Discovery of novel potential diagnostic markers of pancreatic cancer

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Discovery of novel potential diagnostic markers of pancreatic cancer

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of Jae Wan Kim is approved.

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먼저 이 논문이 완성되기까지 많은 관심과 격려로 늘 변함없이 따뜻하게 지도해 주신 송시영 교수님께 진심으로 감사드립니다. 또한 실험에 있어서 과학적인 생각을 갖게 해주신 김한수 교수님께도 진심으로 감사드립니다. 그리고 바쁘신 와중에도 귀중한 시간을 내주시어 논문에 대한 좋은 조언을 해주신 김호근 교수님께 감사드립니다. 그밖에도 석사 2년동안 많은 가르침을 주신 의과학과 모든 교수님들께도 감사의 마음을 전합니다.

어려울때 마다 아낌없는 조언해주신 실험실의 맏형 같은 강진구 선생님, 언제나 따뜻한 말한마디로 위로가 되어주신 태윤이형, 항상 웃는 얼굴로 대해주시고 걱정해주신 지은누나, 경화누나, 건강을 책임져주신 선아누나, 그리고 문정, 다운, 전우 같은 아줌마 이신에게도 고마움을 전합니다. 같은 입학동기인 진희, 실험실 막내 애화에게 좋은 논문을 쓰기를 기원하며, 경선에게도 앞으로 밝은 미래가 오기를 바랍니다.

지금은 다른 실험실에 계시지만 석사생활을 시작함에 있어서 많은 충고를 주신 경주 선생님께 감사드리며, 친형처럼 힘들때마다 언제나 활력소가 되어준 진환이형, 용현이형, 중기형, 용섭이형, 경민이형에게 고마운 마음을 전합니다. 그리고 나의 소중한 벗들 경선, 찬희, 만환, 유근, 재형, 성준, 경석, 현우, 친동생 같은 민수, 창수, 진호, 경문, 가족 같은 선미에게도 다시 한번 고마운 마음을 전하고, 앞으로의 미래에 좋은 일들만 가득하길 바랍니다.

이밖에도 저를 아는 모든 분들께 이제까지의 제 삶에 영향을 준 것에 대해서 깊은 감사의 마음을 전하며 항상 건강하시고 웃음을 잃지 않으시길 바랍니다.

다시 한번 이제까지의 제 삶에 아낌없는 지원과 믿음으로 지켜봐주신 아버지, 어머니 그리고 큰누나와 작은 누나, 큰매형과 작은매형께 머리숙여 감사의 마음을 전하며 이 글을 바칩니다.

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ABSTRACT

Discovery of novel potential diagnostic markers of pancreatic cancer

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Despite several advances in diagnostic technique and in the clinical management of cancer, pancreatic cancer has a high mortality rate with an overall 5-year patient survival of less than 2%. The main issue of pancreatic cancer is about the diagnostic techniques with early detection. Unfortunately, there is no effective diagnostic marker which is very sensitivity and specificity for the detecting of pancreatic cancer at early stage. For this reason, the goal of this study was the identification of novel potential diagnostic markers in pancreatic cancer. To find novel diagnostic markers, oligonucleotide microarray was performed with normal pancreas tissues and pancreatic cancer tissues. Statistical analysis was performed to collect data of overexpressed genes in pancreatic adenocarcinoma tissues than normal tissues using DNA micoarray data. After expression profiles of pancreatic cancer were selected, a PubMed search of each of the collected twenty-three known genes revealed that

seventeen were previously reported as expressed in pancreatic cancer. The six genes were selected; Apoc-I, C1qB, LAMC-2, LTBP-1, MGP, MIA. Reverse transcriptase polymerase chain reaction for six molecules were performed to validate the microarray data. By RT-PCR, mRNA of most of the candidate genes levels were increased in pancreatic cancer cell lines in comparison with normal pancreatic duct cell line(YGIC-6), except LTBP-1. Because there was no previous report regarding Apoc-I, C1q in pancreatic adenocarcinoma, ELISA was performed to compare between the patients with pancreatic adenocarcinoma, pancreatitis, non-malignant and normal control. In pancreatic adenocarcinoma, the Apoc-I expression was increased compared to that of patients with non-malignant, pancreatitis and normal control. C1q, however, was not significantly different between pancreatic adenocarcinoma, non-malignant serum samples and normal control. Rather, C1q was only highly expressed in pancreatitis. Six candidate genes, were compared with the conventional serum tumor marker, CA19-9 in pancreatic adenocarcinoma. In the results of ROC, CA19-9 was more sensitive and specific to detect pancreatic adenocarcinoma than Apoc-I and C1q. Here, the data suggest that Apoc-I not as sensitive and specificity as CA19-9, it may be it will be helpful for deteting the pancreatic cancer with CA19-9 since it clearly differentiate pancreatic cancer from pancreatitis. In the case of C1q, it may be useful marker for distingusing the pancreatitis from pancreatic cancer.

Key words : Pancreatic cancer, oligonucleotide microarray, diagnostic serum marker

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I. INTRODUCTION

Pancreatic cancer remains one of the lethal cancer worldwide with a 5years survival rate below 1%[1, 2]. Because of its propensity for aggressive invasion and early metastasis and the lack of effective early detection strategies, only around 10% of patients are surgical candidates at the time of diagnosis[3]. The vast majority of patients are diagnosed at an advanced stage of disease because currently no tumor markers are known that allow reliable screening for pancreatic cancer at an earlier, potentially curative stage[4, 5]. An early diagnosis of pancreatic cancer with appropriate treatment reduces the risk of death and improves the outcome. Furthermore there are currently no effective biomarkers useful to differentiate between pancreatic adenocarcinoma and another major pancreatic disease, chronic pancreatitis[6]. The clinical diagnosis of pancreatic cancer is often difficult because existing tumor markers such as CA19-9 are not sufficiently specific to reliably differentiate benign from malignant disease

and also because cytological detection of cancer requires invasive and often repeated investigation. Although the accuracy of imaging and endoscopic approaches continues to improve, it is often difficult to identify individuals with small surgically resectable cancer. Thus, a sensitive and specific serum biomarker that could diagnose early stage pancreatic adenocarcinoma would have the potential to improve the prognosis of pancreatic cancer by increasing the number of individuals detected with resectable disease[5]. New tumor markers of pancreatic cancer are urgently needed.

Gene profiling can be used to develop candidate biomarkers and to identify groups of genes involved in specific functional aspects of tumor biology[6]. Microarray technology permits the simultaneous comparison of the expression of thousands of genes in samples to allow identification of those that are differentially expressed. The technique has been applied to the molecular classification of tumors and may also be able to identify overexpressed complementary DNA (cDNA) corresponding to secretory proteins that might serve as serum markers for cancer[7].

In an effort to rephrase novel genes highly expressed in pancreatic cancers with the potential for development of serological markers or therapeutic targets, DNA microarray was performed to compare with surgically resected pancreatic cancer tissues and normal pancreas tissues. These studies have provided important information and have led to the discovery of a number of genes that may be useful for pancreatic cancer detection, diagnosis, or treatment.

In the current study, we screened DNA microarray data for the selection of potential biomarkers for pancreatic cancer and perforemed a series of experiment to validate the data. Most of the genes on this list have no research data about pancreatic

cancer and might have immediate significance as potential diagnostic markers for the differentiation of pancreatic adenocarcinoma and normal group. We selelcted six genes Apoc-I, LTBP-1, LAMC-2, MGP, MIA, C1qB for additional investigation as to their expression in neoplastic components of pancreatic adenocarcinoma. This molecular profile and validation of overexpressed genes in pancreatic adenocarcinoma should help to identify genes elucidate clinical biomarkers and lead to improved understanding of the molecular basis of pancreatic cancer.

II. MATERIALS AND METHODS

1. DNA microarray

All samples were frozen in liquid nitrogen and homogenized with a polytron (Kinematica Inc., Newark, NJ, USA). Total RNA from normal tissues and cancer tissues was isolated by TRIzol reagent (GibcoBRL, Grand Island, NY, USA) and purified by RNeasy mini kit and RNase-free DNase set (Qiagen Valencia, CA, USA) according to the manufacturer's protocols. RNA quality of all samples was tested by RNA electrophoresis and RNA LabChip analysis (Agilent, Palo Alto, CA, USA) to ensure RNA integrity. The purified cRNA was fragmented by incubation in fragmentation buffer [40 mmol/L Tris-accetate (pH 8.1), 100mmol/L KOAc, 30mmol/L MgOAc] at 95(temp) for 35 minutes and chilled on ice. The fragmented labeled cRNA was tested on Test Chip (Affymetrix, Santa Clara, CA, USA) to ensure that the control transcript 3′/5' ratio was approximately 1. Then, the fragmented labeled cRNA was applied to Human Genome U133A,B Array (Affymetrix) and hybridized to the probes in the array. Cell lines DNA microarray describes the expression profile obtained from 20 pancreatic cell lines using cDNA microarrays containing 9,932 human gene elements. All raw data files are available at http://sci.cancerresearchuk.org/axp/mphh/ijc04/[8].

2. Statistical data analysis

The limma package(Bioconductor, Seattle, WA, USA) analysis tool was used to identify genes expressed at least two fold greater in the pancreatic cancers compared to normal tissues.

3. Data filtering

Affymetrix GeneChips were analyzed for all genes with a two fold or greater increase in expression in pancreatic adenocarcinoma tumor tissues or cell lines compared to all normal tissues, using a 95% confidence limit[4]. To use the candidate marker in sera, candidate genes were filtered using GO annotation terms "Extracellular matrix" or "Extracellular region." The data comparison with pancreatic cancer cell line DNA microarray using locus link ID.

4. Cell lines and cell culture

Eight pancreatic cancer cell lines were analyzed in this study: AsPC-1, BxPC-3, CAPAN-1, CAPAN-2, CFPAC-1, HPAC-1, MiaPaCa-2, Panc-1. These cell lines were obtained from the American Type Culture Collection (Manassas, VA). AsPC-1 and BxPC-3 were cultured in RPMI 1640 supplemented (GIBCO, Grand Island, NY, USA). CAPAN-1 was cultured in Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO) with 20% fetal bovine serum (Hyclone, Logan, UT, USA). CAPAN-2 was cultured in McCoy's 5A Medium (GIBCO). CFPAC-1, HPAC-1 and Panc-1 were cultured in Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO), D-MEM/F-12 medium (GIBCO) and Dulbecco's Modified Eagles Medium (DMEM) (GIBCO), respectively. MiaPaCa-2 was cultured in Dulbecco's Modified Eagles Medium (DMEM) (GIBCO) with 2.5% horse serum. All media were supplemented with 10% fetal bovine serum (Hyclone) and 1% antibiotics (100 U/ml penicillin, 100 g/ml streptomycin, 2.5g/ml amphotericin B). Cells were maintained in a 37℃ humidified atmosphere saturated with 5% CO_2.

5. Reverse-Transcription Polymerase Chain Reaction

cDNAs from eight pancreatic cancer cell lines were synthesized from 2ug of total RNA using an oligo dT primer and the Superscript II reverse transcription kit (Gibco). Loading was controlled by the simultaneous PCR of actin cDNA. The primers used were Apoc-I sense, 5'-AACCAAGCCCTCCAGCAA-3'; Apoc-I antisense, 5'-GTTTCTCCT TCACTTTCTG-3'; LAMC-2 sense, 5'-TCTGCTTCTCGCTCCTCCT-3'; LAMC-2 antisense, 5'-AGCCTTCCAGCCATCAAC-3'; LTBP-1 sense, 5'-GGGAACACCACCACTCTCATT-3'; LTBP-1 antisense, 5'-TGTATGGTCTGGGTCTTCTGGA-3'; MGP sense, 5'-TCAGCAGAGATGGAGAGC-3'; MGP antisense, 5'-GTGCCAGCCTCCAGAAA-3'; MIA sense, 5'-CTCTTGCTCACAGTCCACGATG-3'; MIA antisense, 5'-CACTGGGCTGATTGTATTTGC-3'; C1qB sense, 5'-TCTGCCACAAGAACCAT CAACG-3'; C1qB antisense, 5'-AGAGCAGGAACCCGGAAAAGAT-3'. These amplify a 281 bp Apoc-I fragment, a 632 bp LAMC-2 fragment, a 471 bp LTBP-1 fragment, a 220 bp MGP fragment, a 481 bp MIA fragment and a 370 bp C1qB fragment. 0.5 ul of the cDNA was used for each PCR in 25 ul reaction volume containing 2 ul of 10mM dNTPs, 0.5 μM sense and antisense primers, 2.5 μl of 10× PCR buffer and 0.5 unit of TaKaRa Ex Taq DNA polymerase (Takara, Tokyo, Japan). PCR was performed on a Mastercycler® gradient (eppendorf AG, Germany) and the PCR conditions for MGP and LAMC-2 were as follows : 1 cycle of denaturation at 94℃ for 5 min, followed by 34 cycles of 94℃ for 30 sec, 55℃ for 30 sec and 72℃ for 30 sec before a final incubation at 72℃ for 5 min. The PCR conditions were the same for Apoc-I, C1qB and LTBP-1, except that the primers were annealed at 52℃, 58℃ and 54℃, respectively. To reduce mispriming and to increase efficiency, Touchdown PCR was employed in the first amplification round. Following hot start, MIA was subjected to

20 cycles in a TD program (94℃ for 30 s, 62℃ for 30 s and 72℃ for 30 s for 1 cycles, followed by a 2℃ decrease of the annealing temperature every second cycle). Thirty cycles were subsequently run (94℃ for 30 s, 54℃ for 30 s and 72℃ for 30 s) ending with a 5 min extension at 72℃. Aliquots(12μl) of RT-PCR products were separated by electrophoresis in 1.5% or 2% agarose gels, depending on the product size, and were stained with ethidium bromide.

6. Sandwich Enzyme-Linked Immunosorbent Assay

Tweenty five serum samples from pancreatic adenocarcinoma patients and Sixteen healthy donors were subjected to sandwich ELISA to detect Apoc-I, C1q(USBiological, Swampscott, Massachusetts, USA). Briefly, Apoc-I polyclonal antibody(Chemicon, Temecula, CA, USA) was coated onto ELISA plates(Corning.Inc, Corning, NY, USA) in a cold room overnight at 4℃ at a concentration of 1 μg/ml. After three washing, blocking buffer (5% FBS/PBS) was added to the plates (Room temperature/1h) before serially diluted Apoc-I protein standard(Calbiochem, Darmstadt, Germany) (or serum samples). The anti-Apoc-I mouse mononal antibody(Chemicon) (1:5000 diluted in washing buffer) was employed for detection before the plate was incubated with a 1:5000 dilution of the secondary antibody-HRP conjugated goat anti-mouse antibody(Santa Cruz Biotechnology,Inc.). After washing, TMB(BD, USA) was used as the HRP substrate and 1M H_2SO_4 to stop the development reaction. The optical density (OD) at 450nm was evaluated on a micro-plate reader. A standard curve was constructed using the OD values for standard concentrations of Apoc-I protein. The concentration of Apoc-I in the serum was then

calculated using the standard curve.

7. CA19-9 Serum ELISA

CA19-9 levels were measured in serum samples (25 µl) by commercially available ELISA (Alpha Diagnostics Inc., San Antonio, TX, USA) according to the manufacturer's recommendations.

III. RESULTS

1. Selected candidate tumor markers

To candidate genes, we used two approaches. First, we used statistic analysis from DNA microarray data(P value ≤ 0.05, fold change ≥ 2). In the first filtering step, Oligonucleotide probe-sets was filtered to 5,756 genes probe-sets. In a second step, we used the Genome Ontology(GO) consortium annotations to identify genes associated with keyword implying extracellular region and extracellular matrix[9]. As the second filtering results, 5,756 genes probe-sets was filtered to 336 genes probe-sets. And these candidate genes were more highly expressed in both pancreatic adenocarcinoma and pancreatic cancer cell lines compared with noncancerous pancreas[6]. To evaluate early diagnostic marker, we tried to compare those 336 genes with microarray data from these cell lines with primary tumor origin(not liver metastasis, not lymph node metastasis, not ascite) of pancreatic cancer (Fig. 1). The data filtering resulted in the number of selected genes to a final list of twenty-three genes (Table. 1). Most of the genes on this list are novel in pancreatic adenocarcinoma and have significance as potential diagnostic markers. For each of the twenty-three genes identified, a search was performed using the online NCBI database PubMed. Of the twenty-three genes analyzed, six genes were previously reported to be associated with pancreatic cancer, whereas seventeen genes was not[4]. Among the seventeen genes, six genes Apoc-I(apolipoprotein C-I), C1qB(Compleme -nt component 1, q subcomponent, beta polypeptide), LTBP-1(Latent transforming growth factor beta binding protein 1), LAMC-2(laminin, gamma 2), MGP(matrix

gla-protein) and MIA(Melanoma inhibitory activity) were selected.

2. Expression of candidate genes in pancreatic cancer cell lines

To evaluate the differential expression of candidate genes in normal and pancreatic cancer cell lines(AsPC-1;origin of ascite, BxPC-3, CFPAC-1;origin of liver metastasis, CAPAN-1, CAPAN-2;origin of primary tumor, HPAC-1, MIAPaCa-2;origin of primary tumor, PANC-1;origin of primary tumor), Quantitative PCR analysis were performed on immortalized normal pancreatic duct cell line(YGIC-6) and on pancreatic cancer cell lines(fig. 2). The most of candidate genes were overexpressed in pancreatic cancer cell lines than normal pancreatic duct cell line, however LTBP-1 overexpressed in normal pancareatic duct cell line(YGIC-6).

3. Serum Apoc-I, C1q and CA19-9 levels in pancreatic cancer

In order to evaluate Apoc-I, C1q as a diagnostic marker, Sandwich ELISA was performed. In the previous report reported that the concentration of Apoc-I and C1q in normal plasma are about 6 mg/dl[10] and 70 μg/ml[10, 11], respectively. Mean serum Apoc-I levels were elevated ~1.5-fold in sera from patients with pancreatic adenocarcinoma compared to healthy controls(fig. 3), whereas serum C1q levels were not elevated in sera from patients with pancreatic adenocarcinoma(fig. 4). Serum levels of Apoc-I, C1q, CA19-9 in 25 patients with pancreatic adenocarcinoma were 550.9 μg/ml, 183.8 μg/ml and 395 U/ml; 5 with chronic pancreatitis 358.1 μg/ml, 259.7 μg/ml and 110.5 U/ml; 4 with Non-malignant diseases (CBD stones, common bile duct stones) 587.2 μg/ml, 154.9 μg/ml and 15.06 U/ml; 16 with healthy samles

373.9 μg/ml, 178.2 μg/ml and 10.27 U/ml, respectively(Table 2). The receiver operator characteristic (ROC) curve was calculated (fig. 8,9), and the area under the curve (AUC) determined to be Apoc-I 0.68(95% CI, 0.52 to 0.83) and C1q 0.55(95% CI, 0.39 to 0.72), respectively. There was not significant difference for discrimination of pancreatic cancer from nonpancreatic cancer specimens. Because the available antibodies to perform ELISA were limited, LTBP-1 and MGP were not tested. In case of LAMC-2 and MIA, these were already reported in pancreatic adenocarcinoma, so the author was not performed ELISA.

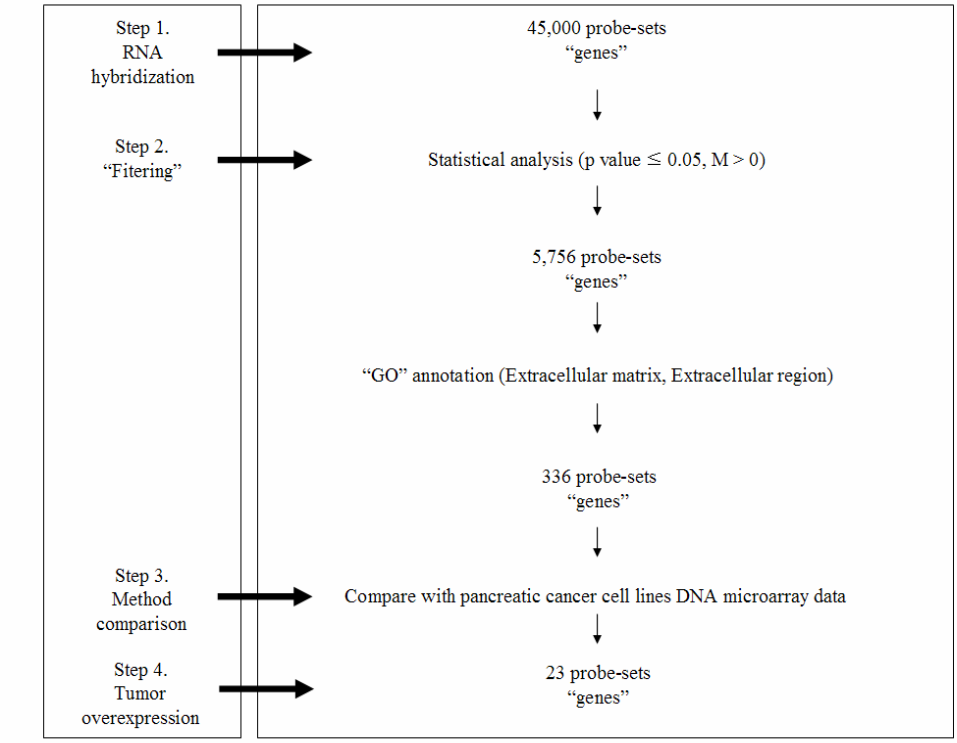

Figure 1. Mining for genes that encode secreted proteins.

Table 1. Highly expression genes identified in pancreatic cancer tissues and cell lines

Affymetrix Fragment name	Known gene name	Fold change	P value	Reported in pancreas	Ref.	Cellular location
209082_s_at	Collagen, type XVIII, alpha 1	1.95	0.00162	N		
221087_s_at	Apolipoprotein L, 3	1.97	0.000236	N		C
202510_s_at	Tumor necrosis factor, alpha-induced protein 2	1.84	0.00242	N		
203167_at	Tissue inhibitor of metalloproteinase 2	2.28	0.00192	N		S
212143_s_at	Insulin-like growth factor binding protein 3	5.53	1.32e-06	Y	6,12	S
203892_at	WAP four-disulfide core domain 2	1.49	0.0494	N		S
206560_s_at	Melanoma inhibitory activity	8.29	0.0083	Y	13	S
201069_at	Matrix metalloproteinase 2 (gelatinase A)	3.57	0.000309	Y	14,15	S
208949_s_at	Lectin, galactoside-binding, soluble, 3 (galectin 3)	3.06	0.000191	Y	16	
203851_at	Insuline-like growth factor binding protein 6	2.67	0.00316	N		
213553_x_at	Apolipoprotein C-I	2.68	0.000137	N		S
218002_s_at	Chemokine (C-X-C motif) ligand 14	8.42	4.2e-05	N		S
211668_s_at	Plasminogen activator, urokinase	3.41	1.53e-05	Y	17	
201438_at	Collagen, type VI, alpha 3	3.26	0.000541	N		
202729_s_at	Latent transforming growth factor beta binding protein 1	3.23	6.6e-05	N		S
211959_at	Insuline-like growth factor binding protein 5	11.4	2.01e-07	N		S
205618_at	Proline rich Gla (G-carboxyglutamic acid) 1	1.97	0.00021	N		ER
202953_at	Complement component 1, q subcomponent, beta polypeptide	2.44	0.0149	N		S
231579_s_at	Tissue inhibitor of metalloproteinase 2	3.31	4.31e-06	Y	14	S
202291_s_at	Matrix Gla protein (MGP)	2.05	0.00693	N		EM
202267_at	Laminin, gamma 2			Y	18,19	EM
228143_at	Ceruloplasmin (ferroxidase)	1.75	0.0135	Y	20	S
241808_at	Interleukin 7	1.87	0.03	N		S

*C,cytoplasmic; S,secreated; EM,extracellular matrix; ER,extracellular region.

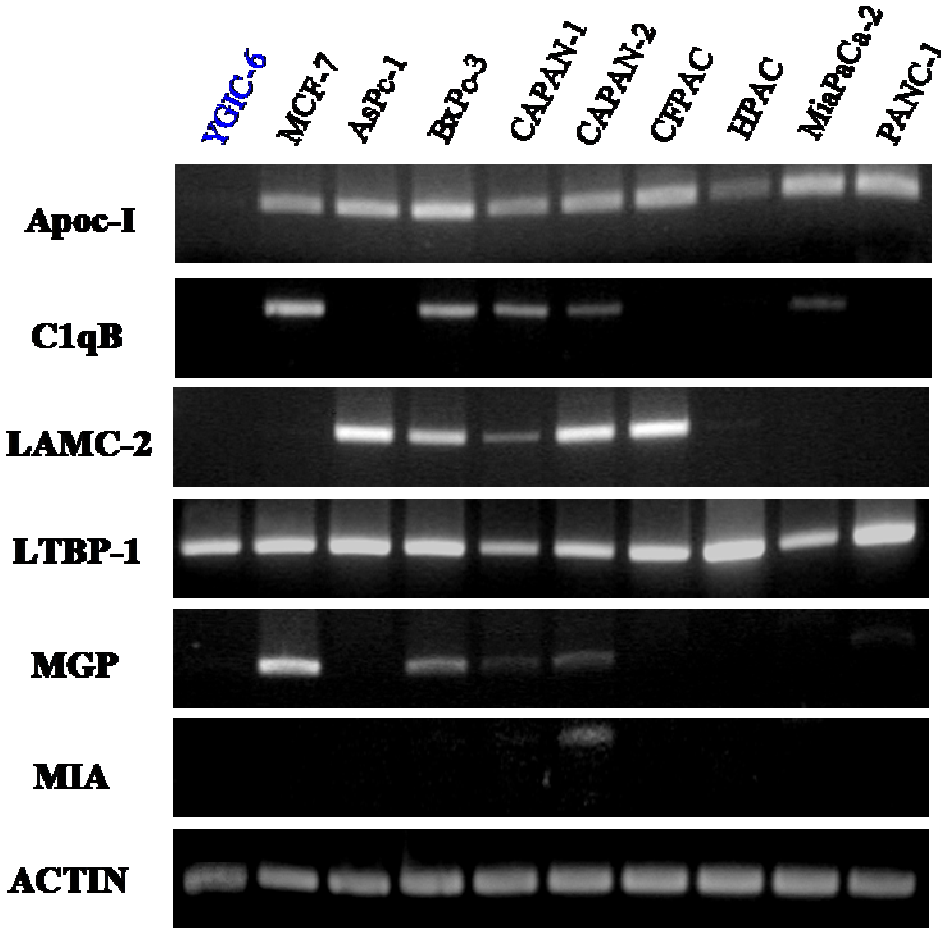

Figure 2. Expression of Apoc-I, C1qB, LAMC-2, LTBP-1 and MIA mRNA was determined by quantitative PCR analysis as described in Materials and Methods. Candidate genes overexpressed in pancreatic cancer cells as compared with normal pancreatic duct cell YGIC-6. MCF-7 is breast cancer cell line.

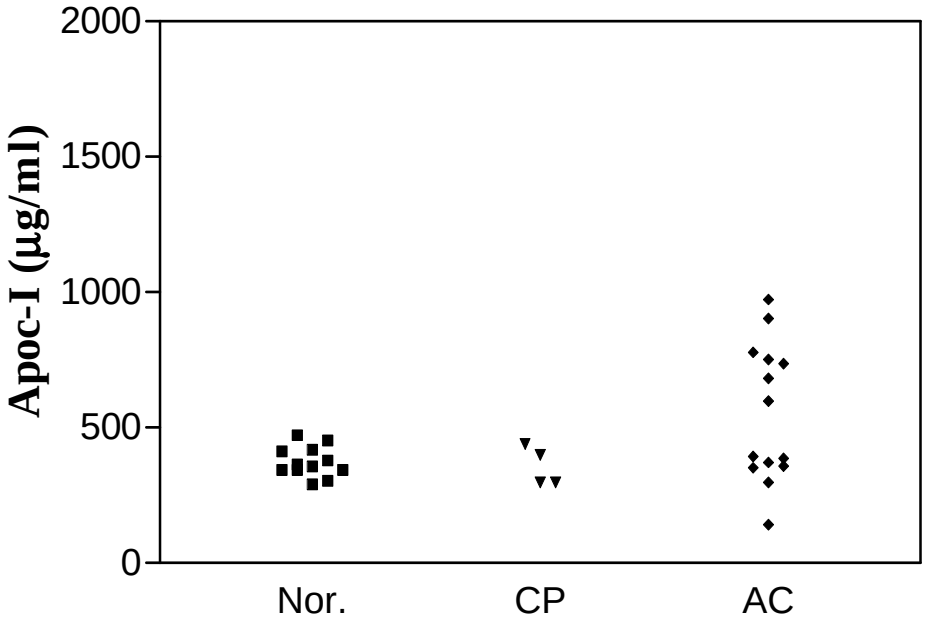

Figure 3. Dot plot of serum Apoc-I levels. Nor, healthy control; CP, chronic pancretitis; AC, pancreatic adenocarcinoma.

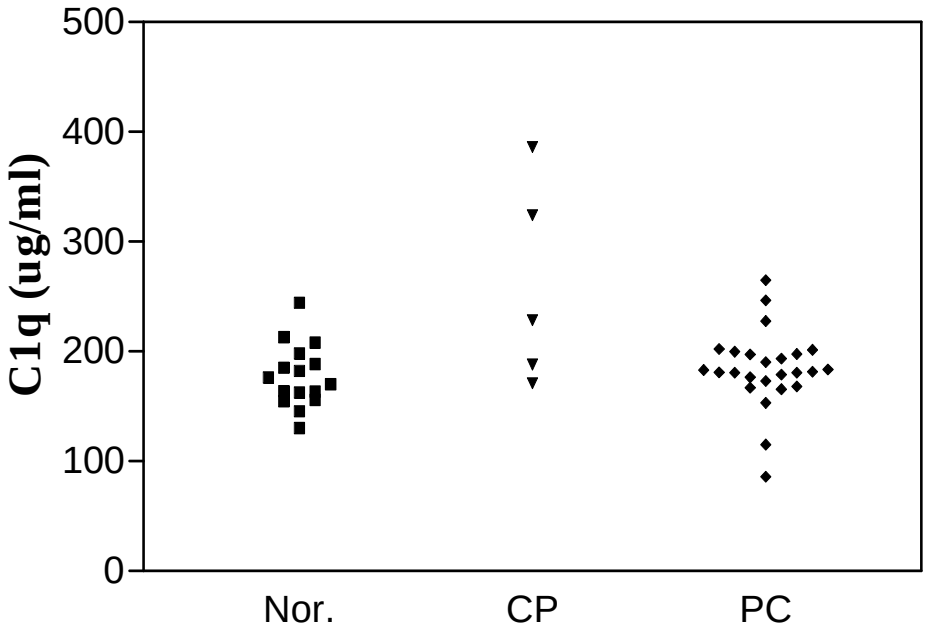

Figure 4. Dot plot of serum C1q levels. Nor, healthy control; CP, chronic pancretitis; PC, pancreatic cancer.

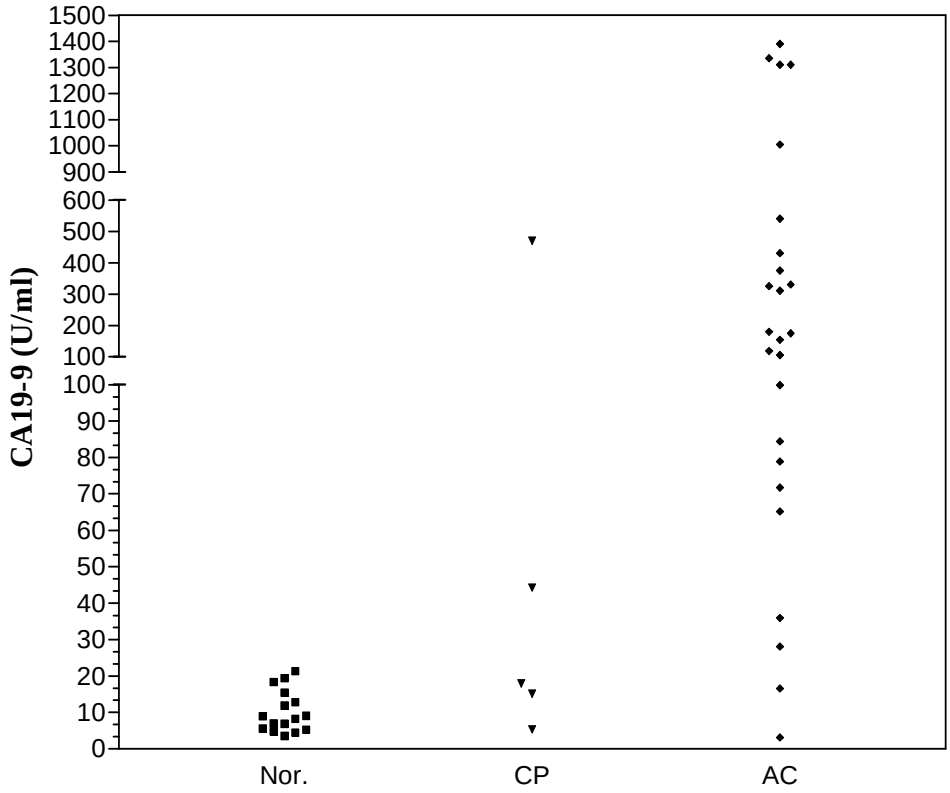

Figure 5. Dot plot of serum CA19-9 levels. Nor, healthy control; CP, chronic pancretitis; PC, pancreatic cancer.

Table 2. Serum levels of biomarkers in normal controls, chronic pancretitis, pancreatic cancer.

	Healthy controls	Chronic pancreatitis	Pancreatic cancer
CA19-9 (units/ml)			
Mean	10.27	110.50	395
Median	8.65	17.95	174.9
SD	5.73	201.50	471.2
Apoc-I (μg/ml)			
Mean	373.9	358.1	550.9
Median	361.5	348.0	494.6
SD	55.82	72.14	254.2
C1q (μg/ml)			
Mean	178.2	259.7	183.8
Median	173.7	228.7	181.3
SD	28.51	92.22	35.42

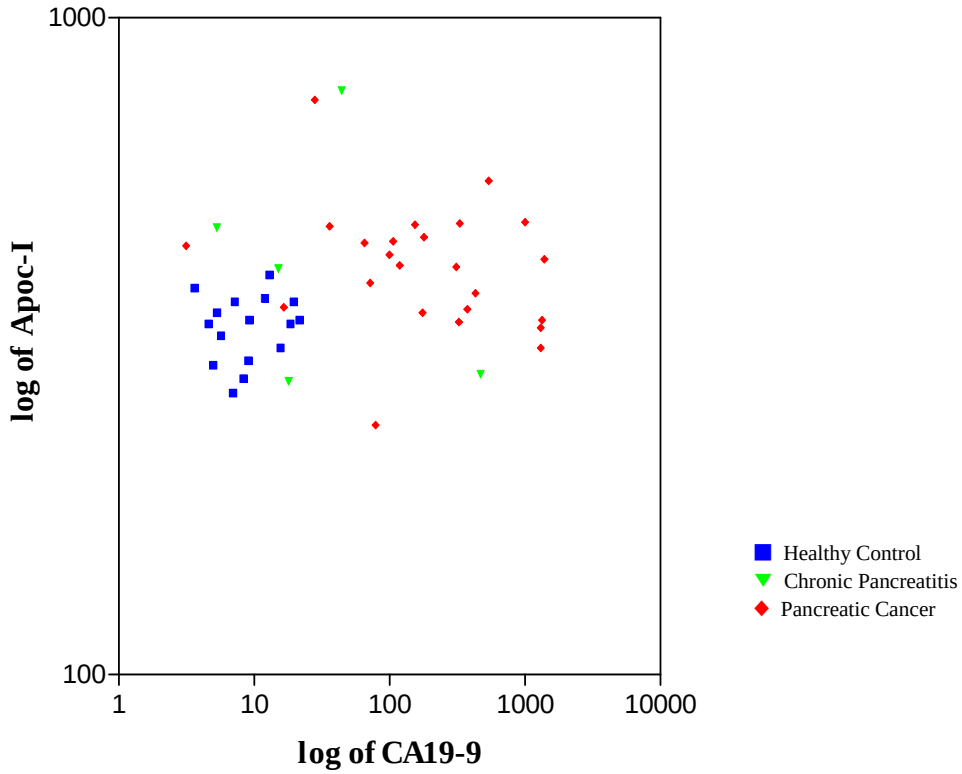

Figure 6. A scatter plot of serum CA19-9 (horizontal axis) and Apoc-I levels (vertical axis).

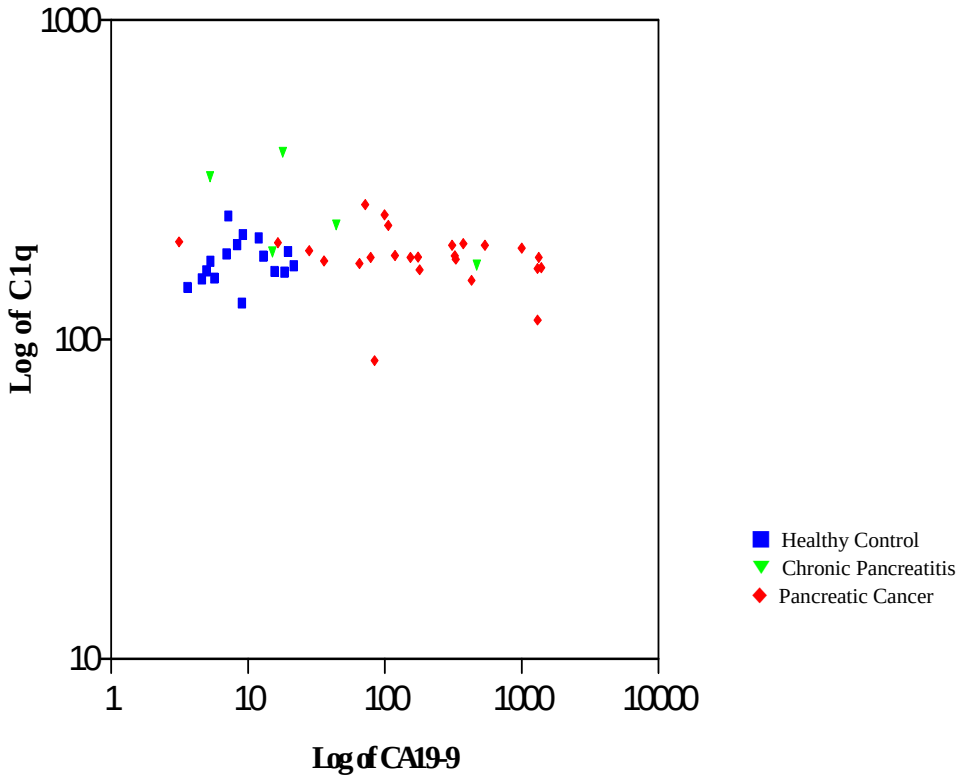

Figure 7. A scatter plot of serum CA19-9 (horizontal axis) and C1q levels (vertical axis). Most samples are plotted on the half (C1q levels), whereas pancreatic cancer samples are plotted on the right (CA19-9 levels). In contrast, almost all patients with pancreatic cancer have elevated in CA19-9, but all samples have not elevated in C1q.

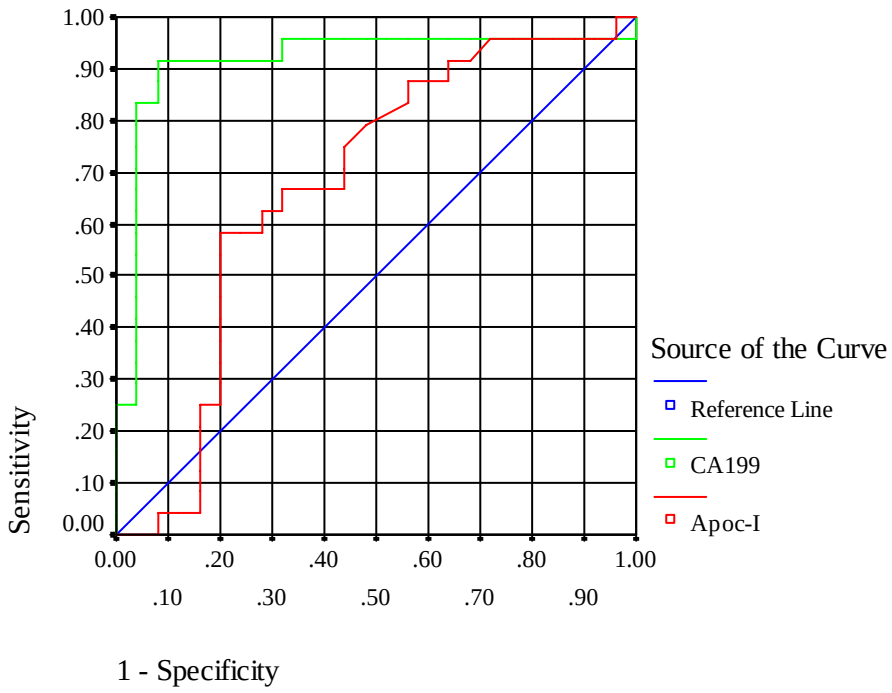

Figure 8. ROC analysis of Apoc-I. pancreatic cancer versus non-cancer: Red, Apoc-I (AUC,0.68); green, CA19-9 (AUC,0.91).

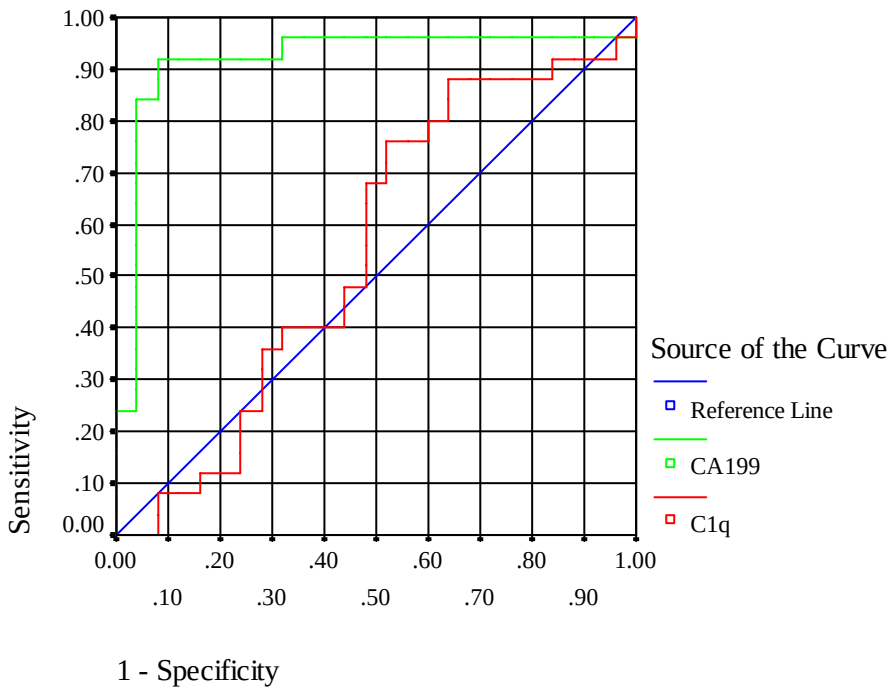

Figure 9. ROC analysis of C1q. pancreatic cancer versus non-cancer: Red, C1q (AUC,0.55) ; green, CA19-9 (AUC,0.91).

IV. Discussion

Pancreatic cancer cells have a highly propensity to infiltrate to the surround tissues and metastasize to distant organs. Only in a minority of patients with pancreatic cancer is the diagnosed at a very early stage, when curative surgery might significantly ameliorate the 5 years survival rate[21].

In this study, DNA microarray was employed as a tool to screen the overexpressed genes in pancreatic cancer, and then data filtering was implemented as a biomarker for pancreatic cancer. Since these genes differentially expressed in the cell lines of primary tumor origin may represent genes selected to early events of cancer development rather than metastasis stage. And RT-PCR was performed to identify the candidate genes express in pancreatic cancer cell lines, and finally, sandwich ELISA was used to evaluate candidate genes as a serological marker for pancreatic cancer.

Apolipoprotein C-I(Apoc-I) is a secreted plasma protein present in the circulation in association with chylomicrons, LDL and VLDL lipoproteins. It is produced by the liver[22]. Recent data reported that human Apoc-I overexpression can modulate the development of obesity and insulin resistance in mice[23]. In this study, Apoc-I serum level of pancreatic adenocarcinoma were higher than healthy control. It was suggested that loss of pancreatic function, causing pancreatic adenocarcinoma, was attended with overexpressed Apoc-I to control chylomicrons, VLDL and LDL level. Subcomponent of complement C1, C1q, is composed of 18 polypeptide chains of three different type, called the A-, B-, and C-chains, with molecular weights of 29, 27, and 23 kd, respectively[24]. In previous study, C1q expression is elevated in the Alzheimer’s disease(AD) brain and in experimental animal models of AD, where it

may be involved in the initiation of proinflammatory cascades[25]. Because of C1q contain C1qB, the study was performed C1qB ELISA using C1q. As a results of ELISA, serum level C1q was revealed that C1q was more expressed in pancreatitis than any other samples, healthy controls or pancreatic adenocarcinoma. Because of C1q mediates complement activation via the classical pathway as a part of inflammatory response in pancreatitis, the results may not be surprisinging. Reduced serum level of C1q, however, in pancreatic adenocarcinoma could not be explained in this study. Although C1q is not useful for distinguishing pancreatic cancer from healthy control, the sandwich ELISA results suggest that serum C1q could be particularly helpful in the detection of pancreatitis. Melanoma inhibitory activity(MIA) increases cell mortality by decreasing the attachment of the cell to the extracellular matrix(ECM). Overexpression of MIA leads to increased metastasis of malignant melanoma cells by enhancing invasion and extravasation[13]. In the recent report was showed by QRT-PCR and immunohistochemistry that MIA is significantly over-expressed in pancreatic cancer in comparison with normal pancreatic tissues. Whereas MIA was not detected either a significant difference of serum levels between pancreatic cancer patients and donors or a significant difference between patients at different stages of pancreatic cancer. Therefore, MIA cannot serve as a diagnostic or prognostic marker in pancreatic cancer[13]. Matrix gammacarboxyglutamate(Gla)-protein(MGP) is a strong inhibitor of soft tissue calcification and is mainly produced by chondrocytes and vascular smooth muscle cells(VSMCs)[26]. Recent study was reported MGP mRNA was downregulation in colorectal adenocarcinoma[27]. In various cartilage diseases and angina pectoris, serum MGP concentrations were significantly

decreased but it was unkown in pancreatic cancer[26]. Laminin γ2 chain(LAMC2) is involved in tumor invasion and metastasis[28]. In pancreatic adenocarcinoma, LAMC-2 had two different staining pattern, cytoplasmic expression and basement membrane expression, at immunohistochemistry study. The cytoplasmic expression type was associated significantly with occurrence of postoperative hepatic metastasis and also was strongest predictive factor for poorer overall survival in patients with pancreatic ductal adenocarcinomas[18]. Latent TGF-β-binding protein-1(LTBP-1) was reported as a stable component of the extracellular matrix that is important in storage of latent TGF-β in the matrix and may be a structural component of connective tissue microfibrils[29]. Recent data indicating that differential expression of LTBP-1 isoforms occurs during the development of coronary heart disease is considered, together with evidence that modulation of LTBP function, and hence of TGF-β activity, is associated with a variety of cancers[30].

In this study, only a few selected genes were studied. The reason for this were; (i) some of the selected genes are not functionally defined, (ii) limited availability of antibodys to perform ELISA(ie, LTBP-1, LAMC-2, MGP), (iii) a few cases with the reports of cancer associate(ie, LAMC-2, MIA), and (iv) validation with RT-PCR of pancreatic cancer cell lines ruled out the occasional overexpressed gene(ie, MIA). So the candidate genes, Apoc-I and C1q, were selected that have not previously been studied in pancreatic adenocarcinoma.

In conclusion, the sensitivity of tumor marker is important to decrease the missing of pancreatic carcinoma and the specificity is also important to decrease the over diagnosis[31]. Although, serum CA19-9 is more sensitive and specific in pancreatic

adenocarcinoma than Apoc-I and C1q, Apoc-I may be helpful for deteting the pancreatic cancer with CA19-9. In case of C1q, it suggest that may be useful marker for detecting the pancreatitis.

V. Conclusion

In summary, the study was researched on overexpressed genes in patient serum with pancreatic adenocarcinoma. For validating whether candidate genes overexpressed actually in pancreatic cancer cell lines or not, reverse transcription polymerase chain reaction was performed. Most of candidate genes were more overexpressed in pancreatic cancer cell lines than normal pancreatic duct cell line, except LTBP-1. Finally, the author demonstrated higher levels of serum Apoc-I, C1q in case patients with pancreatic adenocarcinoma than in healthy control. The level of serum Apoc-I was higer expressed in pancreatic adenocarcinoma than anyother serum samples, healthy controls, Non-malignant samples and pancreatitis samples. But C1q was only higher expressed in pancreatitis serum samples and C1q serum level was not significantly different between pancreatic adenocarcinoma and healthy control. The author also measured CA19-9 serum level in pancreatic adenocarcinoma. Like Apoc-I, serum CA19-9 level were elevated in pancreatic adenocarcinoma but the ROC data was revealed serum CA19-9 level were more sensitive and specific in pancreatic adenocarcinoma than Apoc-I and C1q(fig 8, 9). Apoc-I not as sensitive and specificity as CA19-9, it may be it will be helpful for detetecting the pancreatic cancer with CA19-9 since it clearly differentiate pancreatic cancer from pancreatitis. In the case of C1q, it may be useful marker for distingusing the pancreatitis from pancreatic cancer. Apoc-I and C1q, therefore, is useful tools for deteting pancreatic cancer and pancreatitis, respectively.

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ABSTRACT(IN KOREAN)

혈청을 통한 췌장암의 새로운 진단표지자 동정

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최근 암에 대한 진단법과 치료법이 눈부신 발전을 이룩하였지만, 췌장암은 여전히 높은 치사율과 5 년간 생존율이 2%로 예후가 좋지 않다. 췌장암은 현재 효과적으로 조기에 진단할수 있는 표지자가 아직까지는 없다는 것이 큰 문제점으로 남아 있으며, 따라서 본 연구의 목적은 췌장암을 조기에 발견할수 있는 새로운 진단표지자를 동정/개발하고자 함에 있다. 췌장암의 진단표지자를 동정하기 위한 방법으로 췌장암 조직의 oligonucleotide microarray 실험 결과를 통계적 방법으로 분석하여, 발현양이 정상조직에 비해 증가된 유전자만을 선별하였다. 또한 선발된 유전자가 췌장암 혹은 다른 종류의 암과 연관성이 있는지 알아보기 위해 생물정보학적인 도구를 이용하여 NCBI, NIC, EBI 와 같은 유전자발현 정보가 있는 데이터베이스에서 선별한 유전자를 조사하였다.

선별한 유전자는 총 6 개의 유전자, Apoc-I, C1qB, LAMC-2, LTBP-1, MGP, MIA 이며, 이들 선별유전자들이 췌장암세포주에서 실제적으로 발현하는지를 RT-PCR 를 통하여 mRNA 수준에서 알아보았다. 그결과 LTBP-1 을 제외한 나머지 선별유전자가 정상 세포주와 비교하였을 때 췌장암 세포주에서 발현되었다. 선별된 유전자중 현재까지 췌장암에 대한 연구가 없는 Apoc-I, C1q 가 실제적으로 진단 표지자로 유용한지를 알아보기 위해, 정상인과 췌장암 환자의 혈액에서 발현정도를 ELISA 를 통해 알아보았다. 실험결과 Apoc-I 은 정상인과 비교해 보았을 때 췌장암 환자의 혈액내에서 보다 많이 발현되었으며, 췌장염 환자의 혈액에서는 정상인과 같은 수치를 보였다. 반대로 C1q 의 경우, 정상인과 췌장암환자의 혈액과 비교해 보았을 때 유의할만한 발현 차이를 나타내지 않았지만 췌장염 환자의 경우 다른 군에 비해 C1q 가 높게 발현되는 것이 관찰되었다. ROC 그래프를 이용하여 민감도와 특이도를 기존의 췌장암 진단 표지자인 CA19-9 과 비교하여 보았을 때, Apoc-I 과 C1q 모두 민감도와 특이도가 CA19-9 에 비해 낮았다.

결론적으로 여섯개의 선별 유전자중 현재 췌장암에서 연구가 안된 Apoc-I 과 C1q 는 기존의 진단표지자인 CA19-9 과 비교해 보았을 때 민감도와 특이도가 유의할만한 수준이 아니기 때문에 췌장암의 진단 표지자로 쓰이기에는 적합하지 않았다. 하지만 Apoc-I 의 경우 기존의 진단 표지자인 CA19-9 과 함께 췌장암을 진단하는 데 참고가 될수 있고,

C1q 의 경우 췌장암과 췌장염을 구별하는데 유용한 진단 표지자가 될수 있다 하겠다.

핵심 되는 말 : 췌장암, 유전자 칩, 혈청 진단 표지자