis. It is well known that pancreatic cancer is closely associated with apoptosis [22, 23]. In this study, we found 71 significantly abnormal genes of pancreatic cancer patients were involved in intracellular signal transduction cascade.

Moreover, Bioinformatics analysis of KEGG pathway in this study showed that mTOR signaling pathway and renal cell carcinoma pathway had a most biological significance associated with pancreatic cancer. It has been shown that mTOR pathway functions in the downstream of the phosphatidylinositol 3-kinase (PI3K) /AKT pathway, which is often deregulated in human cancer and the deregulation of mTOR pathway will cause loss of growth control in tumor. In addition, several studies have reported that mTOR pathway whose activity increases in some tumors can be used as the target for cancer treatment [24, 25]. In mTOR pathway, there were nine key genes differentially expressed which included ULK2, PIK3R3, PDPK1, EIF4EBP1, PGF, VEGFB, ULK3, RICTOR and PIK3R5 ($P < 0.05$). Some of these genes have been reported to associate with pancreatic cancer or other cancers. For example, Soroceanu *et al* [26] reported that Insulin-Like Growth Factor II (IGF2)-PIK3R3 signaling axis involved in the promotion of occurrence of human neural glioblastoma. In addition, Zhang *et al* [27]found that knockdown of PIK3R3 significantly increased the apoptosis in cultured ovarian cancer cell lines, and the result suggested that PIK3R3 may be a potential therapeutic target of epithelial ovarian cancer. One Study has found that PDPK1 gene show aberrant expression in gastric cancer, colon cancer and lung cancer [28]. Chakraborty *et al* [29] reported to confirme that mTOR signaling pathway and PDPK1 up-regulation play an important role in oral squamous cell carcinoma and several key members of this pathway can provide useful therapeutic targets. Our study has reported PDPK1 was significantly up-regulated in pancreatic cancer which was consistent with the previous research. Similarly, EIF4EBP1 [30], PGF [31], VEGFB [32] and RICTOR have associated with a variety of tumors, the former has tumor suppressor function and can be used as an indicator of prognostic evaluation of gastrointestinal tumors. However, the relationships of PIK3R5, ULK2 and ULK3 with tumor have not been reported previously.

Additionally, In renal cell carcinoma pathway, which involves multiple genes and interactive pathway, nine key genes including TGFB3, EPAS1, PIK3R3, EGLN1, PGF, ETS1, VEGFB, CREBBP and PIK3R5 ($P < 0.05$) were significantly differentially expressed in present study. Among those genes, Some previous studies have reported that the activity of PIK3R3, PGF and VEGFB increased in some tumors and these genes can be used as targets for the tumor treatment [26, 27, 32]. TGFB3 inhibits tumor formation. There are several reports about its decreased expression associated with cancer diagnosis and therapy [33, 34]. Fukumura *et al* [35] also considered TGFB3 was associated with chronic, cancer-related pancreatitis fibrosis. This study found that TGFB3 was significantly reduced in pancreatic cancer. Collado *et al* [36] found that endothelial

PAS domain protein 1 (EPAS1) was significantly increased in colorectal cancer tissue, supervised class prediction using EPAS1, UBE2D3 and KIAA0101 correctly (77%) assigned pre-surgery samples to the CRC group and assigned post-surgery samples from the same patients to the healthy group. In our result, EPAS1 and EGLN1 were found to be over-expressed in pancreatic cancer, which were consistent with some previous studies [37, 38]. Moreover, ETS1 was found over-expressed in pancreatic cancer and to be involved in the chemoresistance to gemcitabine in pancreatic cancer [39]. Dysregulation of CREBBP also contributes to various diseases including cancer [40], but there is no report about CREBBP activity in Pancreatic cancer. We found ETS1 ($P < 0.05$) and CREBBP ($P < 0.01$) were significantly increased in pancreatic cancer on whole genome detection. Their relationship with the biological behavior of pancreatic cancer is being further explored.

Taken together, our present study confirmed that the occurrence of pancreatic cancer was involved in multiple-gene interaction. Moreover, our study found that mTOR pathway and renal cell carcinoma pathway were closely related to pancreatic cancer. The research of these signal systems and the interactions of genes in pancreatic cancer research will contribute to the early diagnosis and targeted therapy cancer. In addition, the action of these differentially expressed genes in the progress of pancreatic cancer pathogenesis and development needs further excavation and research.

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References

[1] Jemal A, Siegel R, Xu JQ and Ward E. Cancer Statistics, 2010. Ca-a Cancer Journal for Clinicians 2010; 60: 277-300.

[2] Li DH and Abbruzzese JL. New Strategies in Pancreatic Cancer: Emerging Epidemiologic and Therapeutic Concepts. Clinical Cancer Research 2010; 16: 4313-4318.

[3] Remmers N, Bailey J, Mohr A and Hollingsworth MA. Molecular pathology of early pancreatic cancer. Cancer Biomarkers 2011; 9: 421-440.

[4] Lauth M and Toftgard R. Hedgehog Signaling and Pancreatic Tumor Development. Advances in Cancer Research 2011; 110: 1-17.

[5] Morris JP, Wang SC and Hebrok M. KRAS, Hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma. Nature Reviews Cancer 2010; 10: 683-695.

[6] Wang Z, Banerjee S, Ahmad A, Li Y, Azmi AS, Gunn JR, Kong D, Bao B, Ali S, Gao J, Mohammad RM, Miele L, Korc M and Sarkar FH. Activated K-ras and INK4a/Arf deficiency cooperate during the development of pancreatic cancer by activation of Notch and NF-kappaB signaling pathways. Plos One 2011; 6: e20537.

[7] Liotta LA and Kohn EC. The microenvironment of the tumour-host interface. Nature 2001; 411: 375-379.

[8] Wong HH and Lemoine NR. Pancreatic cancer: molecular pathogenesis and new therapeutic targets (vol 6, pg 412, 2009). Nature Reviews Gastroenterology & Hepatology 2009; 6: 440-440.

[9] Huang MZ, Li YQ, Wu GH and Zhang FC. Whole Genome Expression Profiling Reveals a Significant Role for the Cell Junction and Apoptosis Pathways in Breast Cancer Stem Cells. Molecular Biotechnology 2010; 45: 39-48.

[10] Nancarrow DJ, Clouston AD, Smithers BM, Gotley DC, Drew PA, Watson DI, Tyagi S, Hayward NK and Whiteman DC. Whole genome expression array profiling highlights differences in mucosal defense genes in Barrett's esophagus and esophageal adenocarcinoma. Plos One 2011; 6: e22513.

[11] Kang HN, Wilson CS, Harvey RC, Chen IM, Murphy MH, Atlas SR, Bedrick EJ, Devidas M, Carroll AJ, Robinson BW, Stam RW, Valsecchi MG, Pieters R, Heerema NA, Hilden JM, Felix CA, Reaman GH, Camitta B, Winick N, Carroll WL, Dreyer ZE, Hunger SP and Willman CL. Gene

expression profiles predictive of outcome and age in infant acute lymphoblastic leukemia: a Children's Oncology Group study. Blood 2012; 119: 1872-1881.

[12] Salazar R, Roepman P, Capella G, Moreno V, Simon I, Dreezen C, Lopez-Doriga A, Santos C, Marijnen C, Westerga J, Bruin S, Kerr D, Kuppen P, van de Velde C, Morreau H, Van Velthuysen L, Glas AM, Van't Veer LJ and Tollenaar R. Gene Expression Signature to Improve Prognosis Prediction of Stage II and III Colorectal Cancer. Journal of Clinical Oncology 2011; 29: 17-24.

[13] Jennen DGJ, Magkoufopoulou C, Ketelslegers HB, van Herwijnen MHM, Kleinjans JCS and van Delft JHM. Comparison of HepG2 and HepaRG by Whole-Genome Gene Expression Analysis for the Purpose of Chemical Hazard Identification. Toxicological Sciences 2010; 115: 66-79.

[14] Zhang YZ, Zhang LH, Gao Y, Li CH, Jia SQ, Liu N, Cheng F, Niu DY, Cho WCS, Ji JF and Zeng CQ. Discovery and validation of prognostic markers in gastric cancer by genome-wide expression profiling. World Journal of Gastroenterology 2011; 17: 1710-1717.

[15] Morse DL, Balagurunathan Y, Hostetter G, Trissal M, Tafreshi NK, Burke N, Lloyd M, Enkemann S, Coppola D, Hruby VJ, Gillies RJ and Han HY. Identification of novel pancreatic adenocarcinoma cell-surface targets by gene expression profiling and tissue microarray. Biochemical Pharmacology 2010; 80: 748-754.

[16] Hirono S, Yamaue H, Hoshikawa Y, Ina S, Tani M, Kawai M, Ushijima M, Matsuura M, Saiki Y, Saiura A, Yamamoto J, Miki Y and Noda T. Molecular markers associated with lymph node metastasis in pancreatic ductal adenocarcinoma by genome-wide expression profiling. Cancer Science 2010; 101: 259-266.

[17] Karanjawala ZE, Illei PB, Ashfaq R, Infante JR, Murphy K, Pandey A, Schulick R, Winter J, Sharma R, Maitra A, Goggins M and Hruban RH. New markers of pancreatic cancer identified through differential gene expression analyses: Claudin 18 and annexin A8. American Journal of Surgical Pathology 2008; 32: 188-196.

[18] Huang H, Dong X, Kang MX, Xu B, Chen Y, Zhang B, Chen J, Xie QP and Wu YL. Novel blood biomarkers of pancreatic cancer-associated diabetes mellitus identified by peripheral blood-based gene expression profiles. The American journal of gastroenterology 2010; 105: 1661-1669.

[19] 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. 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 2004; 23: 2385-2400.

[20] Mouratidis PXE, Colston KW, Bartlett JB, Muller GW, Man HW, Stirling D and Dalgleish AG. Antiproliferative Effects of CC-8062 and CC-8075 in Pancreatic Cancer Cells. Pancreas 2009; 38: 78-84.

[21] Fotin-Mleczek M, Welte S, Mader O, Duchardt F, Fischer R, Hufnagel H, Scheurich P and Brock R. Cationic cell-penetrating peptides interfere with TNF signalling by induction of TNF receptor internalization. Journal of Cell Science 2005; 118: 3339-3351.

[22] Angst E, Dawson DW, Stroka D, Gloor B, Park J, Candinas D, Reber HA, Hines OJ and Eibl G. N-myc downstream regulated gene-1 expression correlates with reduced pancreatic cancer growth and increased apoptosis in vitro and in vivo. Surgery 2011; 149: 614-624.

[23] Thoennissen NH, Iwanski GB, Doan NB, Okamoto R, Lin P, Abbassi S, Song JH, Yin D, Toh M, Xie WD, Said JW and Koeffler HP. Cucurbitacin B Induces Apoptosis by Inhibition of the JAK/STAT Pathway and Potentiates Antiproliferative Effects of Gemcitabine on Pancreatic Cancer Cells (vol 69, pg 5876, 2009). Cancer Research 2009; 69: 8527-8527.

[24] Akcakanat A, Sahin A, Shaye AN, Velasco MA and Meric-Bernstam F. Comparison of Akt/mTOR signaling in primary breast tumors and matched distant metastases. Cancer 2008; 112: 2352-2358.

[25] Chan S. Targeting the mammalian target of rapamycin (mTOR): a new approach to treating cancer. British Journal of Cancer 2004; 91: 1420-1424.

[26] Soroceanu L, Kharbanda S, Chen RH, Soriano RH, Aldape K, Misra A, Zha JP, Forrest WF, Nigro JM, Modrusan Z, Feuerstein BG and Phillips HS. Identification of IGF2 signaling through phosphoinositide-3-kinase regulatory subunit 3 as a growth-promoting axis in glioblastoma. Proceedings of the National Academy of Sciences of the United States of America 2007; 104: 3466-3471.

[27] Zhang L, Huang J, Yang N, Greshock J, Liang S, Hasegawa K, Giannakakis A, Poulos N, O'Brien-Jenkins A, Katsaros D, Butzow R, Weber BL and Coukos G. Integrative genomic analysis of phosphatidylinositol 3 '-kinase family identifies PIK3R3 as a potential therapeutic target in epithelial ovarian cancer. Clinical Cancer Research 2007; 13: 5314-5321.

[28] Lee JW, Soung YH, Kim SY, Nam SW, Park WS, Lee JY, Yoo NJ and Lee SH. Mutational analysis of PDPK1 kinase domain in gastric, colorectal and lung carcinomas. Acta Oncologica 2006; 45: 340-341.

[29] Chakraborty S, Mohiyuddin SMA, Gopinath KS and Kumar A. Involvement of TSC genes and differential expression of other members of the mTOR signaling pathway in oral squamous cell carcinoma. Bmc Cancer 2008; 8:

[30] Martin ME, Perez MI, Redondo C, Alvarez MI, Salinas M and Fando JL. 4E binding protein 1 expression is inversely correlated to the progression of gastrointestinal cancers. International Journal of Biochemistry & Cell Biology 2000; 32: 633-642.

[31] Jinawath N, Chamgramol Y, Furukawa Y, Obama K, Tsunoda T, Sripa B, Pairojkul C and Nakamura Y. Comparison of gene expression profiles between Opisthorchis viverrini and non-Opisthorchis viverrini associated human intrahepatic cholangiocarcinoma. Hepatology 2006; 44: 1025-1038.

[32] Abdelrahim M, Baker CH, Abbruzzese JL, Sheikh-Hamad D, Liu SX, Cho SD, Yoon K and Safe S. Regulation of vascular endothelial growth factor receptor-1 expression by specificity proteins 1, 3, and 4 in pancreatic cancer cells. Cancer Research 2007; 67: 3286-3294.

[33] Vagenas K, Spyropoulos C, Gavala V and Tsamandas AC. TGFbeta1, TGFbeta2, and TGFbeta3 protein expression in gastric carcinomas: correlation with prognostics factors and patient survival. The Journal of surgical research 2007; 139: 182-188.

[34] Peng L, Mayhew CN, Schnekenburger M, Knudsen ES and Puga A. Repression of Ah receptor and induction of transforming growth factor-beta genes in DEN-induced mouse liver tumors. Toxicology 2008; 246: 242-247.

[35] Fukumura Y, Kumasaka T, Mitani K, Karita K and Suda K. Expression of transforming growth factor beta1, beta2, and beta3 in chronic, cancer-associated, obstructive pancreatitis. Archives of pathology & laboratory medicine 2006; 130: 356-361.

[36] Collado M, Garcia V, Garcia JM, Alonso I, Lombardia L, Diaz-Uriarte R, Fernandez LA, Zaballos A, Bonilla F and Serrano M. Genomic profilingof circulating plasma RNA for the analysis of cancer. Clinical chemistry 2007; 53: 1860-1863.

[37] Zhu DM, Li DC, Zhang ZX and Zhang XY. Effect of endothelial PAS domain protein 1 and hypoxia inducible factor 1alpha on vascular endothelial growth factor expression in human pancreatic carcinoma. Chinese medical journal 2008; 121: 2258-2264.

[38] Lee KA, Lynd JD, O'Reilly S, Kiupel M, McCormick JJ and LaPres JJ. The biphasic role of the hypoxia-inducible factor prolyl-4-hydroxylase, PHD2, in modulating tumor-forming potential. Molecular cancer research : MCR 2008; 6: 829-842.

[39] Khanna A, Mahalingam K, Chakrabarti D and Periyasamy G. Ets-1 expression and gemcitabine chemoresistance in pancreatic cancer cells. Cellular & molecular biology letters 2011; 16: 101-113.

[40] Piwkham D, Gelfond JA, Rerkamnuaychoke B, Pakakasama S, Rebel VI, Pollock BH, Winick NJ, Collier AB, 3rd, Tomlinson GE and Beuten J. Multilocus association of genetic variants in MLL, CREBBP, EP300, and TOP2A with childhood acute lymphoblastic leukemia in Hispanics from Texas. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2011; 20: 1204-1212.