# Identification of a new immune escape related gene in pancreatic cancer

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# Identification of a new immune escape related gene in pancreatic cancer

**Directed by Professor Si Yong Song**

**The Master's Thesis to the Department of Medical Science, the Graduate School of Yonsei University in Partial Fulfillment of the Requirements for the Degree of Master of Medical Science**

**Kyoung Sun Park**

**June 2006**

**This certifies that the Master's Thesis of Kyoung Sun Park is approved.**

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**June 2006**

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부족한 자식인데도 항상 최고라고 생각하시고 늘 당신들의 자랑으로 여기시는 존경하는 아버지, 어머니께도 두 손 모아 이 논문을 바칩니다. 당신들의 기대에 부끄럽지 않고 언제나 자랑으로 남을 수 있는 자식이 되도록 열심히 살겠습니다. 언제나 든든한 오빠와 다름없는 동생에게도 고마움을 전합니다. 부족한 지면이라 일일이 인사드리지 못하지만 늘 지켜봐 주시고 격려해 주신 친가, 외가, 어른들과 많은 분들께도 항상 고마운 마음을 간직하고 있습니다.

앞으로도 학문과 교육에 대한 열정에 있어 늘 처음처럼 가슴 뛰는 삶을 살고자 합니다. 또한 늘 부족함 없이 채주신 하나님께 이 작은 논문을 통해 영광을 돌리기 원합니다.

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ABSTRACT

# Identification of a new immune escape related gene in pancreatic cancer

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In pancreatic cancer, soluble factors produced by and for the protection of the tumor environment have been detected and are often distributed to the patient's circulatory system where they may cause a more generalized immunosuppression. Tumors exploit several strategies to evade immune recognition, including the production of a large number of immunosuppressive factors, which lead to reduced numbers and impaired functions of dendritic cells (DCs) in the vicinity of tumors.

In this study, several immune related genes were used to obtain gene expression profiles of pancreatic cancer for immune escape, and validation of gene expression in pancreatic cancer cell lines and patient sera. The inhibitory function of

immune cells in vitro was then confirmed and the selection genes controlling susceptibility to induced immunosuppression were identified.

We used Affymetrix GeneChip arrays to identify genes differentially expressed in pancreatic cancer. Samples were hybridized to the complete Affymetrix U133A oligonucleotide microarray for simultaneous analysis of 45,000 fragments corresponding to 33,000 known genes and 6,000 ESTs. Genes with a 2-fold greater increase in expression in the 8 pancreatic adenocarcinoma tissues compared to 17 normal tissues were identified. We primarily selected fragments that were expressed at least 2-fold more in pancreatic cancer samples compared to normal tissues, of which 4 corresponded to known gene fragments ranged from less than $p\ value < 0.05$ (modified Welch $t$ test). Next, we selected genes related to secretory proteins, growth factors, receptor, ligands, and extra-matrix proteins. For each of the 225 genes identified, a search was performed by means of the online NCBI database PubMed using search parameters of the gene name together with the terms “pancreas cancer” or “immune.” Of the 225 genes analyzed, most of the genes and gene products were reported to be associated with pancreatic cancers. Many of them were typically associated with the general phenomenon of inflammation and pancreatitis. A few assessed good geness were primarily reported to be associated with immune suppression. Finally, four genes that were not reported to be associated with either pancreatic cancer or the immune system were selected for the further

study. Candidate genes were selected for verification of expression in samples of pancreatic cancer cell lines. These four genes were expressed in approximately 50% of pancreatic cancer cells, in support of their initial identification as differentially expressed genes by Affymetrix GeneChip.

In addition, this study has provided evidence that the selected genes are involved in immune function. We used recombinant protein to study these genes in DC. We demonstrated that Neuromedin U (NMU) and Trefoil Factor 2 (TFF2) interfere with the differentiation of monocytes into DC *in vitro*, resulting in the down-regulation of CD40, CD80, and HLA-DR. These results suggested that NMU and TFF2 had little effect on the expression of cell surface molecules in antigen presenting cells (APC). Moreover, we found that DC treated with NMU and IL-10 had a higher capacity than control DC for uptake of FITC-dextran. These results suggested that NMU-treated DC generated in the presence of the absence of IL-10 had a similar degree of endocytic activity. In addition, we found that in our cultures, migration of immature DC was induced by both NMU and TFF2.

In conclusion, we provide evidence here that NMU and TFF2 modulate DC function. The identification of signal-transduction events that participate in the modulation of DC function via NMU and TFF2 would further contribute to the elucidation of the mechanisms underlying the complex anti-inflammatory effects of NMU, and TFF2 and would enable the construction of a theoretical framework for

its eventual therapeutic use. The results described here suggest that the presence of NMU and TFF2 from the onset of the tumor can promote progressive induction of immune silencing in synergy with other described immunosuppressive factors.

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Key words : pancreatic cancer, immune escape, oligonucleotide microarray

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## I. INTRODUCTION

Pancreatic cancer is the fourth leading cause of death from cancer in North America. With an overall 5-year survival rate of 3% , pancreatic cancer has one of the poorest prognoses among all cancers[1, 2]. Aside from its silent nature and tendency for late discovery, pancreatic cancer also shows unusual resistance to chemotherapy and radiation therapy. Only 20% of pancreatic cancer patients are eligible for surgical resection, which currently remains the only potentially curative therapy[3]. In pancreatic cancer, soluble factors produced by and for the protection of the tumor environment have been detected and are often distributed to the patient's circulatory system where they may affect a more generalized immunosuppression[4].

During tumor transformation, genomic instability creates a vast repertoire of

tumor cells that are selected by factors present in the microenvironment, such as growth factors, nutrient supply, and immune pressure[5]. Many mechanisms have been found to contribute to the failure of the immune system to control tumor growth. Tumor cells often have decreased expression of major histocompatibility complex (MHC) molecules on their cell surface[6]. Most tumor cells lack critical co-stimulatory molecules, such as CD40, CD80, and CD86, or adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and leukocyte function associated antigen-3, which can contribute to activating T cells[7]. Recent data show that tumor cells produce and secrete many factors, such as transforming growth factor beta (TGF-β), interleukin-10 (IL-10), prostaglandin E2 (PGE2), and the vascular endothelial growth factor (VEGF), which serve to inhibit the function of antigen presenting cells and immune effector cells[8]. In pancreatic adenocarcinoma, there are some reports that cancer cells escape immunity by secreting cytokines such as IL-10 and TGF- , or by producing nonfunctional Fas receptors (e.g. RCAS1)[9].

Tumor cells have multiple strategies to elude both innate and adaptive immune responses. Tumor cells can also affect the function of APCs, in particular DCs, which play a pivotal role in the induction and maintenance of an effective immune response. DCs are the most potent APCs[10]. Their function and polarizing capacities are decisive for the outcome of Th-mediated immunity. In the T-cell zone of lymph nodes, they function as APCs, which prime naive antigen-specific T cells

and drive their differentiation toward Th1, Th2, or regulatory T cells[11]. DCs pulsed with tumor-derived peptide are able to substantially augment the anti-tumor immune responses. There is a positive correlation between a favorable prognosis and the presence of DCs in several human tumors, including head and neck tumors, melanoma, lingual carcinoma, gastric carcinoma, thyroid carcinoma, and uterine cervical carcinoma[12-17].

In cancer patients, the number of circulating DCs is reduced, and their function is impaired. An increased proportion of immature DCs has been associated with the suppression of Ag-specific T cell responses[18]. Transformed cells produce a variety of immunosuppressive cytokines and chemokines such as IL-10, vascular endothelial growth factor, and TGF-β, that can negatively affect the maturation and function of APCs[19]. High concentrations of some of these result in decreased or absent expression of MHC molecules, co-stimulatory molecules, or secretion of immunosuppressive factors by tumors[20]. The purpose of this paper is to analyze immune reactivity and the DC network in relation to tumor development and the possible mechanisms by which tumors inhibit DC and escape from immune surveillance.

## II. MATERIALS AND METHODS

### 1. Reagents

Culture media used were RPMI-1640, Iscove's modified Dulbecco's medium, McCoy's 5a, Dulbecco's modified Eagle's medium, Ham's F12 medium (Invitrogen Life Technologies, Carlsbad, CA) Waymouth MB 756/1 medium (Sigma-Aldrich)and M199 (Gibco BRL, Grand Island, NY, USA), all supplemented with penicillin/streptomycin (1,000 unit/mL; Gibco BRL, Grand Island, NY, USA), 2 mM L-glutamine, 20 nM HEPES (Sigma, St. Louis, MO, USA) and 10% fetal bovine serum (FBS; HyClone, Logan, Utah, USA).

Recombinant human NMU was obtained from United States Biological (USA). Recombinant human TFF2 was obtained from Cytolab (USA). Il-8 and MIP-3β were obtained from Prospec-Tany TechnoGene Ltd (Israel).

Growth factors for DC generation were GM-CSF (200 ng/mL; LG. Co., Daejeon, Korea), IL-4 (1,000 unit/mL; BD Pharmingen, San Diego, CA, USA). Thw following fluorochrome-labelled monoclonal antibodies were used to analyze phenotypes of cells in peripheral blood mononuclear cells (PBMC) or cultured DC: CD1a-PE, CD80-PE, CD83-FITC, CD86-FITC (BD Pharmingen, San Jose, CA, USA).

**2. Tissues**

Samples of normal pancreas (n =17) and pancreatic adenocarcinoma (n =8) were collected from surgical specimens from patients at the Yonsei University Medical Center, and all procedures were carried out in accordance to the Guidelines and Regulations for Use and Care of IRB (Institutional Review Board) in Severance Hospital. In each case, the specimens were harvested within 10 minutes of resection from the patient and snap-frozen in liquid nitrogen before storage at 80°C. The resected cancers were not microdissected because we were interested not only in identifying the genes expressed by neoplastic epithelial calls, but also the genes expressed as a result of the neoplastic cell-stroma interaction. Hematoxylin and eosin-stained sections of adjacent sections of the tissue were prepared before snap-freezing to confirm the diagnosis. The neoplastic cellularity of these tissue samples ranged from 5 to 55%. Normal gastrointestinal mucosa was included in the analyses to facilitate the identification of markers of pancreatic cancer that would be useful in screening secondary sources.

**3. Cell lines**

Human pancreatic cancer cell lines AsPc-1, BxPc-3, CAPAN-1, CAPAN-2, CFPAC-1, Hpac, MiaPaCa2, Panc-1 and the immortalized normal pancreatic cell line YGIC-6, were obtained from the American Type Culture Collection, Rockville,

MD. The ascites derived human pancreatic cancer cell line, ASPC-1, the primary tumor derived human pancreatic cancer cell line, BXPC-3, and the human chronic myelogenous leukemia cell line, K-562, were cultured in RPMI-1640 medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% FBS and 1% antibiotic-antimycotic solution (Invitrogen Life Technologies). The liver metastasis derived human pancreatic cancer cell lines, Capan-1 and CFPAC-1 were cultured in Iscove's modified Dulbecco's medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% FBS and 1% antibiotics (Invitrogen Life Technologies). The primary tumor derived human pancreatic cancer cell line, Capan-2, was cultured in McCoy's 5a medium. The primary tumor derived human pancreatic cancer cell line, panc1, was cultured in Dulbecco's modified Eagle's medium. The primary tumor derived human pancreatic cancer cell line, Hpac, and the human breast cancer cell line, MCF-7, were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium. The primary tumor derived human pancreatic cancer cell line, Mia-Paca-2, and the gastric adenoma cell line, MKN-45, were cultured in Dulbecco's modified Eagle's medium with the extra addition of 2.5% horse serum. The immortalized normal pancreatic cell line YGIC-6 was cultured in Waymouth MB 756/1 medium. All cultures were maintained at 37°C in a humidified atmosphere containing 95% air and 5% $CO_2$. For mRNA isolation, cells were washed three times with phosphate buffered saline (PBS) and

harvested with trypsin (0.05% (w/v), Life Technology) and EDTA (0.02% w/v) for total RNA isolation.

**4. mRNA extractions and affymetrix genechip hybridization**

Sample preparation and processing were performed as described in detail in the Affymetrix GeneChip expression analysis manual (Santa Clara, CA) with the exception that the labeled cRNA samples were hybridized to the complete human U133 GeneChip set (Affymetrix U133A oligonucleotide microarray).

**5. Statistical data analysis**

The GeneExpress Software System Fold Change Analysis tool was used to identify all genes expressed at least 2-fold greater in the pancreatic cancer cells compared to normal tissues. For each gene fragment, the ratio of the geometric means of the expression intensities in the normal control tissues and the pancreatic cancer samples was calculated, and the fold change was then calculated on a per fragment basis. Confidence limits were calculated using a two-sided Welch modified *t* test on the difference of the means of the logs of the intensities.

**6. Reverse transcriptase-polymerase chain reaction (RT-PCR )**

mRNA was isolated using a QIAshredder and the RNeasy kit (Qiagen).

mRNA, Moloney murine leukemia virus reverse transcriptase, and pd$(N)_6$ primers (Invitrogen Life Technologies) were used to obtain cDNA. Sequences of all the primers used in the present study, sizes of PCR products, and PCR conditions are listed in Table 1. Amplification was performed over 35 cycles (94°C/5 min (denaturation), 58°C/30 sec (annealing), and 72°C/30 sec (extension). PCR products were electrophoresed on a 2% agarose gel and were visualized by ethidium bromide staining.

**Table 1.** Primers and PCR conditions used for RT-PCR studies

| Gene Symbol | sequnce | Tm | product size | positive control |
|---|---|---|---|---|
| spp1 | TGAGAGCAATGAGCATTCCGATG<br>CAGGGAGTTTCCATGAAGCCAC | 58 | 375bp | MCF-7 |
| GRN | CCAAGACACTGTGTGTGACCTGA<br>AGACAGCCTCTGGGATTGGAC | 58 | 443bp | MCF-7 |
| NMU | GAGATGCTGCGAACAGAGAGCT<br>GATGCACAACTGACGACACAACA | 58 | 379bp | K562 |
| TFF2 | AGCCCCCATAACAGGACGAA<br>GACTTCGGGAAGAAGCACCA | 58 | 266bp | MKN45 |

**7. Culture of monocyte derived dendritic cells**

**A. Preparation of peripheral blood mononuclear cells**

PBMCs were isolated from buffy coat produced by Ficoll-Hypaque gradient centrifugation of blood donated kindly from healthy volunteers. After centrifugation, PBMCs were collected from interphase between buffer and Ficoll and then washed with PBS.

**B. Isolation of monocyte from peripheral blood mononuclear cells**

Prepared PBMCs were resuspended with 500 μL PBS containing 0.5% BSA and were incubated with magnetic bead conjugated CD14+ antibody (100 μL/1 x $10^8$ PBMCs; Miltenyi Biotech, Auburn, CA, USA) at 4°C for 15 min. PBMCs at the interface were pelleted and washed twice with PBS. $CD14^+$ monocytes were isolated from mononuclear fractions through positive selection with microbeads coated with anti-CD14 antibody and Midi-Macs separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Purity was checked by flow cytometry with anti-CD14 MAb and was >95%.

**C. Generation of monocyte derived dendritic cells**

$CD14^+$ cells (2 x $10^6$cells/mL) in 5 ml RPMI 1640 were cultured in 6 well plates and incubated at 37°C and 5% $CO_2$. On days 1, 3 and 5 GM-CSF (100 ng/mL) and IL-4 (500 U/mL) were added. On day 6 DCs were stimulated with LPS (500 ng/mL) to mature dendritic cells. In some experiments, NMU (3 ug/mL), TFF2 (1 ug/mL) or IL-10 (10 ng/mL) were added to investigate their inhibitory effect on DC maturation.

**8. Flow cytometry analysis**

Cell staining was performed using the monoclonal antibodies listed in above. 5 x $10^5$ cells were stained with 10 μL monoclonal antibodies (MoABs). Cells were incubated for 15 min at 4°C with MoABs in PBS. After incubation, single and two-color flow cytometric analyses were performed using a FACScan flow cytometer (Becton Dickinson). Data were analyzed with the WINMdi program. Each analysis included at least 10,000 events.

**9. Allogeneic mixed leukocyte reaction**

To test T cell stimulatory functions, serial dilutions of DCs (2 x $10^5$ cells/well) were added to allogeneic T cells (2 x $10^6$ cells/well) and co-incubated for 4 days in RPMI-1640 in a 96-well plate. After 4 days, proliferation was determined

by addition of 0.5 μCi/well $^{3}$H-thymidine (Amersham Bioscience) final concentration for 16 h in triplicate. $^{3}$H-thymidine incorporation was measured using a liquid scintillation counter (Beckman, Palo Alto, CA, USA).

**10. FITC-dextran endocytosis by DC**

To measure the endocytotic activity of DC, endocytosis was measured after 24 h treatment with NMU (3 ug/mL), TFF2 (1 ug) or IL-10 (10 ng/mL) on day 6. Cells were incubated in 200 μL PBS containing 5% human serum with 1 mg/ml fluorescein isothiocyanate (FITC)-dextran. After 30 min incubation at 37°C, cells were washed three times in ice-cold PBS and analyzed with a flow cytometer. Cells from each culture condition were also maintained in the same solution for 30 min at 4°C as control.

**11. *In vitro* chemotaxis assay**

Cellular chemotaxis was measured by migration through 5 μm pore polycarbonate filters in 24-well transwell culture chambers (Corning Costar, Cambridge, MA). Culture medium containing different concentrations of chemokines (10 ng/mL NMU; 1 μg/mL TFF2; 50 ng/mL IL-8; and 10 ng/mL MIP-3β) was added to the lower chamber, and 5 x $10^{5}$ cells suspended in complete RPMI-1640 were then added to the upper chamber. After 4 h incubation at 37°C, the

cells in the lower chambers were harvested, concentrated to 50 µL volumes in Eppendorf tubes, and counted with a hemocytometer.

## III. RESULTS

### 1. Data filtering

Samples were hybridized to the Affymetrix U133A oligonucleotide microarray for simultaneous analysis of 45,000 fragments corresponding to 33,000 known genes and 6,000 ESTs. Genes with a 2-fold greater increase in expression in the 8 pancreatic adenocarcinoma tissues compared with 17 normal tissues were identified. We selected fragments expressed at least 2-fold greater in pancreatic cancer samples as compared with normal tissues, of which 4 corresponded to known gene fragment ranged from less than $p\ value<0.05$ (modified Welch $t$ test). Also, we selected genes that were related to secretory proteins, growth factors, receptors, ligands, and extra-matrix proteins. We then attempted to divide the genes into two groups according to whether they were secretory or metabolic. From there, we selected 225 genes up-regulated in pancreatic cancer compared to normal tissue (Table 2).

**Table 2.** Highly expressed genes identified in pancreatic cancer tissues

| Known gene name | Known gene name | Known gene name | Known gene name |
|---|---|---|---|
| lectin, galactoside-binding, soluble, 1 (galectin 1) | CD97 antigen | triple functional domain (PTPRF interacting) | major histocompatibility complex, class II, DQ alpha 1 |
| insulin-like growth factor binding protein 7 | apolipoprotein E | phosphoprotein enriched in astrocytes 15 | calpain, small subunit 1 |
| interferon, gamma-inducible protein 30 | Fc fragment of IgG, low affinity IIa, receptor for (CD32) | annexin A1 | MRNA; cDNA DKFZp686G03142 (from clone DKFZp686G03142) |
| bone marrow stromal cell antigen 2 | N-myc (and STAT) interactor | glycoprotein (transmembrane) nmb | chemokine (C-X-C motif) receptor 4 |
| D4, zinc and double PHD fingers family 2 | TYRO protein tyrosine kinase binding protein | complement component 1, q subcomponent, beta polypeptide | complement component 3 |
| sulfatase 1 | GTP binding protein overexpressed in skeletal muscle | CSE1 chromosome segregation 1-like (yeast) | FXYD domain containing ion transport regulator 5 |
| fibronectin 1 | plasminogen activator, urokinase | calpain, small subunit 1 | signal transducer and activator of transcription 1, 91kDa |
| transforming growth factor, beta-induced, 68kDa | chemokine-like factor | tryptophanyl-tRNA synthetase | lymphocyte antigen 6 complex, locus E |
| tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) | transporter 2, ATP-binding cassette, sub-family B (MDR/TAP) | regulator of G-protein signalling 1 | cadherin 11, type 2, OB-cadherin (osteoblast) |
| adenosine deaminase, RNA-specific | fibrinogen, gamma polypeptide | interleukin 4 receptor | chemokine (C-X-C motif) ligand 5 |
| dimethylarginine dimethylaminohydrolase 2 | SON DNA binding protein | CD58 antigen, (lymphocyte function-associated antigen 3) | major histocompatibility complex, class II, DP alpha 1 |
| inhibin, beta A (activin A, activin AB alpha polypeptide) | integrin beta 1 binding protein 1 | 2',5'-oligoadenylate synthetase 1, 40/46kDa | amyloid beta (A4) precursor-like protein 2 |
| platelet-derived growth factor receptor, beta polypeptide | CASP8 and FADD-like apoptosis regulator | activin A receptor, type I | tumor necrosis factor, alpha-induced protein 6 |
| cathepsin C | copper chaperone for superoxide dismutase | met proto-oncogene (hepatocyte growth factor receptor) | interleukin 6 receptor |
| myeloid cell nuclear differentiation antigen | major histocompatibility complex, class II, DR beta 3 | heparin-binding growth factor binding protein | proteasome (prosome, macropain) activator subunit 2 (PA28 beta) |
| SMAD, mothers against DPP homolog 3 (Drosophila) | proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7) | lymphocyte antigen 96 | transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) |
| lymphocyte antigen 75 | interferon-induced protein 44 | myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) | plasminogen activator, urokinase receptor |
| secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1) | caspase 2, apoptosis-related cysteine protease (neural precursor cell expressed, developmentally down-regulated 2) | aryl hydrocarbon receptor | serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| death associated transcription factor 1 | S100 calcium binding protein A11 (calgizzarin) | lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa) | UL16 binding protein 2 |
| chondroitin sulfate proteoglycan 2 (versican) | lectin, galactoside-binding, soluble, 4 (galectin 4) | granulin | non-metastatic cells 3, protein expressed in |

**Table 2. Continued**

| Known gene name | Known gene name | Known gene name | Known gene name |
|---|---|---|---|
| dual specificity phosphatase 6 | tumor necrosis factor receptor superfamily, member 1B | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae) | retinoic acid receptor responder (tazarotene induced) 1 |
| leukocyte specific transcript 1 | CAP, adenylate cyclase-associated protein 1 (yeast) | Rho GDP dissociation inhibitor (GDI) beta | cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, Drosophila) |
| C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced) | arachidonate 5-lipoxygenase | TAP binding protein (tapasin) | caspase recruitment domain family, member 6 |
| transforming growth factor beta 1 induced transcript 1 | ral guanine nucleotide dissociation stimulator | chemokine (C-X-C motif) ligand 14 | nuclear factor (erythroid-derived 2)-like 1 |
| collagen, type XI, alpha 1 | major histocompatibility complex, class II, DP beta 1 | macrophage stimulating 1 receptor (c-met-related tyrosine kinase) | major histocompatibility complex, class II, DR alpha |
| cadherin-like 26 | chemokine (C-X-C motif) ligand 16 | latent transforming growth factor beta binding protein 2 | axin 2 (conductin, axil) |
| insulin-like growth factor binding protein 6 | ADAM-like, decysin 1 | CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated) | sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C |
| arachidonate 5-lipoxygenase-activating protein | protein phosphatase 1, regulatory (inhibitor) subunit 15A | synovial sarcoma translocation, chromosome 18 | protein tyrosine phosphatase, receptor type, C |
| transcription factor 8 (represses interleukin 2 expression) | tubulin, beta, 4 | monoglyceride lipase | lectin, galactoside-binding, soluble, 3 (galectin 3) |
| caspase 8, apoptosis-related cysteine protease | trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in) | KIAA1715 | protease, serine, 25 |
| frizzled homolog 7 (Drosophila) | collagen, type XVII, alpha 1 | interferon-induced protein with tetratricopeptide repeats 4 | endothelial cell growth factor 1 (platelet-derived) |
| v-ets erythroblastosis virus E26 oncogene homolog 1 (avian) | GATA binding protein 3 | G protein-coupled receptor 91 | apoptosis-associated speck-like protein containing a CARD |
| lectin, galactoside-binding, soluble, 9 (galectin 9) | SHC (Src homology 2 domain containing) transforming protein 1 | endothelin receptor type A | epithelial membrane protein 3 |
| ectonucleoside triphosphate diphosphohydrolase 1 | integrin, beta-like 1 (with EGF-like repeat domains) | integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) | complement component 1, r subcomponent |
| tumor necrosis factor (ligand) superfamily, member 10 | egl nine homolog 3 (C. elegans) | capping protein (actin filament), gelsolin-like | retinitis pigmentosa 26 (autosomal recessive) |
| integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor) | nidogen (enactin) | major histocompatibility complex, class I, B | potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4 |
| filamin A, alpha (actin binding protein 280) | calcineurin binding protein 1 | mucin 4, tracheobronchial | cathepsin S |
| nucleotide-binding oligomerization domains 27 | cadherin 3, type 1, P-cadherin (placental) | H factor 1 (complement) | epithelial V-like antigen 1 |
| protein tyrosine phosphatase, receptor type, F | CD83 antigen (activated B lymphocytes, immunoglobulin superfamily) | Ras association (RalGDS/AF-6) and pleckstrin homology domains 1 | lectin, galactoside-binding, soluble, 3 binding protein |
| tryptase beta 2 | collagen, type VI, alpha 2 | carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) | major histocompatibility complex, class II, DM alpha |

**Table 2. Continued**

| Known gene name | Known gene name | Known gene name | Known gene name |
|---|---|---|---|
| CD38 antigen (p45) | I-mfa domain-containing protein | major histocompatibility complex, class I, F | interferon consensus sequence binding protein 1 |
| purinergic receptor P2Y, G-protein coupled, 5 | frizzled-related protein | guanine nucleotide binding protein (G protein), gamma 11 | dual oxidase 2 |
| chemokine (C-C motif) ligand 5 | lysozyme (renal amyloidosis) | cell division cycle 2-like 1 (PITSLRE proteins) | HLA-G histocompatibility antigen, class I, G |
| plexin domain containing 1 | tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator) | peripheral myelin protein 22 | major histocompatibility complex, class I, E |
| regulator of Fas-induced apoptosis | tumor necrosis factor receptor superfamily, member 21 | tumor protein p53 inducible protein 3 | |
| angiotensinogen (serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 8) | major histocompatibility complex, class I-related | S100 calcium binding protein A6 (calcyclin) | |
| protocadherin 1 (cadherin-like 1) | integrin, beta 4 | integrin, beta 5 | |
| Ras association (RalGDS/AF-6) domain family 2 | basic leucine zipper transcription factor, ATF-like | G protein-coupled receptor kinase 5 | |
| BCL2-interacting killer (apoptosis-inducing) | G protein-coupled receptor 124 | caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase) | |
| G protein-coupled receptor 126 | opsin 3 (encephalopsin, panopsin) | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2 | |
| deleted in liver cancer 1 | BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase) | platelet-derived growth factor receptor, alpha polypeptide | |
| cystatin F (leukocystatin) | TNF receptor-associated factor 4 | leucine-rich repeat-containing G protein-coupled receptor 6 | |
| nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3 | interferon, alpha-inducible protein (clone IFI-6-16) | insulin-like growth factor 1 (somatomedin C) | |
| stem cell growth factor; lymphocyte secreted C-type lectin | integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit) | caspase recruitment domain family, member 11 | |
| Cas-Br-M (murine) ecotropic retroviral transforming sequence b | chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated) | chromosome 11 open reading frame 13 | |
| glutathione synthetase | prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | tumor necrosis factor receptor superfamily, member 1A | |
| epidermal growth factor receptor pathway substrate 8 | trefoil factor 2 (spasmolytic protein 1) | protein tyrosine phosphatase, receptor type, U | |
| neuromedin U | zonadhesin | interleukin 2 receptor, gamma (severe combined immunodeficiency) | |
| likely ortholog of mouse gene rich cluster, C10 gene | EphA3 | protocadherin beta 10 | |
| laminin, alpha 3 | POU domain, class 2, associating factor 1 | discoidin domain receptor family, member 2 | |

**2. Literature search of genes highly expressed in pancreatic cancer**

For each of the 225 genes identified, a search was performed using the online NCBI database PubMed using the unknown gene name together with the terms “pancreas cancer” or “immune system.” Of the 225 genes analyzed, most of the genes and gene products were reported to be associated with pancreatic cancers. Many of them were typically associated with the general phenomena of inflammation and pancreatitis. A few assessed good genes were primarily reported to be associated in immune suppression. Finally, 4 genes that were not reported to be associated with pancreatic cancer and immune systems were selected for further study (Table 3).

**Table 3. Selected candidate immune escape gene**

| Probe Set ID | Known gene name | Gene Symbol | FC(PAN) | p-value (PAN) |
|---|---|---|---|---|
| 209875_s_at | secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1) | SPP1 | 3 | 0 |
| 211284_s_at | granulin | GRN | 2 | 0 |
| 206023_at | neuromedin U | NMU | 3 | 0 |
| 214476_at | trefoil factor 2 (spasmolytic protein 1) | TFF2 | 5 | 0.01 |

**3. Verification of selected candidate immune escape gene**

We used pancreatic cancer cell lines for validation of the selected candidate immune escape genes which explains why we found the immune suppression gene that was directly secreted by cancer cells, as opposed to being present in the tissue surrounding tumor cells. Candidate genes were selected for verification of expression in samples of pancreatic cancer cell lines (Fig. 1). Four genes were selected for validation by an RT-PCR study of 8 pancreas cancer cell lines and the immortal normal human cell line (YGIC-6) (Fig. 1). Genes selected for validation using RT-PCR were secreted phosphoprotein 1 (SPP1), granuin (GRN), NMU, and TFF2. These four genes were expressed in approximately 50% of pancreatic cancer cells, in support of their initial identification as differentially expressed genes by Affymetrix GeneChip.

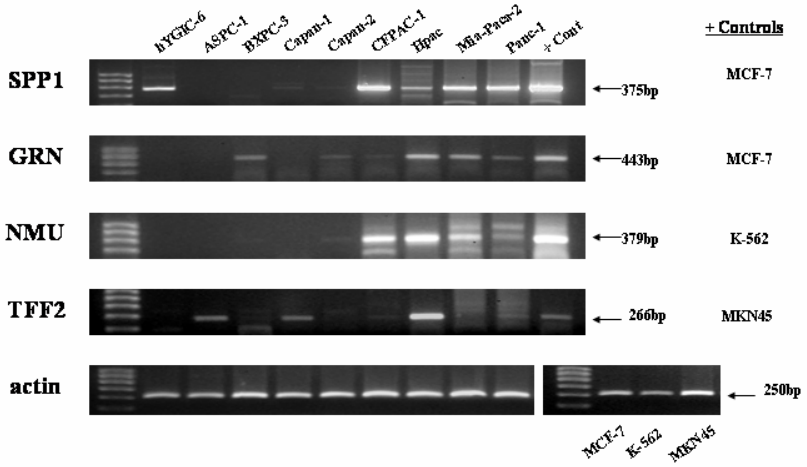


**Figure 1.** Validation of gene expression by RT-PCR in 8 pancreatic cancer cell lines, an immortal normal human pancreatic cell line (YGIC-6), and a positive control. Actin serves as an RNA control. genes are overexpressed in most of the pancreatic cancer cell lines.

**4. NMU and TFF 2 impair dendritic cell differentiation**

NMU and TFF2 are suppressed during differentiation from monocytes to immature DCs as immune escape gene, so we hypothesized that NMU and TFF2 prevent differentiation and maturation of monocytes to DC, especially during the early stage of differentiation. To examine the function of NMU and TFF2, purified $CD14^+$ monocytes were cultured with GM-CSF (100 ng/mL) and IL-4 (500 U/mL) with or without NMU (3 μg/mL) or TFF2 (1 μg/mL).

DCs were analyzed by microscopy and flow cytometry at different time points during *in vitro* maturation. Immature DCs were either cultured without stimulus (Fig. 2A) or treated with LPS (Fig. 2B), IL-10 (Fig. 2c), NMU (Fig. 2D), or TFF2 (Figu. 2E), established factors that initiate terminal DC maturation from day 6 of culture. In Fig. 2, images were taken on day 8. When analyzing DC cultures by light microscopy, it was noted that OPN induced the formation of DC clusters (Fig. 2). In contrast to unstimulated cultures (Fig. 2A), addition of NMU or TFF2 induced DC clustering that began 24 hours after stimulation (Fig.2 D-E). DCs cultured in the presence of OPN showed no changes in the characteristic dendrites of activated DCs.

When antigen expression was assessed by FACS analysis, CD1a, CD86, and CD83 expression in NMU-treated and TFF2-treated cells appeared to have dramatically no decreased in comparison with control DC (Fig. 3). However, exposure of maturing DC to NMU or TFF2 significantly decreased their expression

of cell surface molecules associated with antigen presentation including CD40, CD80, and HLA-DR. These results suggested that NMU and TFF2 had little effect on the expression of APC cell surface molecules.

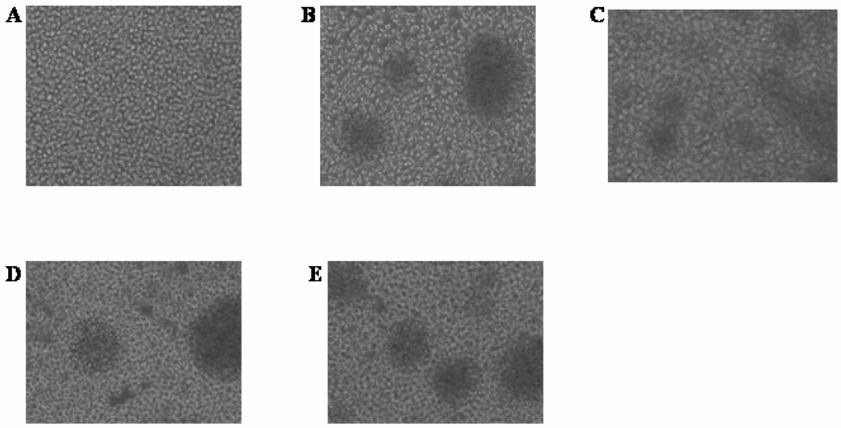


**Figure 2.** NMU/TFF2--stimulated DCs show morphologic signs of activation. DCs derived from peripheral blood monocytes were left untreated (A), or stimulated with 500ng/mL LPS(B) or 10ng/mL IL-10, 3μ g/mL NMU, 1 μg/mL TFF2 (C-E) on day 6 of culture. Photographs of cultures were taken 48 hours after addition of the stimulus.

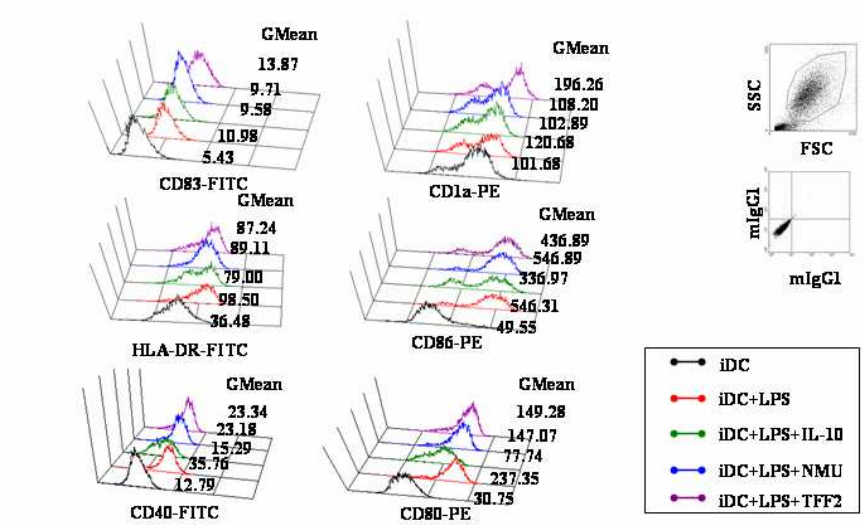


**Figure 3.** NMU, TFF2 induce terminal DC differentiation with expression of MHC class II, costimulatory, and adhesion molecules. DCs (1 x $10^6$) were cultured in the presence or absence of either LPS, IL-10, NMU or TFF2 from days 5 to 7 of culture. Cells were stained with antibodies against HLA-DR, CD40, CD80,and CD86 with antibodies against CD1a and CD83 to determine their DC phenotype.

**5. Effect of NMU and TFF2 on allogeneic T cell proliferation**

Matured DC function can be characterized, in part, by the ability of the cell to stimulate alloreactive T cells in a mixed leukocyte reaction (MLR). To determine whether co-stimulation of DCs with NMU or TFF2 plus LPS affects the allostimulatory capacity conferred by LPS, we performed an MLR assay. As shown in Fig. 4, treatment with LPS (500 ng/mL) resulted in the allostimulatory activity, and this was significantly reduced by treating DCs with IL-10 (10 ng/mL) plus LPS (500 ng/mL). However, treating DCs with NMU (3 ug/mL) or TFF2 (1 ug/mL) plus LPS (500 ng/mL) had no effect on the allostimulatory activity conferred by LPS. In addition, it was found that NMU and TFF2 had no effect on the allostimulatory activity of peripheral blood DCs.

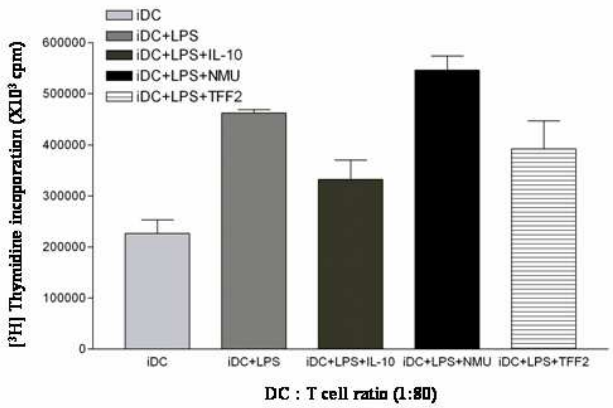


**Figure 4.** Effect of NMU,TFF2 on allogeneic T cell stimulation by LPS stimulated DCs. DCs (2 x $10^5$) were cultured in the presence or absence of either LPS, IL-10, NMU or TFF2 from days 5 to 7 of culture. Cells were then harvested and used to stimulate allogeneic T cells (2 x $10^6$) purified from human PBMCs in a 96-well plate. The stimulator cells were co-cultured with responder cells as 1: 80 ratio. Cells were pulsed with [$^3$H]thymidine on day 4, and [$^3$H]thymidine incorporation was assessed after 12 h. Results represent means ± SE cpm of three independent experiments performed.

**6. Endocytosis of FITC-dextran by DC**

Immature DCs capture and process antigens as a consequence of their high endocytic activity, a feature that is lost during maturation. To determine the ability of NMU and TFF2 to capture exogenous antigen, we examined the effect of NMU and TFF2 on FITC-dextran uptake.

As shown in Fig. 6, we found that DCs treated with NMU + IL-10 had a higher capacity than control DC's for uptake of FITC-dextran. However, TFF2 treated DC showed a slight decrease. These results suggest that NMU treated DC generated in the presence or the absence of IL-10 have a similar degree of endocytic activity.

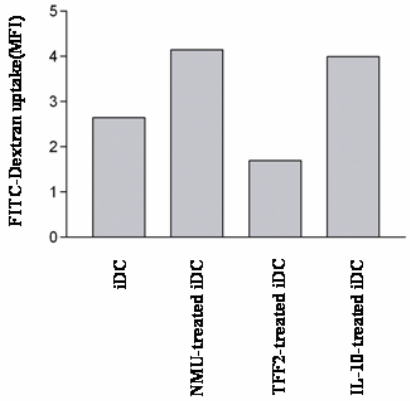


**Figure 5.** Endocytosis of FITC-dextran by dendritic cells (DC). DC were stimulated with NMU(3ug/mL), TFF2(1ug) or IL-10(10ng/mL) for 24 h. The DC in each group were incubated with 1 mg/ml FITC-dextran for 30 min at 37 °C, respectively. Uptake of FITC-dextran by DC was examined by flow cytometry. For a control, cells from each culture condition were also maintained in the same solution for 30 min at 4 °C. Phagocytic activity was quantified by measuring mean fluorescence intensity (MFI).

**7. Migration of immature and mature DC toward NMU and TFF2**

Maturation of DC results not only in loss of Ag uptake capacity but also in substantial changes in the expression of chemokine receptors and the ability to migrate toward chemokine gradients. The capacity of the 3 types of stimuli to induce DC migratory capacity toward chemokines was therefore evaluated, and several important characteristics of this migratory capacity were observed. First, immature and mature DC were highly sensitive to CXCL8 (IL-8) and CCL19 (MIP-3β) (Table 4, Fig. 6A&B), migrating towards 10 to 50 ng/mL of either chemokine. Second, when immature DCs were stimulated with NMU and TFF2, they became migratory (Table 4, Fig. 6A). Third, mature DC stimulated with NMU and TFF2 did not migrate (Table 4, Fig. 6B). These results demonstrate that in our cultures, migration of immature DC was induced by NMU and by TFF2.

**Table 4**. NMU, TFF2 induce chemotactic DC migration

| | Number of migrated cell | |
|---|---|---|
| | Upper chamber | |
| Lower chamber | iDC | mDC |
| Control | $1.32X10^4$ | $1.4X10^4$ |
| IL-8 | $2.64X10^4$ | $1.32X10^4$ |
| MIP-3β | $1.32X10^4$ | $5.28X10^4$ |
| NMU | $1.86X10^4$ | $1.7X10^4$ |
| TFF2 | $2.1X10^4$ | $1.6X10^4$ |

$10^6$ cells/ml DCs were generated as described in Materials and Methods and applied to the upper chamber of modified Boyden chambers. The chamber was incubated for 4h at 37℃ and cells that had migrated to the lower chamber were evaluated as described in Materials an Methods.

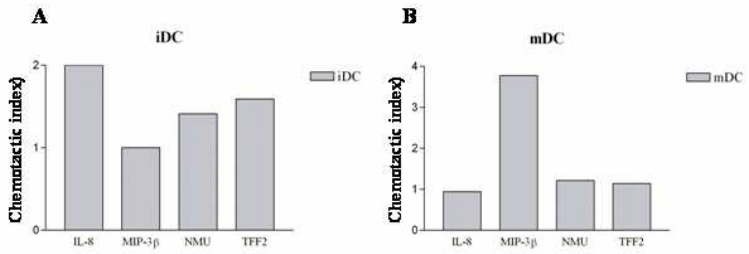


**Figure 6.** Migration of immature and mature DC toward NMU and TFF2. Immature DC (GM-CSF + IL-4) or DC stimulated with the indicated stimuli for 2 days were examined for their migratory capacity toward either CXCL8 (IL-8) or CCL19 (MIP-3β) in transwell assays. (A) Migration toward CXCL8(50 ng/mL), NMU and TFF2 by immature DC (5 X $10^5$). (B) Migration toward either CCL19 (10 ng/mL) by mature DC.

## IV. DISCUSSION

Several reports have described the tumor habitat as an immunocompromised environment, with circulating and tumor-infiltrating DCs being functionally defective in tumor-bearing hosts. It is well established that tumor cells produce and shed several molecules that can negatively affect the maturation and function of immune cells. The majority of molecules that have been identified thus far are chemokines and cytokines such as vascular endothelial growth factor, IL-10, and TGF-β that impair DC function by altering the phenotype or by enhancing spontaneous apoptosis. Some of these findings have been associated with poor prognosis in patients. However, the shedding of soluble factors distinct from cytokines by the tumor has also been shown to be a relevant mechanism of immunosuppression *in vivo* and *in vitro*.

In this study, selection of immune related genes was used to obtain gene expression profiles of pancreatic cancer for immune escape, and for validation of gene expression in pancreatic cancer cell lines and patients' sera. The inhibitory function of immune cells *in vitro* was then confirmed. The genes selected for the ability to control susceptibility to induced immunosuppression were identified.

Here we demonstrate that NMU and TFF2 are expressed abundantly in pancreatic cancers, and they inhibit the differentiation of monocyte-derived DCs,

switching them to a phenotype that has defective Ag presentation. This is the first report that demonstrates an effect of NMU and TFF2 on cells of the immune system. Several immunomodulatory agents, including glucocorticoids, $PGE_2$, IL-10, and vitamin $D_3$, have also been shown to exert suppressive effects on DC .

In this study, we have demonstrated that NMU and TFF2 interfere with the differentiation of monocytes into DC *in vitro*, resulting in the down-regulation of CD40, CD80, and HLA-DR. These results suggest that NMU and TFF2 had little effect on the expression of APC cell surface molecules. In addition, it was found that NMU and TFF2 had no affect on the allostimulatory activity of peripheral blood DCs. We found that NMU or IL-10 treated DCs had a higher capacity than control DC for uptake of FITC-dextran. However, TFF2 treated DC showed a slight decrease. These results indicate that NMU treated DC generated in the presence or the absence of IL-10 had a similar degree of endocytic. It is also well known that IL-8 is a key factor in the surface expression of CXCR1/2, and the migration of immature DC. We demonstrated that in our cultures migration of immature DC was induced by NMU and TFF2.

Three TFF peptides have been characterized in mammals, including humans: TFF1 (formerly pS2), TFF2 (formerly hSP), and TFF3 (formerly hP1.B/hITF). They are characterized by the TFF motif, a three-looped structure held tightly together by disulfide bonds based on six cysteine residues[21]. Human spasmolytic polypeptide

(hSP) was expressed in 23% of pancreatic duct cell carcinomas, and hSP protein was more frequently detected in cases of early-stage or histologically low-grade duct cell carcinomas than in cases of late-stage or histologically high-grade carcinomas[22]. Azarschab *et al*. also showed that aspirin upregulates TFF2 expression in human gastric cell lines[23]. May *et al*. concluded that TFF2 is expressed in normal and malignant breast epithelial cells and that it stimulates the migration of breast cancer cells[24]. Cook et al. showed that the TFF2 and 3 are expressed by the main organs (lymphoid tissues, spleen) involved in immune regulation, that they can be induced by bacterial endotoxin, and are able to stimulate monocyte migration[25]. Together, these observations suggest a potential role for the trefoil peptides in the immunological response to tissue.

Neuromedin U (NmU) is a smooth muscle contracting peptide first isolated from porcine spinal cord and later from other species and organs[26]. Substantial evidence suggests that this animal model mimics human breast cancer. The rat tumor's histopathology, origination from mammary ductal epithelial cells, and dependency on ovarian hormones for tumor development all correlate with human breast cancer[27]. Hedrick *et al*. also showed that whereas NMU is expressed by monocytes and dendritic cells, GPR66/FM-3 is expressed by T cells and NK cells[28]. These data suggest a previously unrecognized role for NMU as an immunoregulatory molecule. NMU also significantly increased the synthesis and

release of cytokines including IL-4, IL-5, IL-6, IL-10, and IL-13. Studies using pharmacological inhibitors indicated that maximal NMU-evoked cytokine release required functional phospholipase C, calcineurin, MEK, and PI3K pathways. These data indicate a role for NMU in inflammation by stimulating cytokine production by T cells[29]. NMU-R1 was highly expressed in primary mast cells, and NMU induced Ca (2+) mobilization and degranulation in peritoneal mast cells[30]. These data imply that NMU promotes mast cell-mediated inflammation. However, it had not been reported whether or not NMU and TFF2 inhibit the differentiation of DC from monocytes, and DC function.

In conclusion, we provide evidence that NMU and TFF2 modulate DC function. This may be a general protective mechanism by NMU and TFF2 against the differentiation or activation of DC in atherogenic inflammatory conditions. In this regard, the identification of signal-transduction events that participate in the differentiation inhibition and modulation of DC function by NMU and TFF2 would further contribute to the elucidation of the mechanisms underlying the complex anti-inflammatory effects of NMU and TFF2 and would allow the construction of a theoretical framework for its eventual therapeutic use. The results described here suggest that the presence of NMU or TFF2 from the onset of the tumor can promote progressive induction of immune silencing in synergy with other described immuno-

suppressive factors. The early impairment of DCs can strongly deviate and compromise the possible immune responses leading to progression of the disease.

## V. CONCLUSION

In this study, selection of immune related genes was used to obtain gene expression profiles of pancreatic cancer for immune escape, and validation of gene expression in pancreatic cancer cell lines and patients' sera. The inhibitory function of immune cells *in vitro* was then confirmed. The genes selected for the ability to control susceptibility to induced immunosuppression were identified.

Here we demonstrated that NMU and TFF2 are expressed abundantly in pancreatic cancers. We have demonstrated that NMU and TFF2 interfere with the differentiation of monocytes into DC *in vitro*, resulting in the down-regulation of CD40, CD80, and HLA-DR. These results suggest that NMU and TFF2 have little effect on the expression of APC cell surface molecules. In addition, it was found that NMU and TFF2 did not alter the allostimulatory activity of peripheral blood DCs. Moreover, we found that NMU, or IL-10 treated DCs had a higher capacity than control DC in uptake of FITC-dextran. These results suggested that NMU treated DC generated in the presence of the absence of IL-10 had similar degree of endocytic activity. It is also well known that IL-8 is a key factor in the surface expression of CXCR1/2, and the migration of immature DC. We found in our cultures that migration of immature DC was induced by NMU and by TFF2.

In this regard, the identification of signal-transduction events that participate in the modulation of DC function by NMU and TFF2 will further contribute to the elucidation of the mechanisms underlying the complex anti-inflammatory effects of NMU and TFF2 and will allow the construction of a theoretical framework for its eventual therapeutic use.

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ABSTRACT(IN KOREAN)

# 췌장암에서 면역 회피 관련 신규 유전자의 규명

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췌장암은 면역학적으로 가장 면역원성이 낮은 암 종 중의 하나이다. 현재 환자의 면역체계를 활성화하여 면역치료법을 통해 췌장암을 치료하려는 시도가 활발히 연구되고 있으며, 아울러 췌장암의 연구에서 확보할 수 있는 면역 억제 현상과 관련된 인자들이 규명이 될 수 있다면 향후 종양면역학에 커다란 기여를 할 수 있음과 아울러 새로운 치료법 개발에 커다란 도움이 될 수 있을 것이다. 종양환자들에 있어 면역세포들의 양적, 질적인 억제현상이 유도되어있다는 사실은 이미 밝혀져 있으나, 아직도 구체적인 mechanism 의 규명과 함께 관여하는 인자들의 동정은 충분하지 못한 상황이다.

본 연구는 췌장암에 있어서 면역 회피를 위해 췌장암 세포들이 이용하는 체액성 인자를 (1) 유전체 정보로부터 동정하고, (2) 동정된

인자들의 존재와 함량을 환자의 체액과 조직 등에서 확인(validation)하며, (3) 체외 배양 조건에서 선발된 인자들이 기본적인 면역세포의 기능에 억제효과를 발휘하는지를 확인하여 종양에 의한 면역억제의 원인들을 규명하는 것을 목적으로 한다. 이러한 정보는 궁극적으로 췌장암에 있어 새로운 치료법을 제시하는 동시에, 여러 종양과 면역 억제의 mechanism 을 규명하여 질병 치료에 응용할 수 있게 한다.

인체 암 조직으로부터 얻어진 mRNA 를 Affymetrix 사의 U133 human chip 을 이용하여 microarray 실험을 수행하였다. 그 결과 Microarray chip data 의 유전자들 중, 정상인보다 췌장암 환자에서 'FC (fold change) >2, p-value<0.05' 조건의 유전자들을 선택하고, 그들 중 GO (gene othology) term 에 따른 gene grouping 을 거쳐 secretory protein, growth factor, receptor, ligand, extra-cellular matrix 와 관련된 유전자들을 선정하여, 췌장암 환자에서 발현이 증가한 유전자들을 선정하였다. 이들 중 췌장암, immune system 에 관한 연구 보고가 없는 유전자들만 1 차 선발하여, 최종 4 개 이내의 유전자를 선정하였다.

최종 선택된 gene 들이 췌장암 세포주와 환자 혈청에서의 발현여부를 확인하기 위해, 각 유전자를 췌장암 세포주(Mia PaCa-2, CFPAC-1, PANC-1, AsPC-1, HPAC, Capan-1, Capan-2, BxPC-3)에

적용하여, RT-PCR 을 수행한 결과, 대부분의 췌장암 세포주에서 발현함을 확인하였다.

선택된 NMU, TFF2 유전자들이 갖는 면역기능을 조사하기 위해, 이 재조합 단백질들을 처리하여 실험관에서 대표적인 immune cell 인 강력한 전문항원제시세포인 수지상세포 (dendritic cells) 에 미치는 영향을 분석하였다. 그 결과, 수지상세포의 migration 유도됨을 보였다. 따라서 본 연구는 면역관련 유전자 (NMU, TFF2) 들이 갖는 면역기능을 이용하여 이들이 췌장암의 면역 억제 유전자로써 역할을 할 수 있는지 검증할 수 있었다. 또한 상기의 인자들 이외에도 추가적인 면역억제 능력을 갖는 인자들의 동정은 향후의 면역치료법이나 환자의 면역결핍 증상을 완화시키는데 정보를 제공할 것이다.

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핵심되는 말 : 췌장암, 수지상 세포, 면역 억제 유전자