

The genetic background of pancreatic cancer: Genes that might be biomarkers or indicators of metastasis to the lung.

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Background: Pancreatic cancer is a malignant neoplasm arising from malignant cells which form the pancreas. It is the fourth leading cause of cancer deaths, being responsible for 6% of all cancer-related deaths. Pancreatic cancer is difficult to be diagnosed in its early stages. One major problem is that it metastasizes to regional lymph nodes and later to the liver or to the peritoneal activity and other organs, like lungs. The discovery of new biomarkers for early prognosis, and/or for predicting metastasis to lungs is essential. The present study aims to determine possible genes that are correlated with pancreatic cancer, as well as with other types of malignancies, including lung cancer.

Methods: mRNA was extracted from cancer cell lines representing pancreatic (PANC-1 and BxPC-3), breast (T47D), lung (COLO699N) and colorectal (HCT-116) cancer by using oligo-dTs magnetic beads. mRNA was also extracted from breast, lung and cancer stem cells (CSCs) as well as from PBMCs from a non-cancer donor. DNA microarrays were performed in the human Caucasian pancreatic cell line PANC-1 and in the human primary pancreatic adenocarcinoma cell line BxPC-3 by using the Human MI Ready Array platform. Gene expression analysis, for these genes that were over-expressed in both cell lines, was performed by using RT-qPCR in all the above mentioned cell lines. All the reactions were performed in triplicates. A p-value < 0.05 was considered significant.

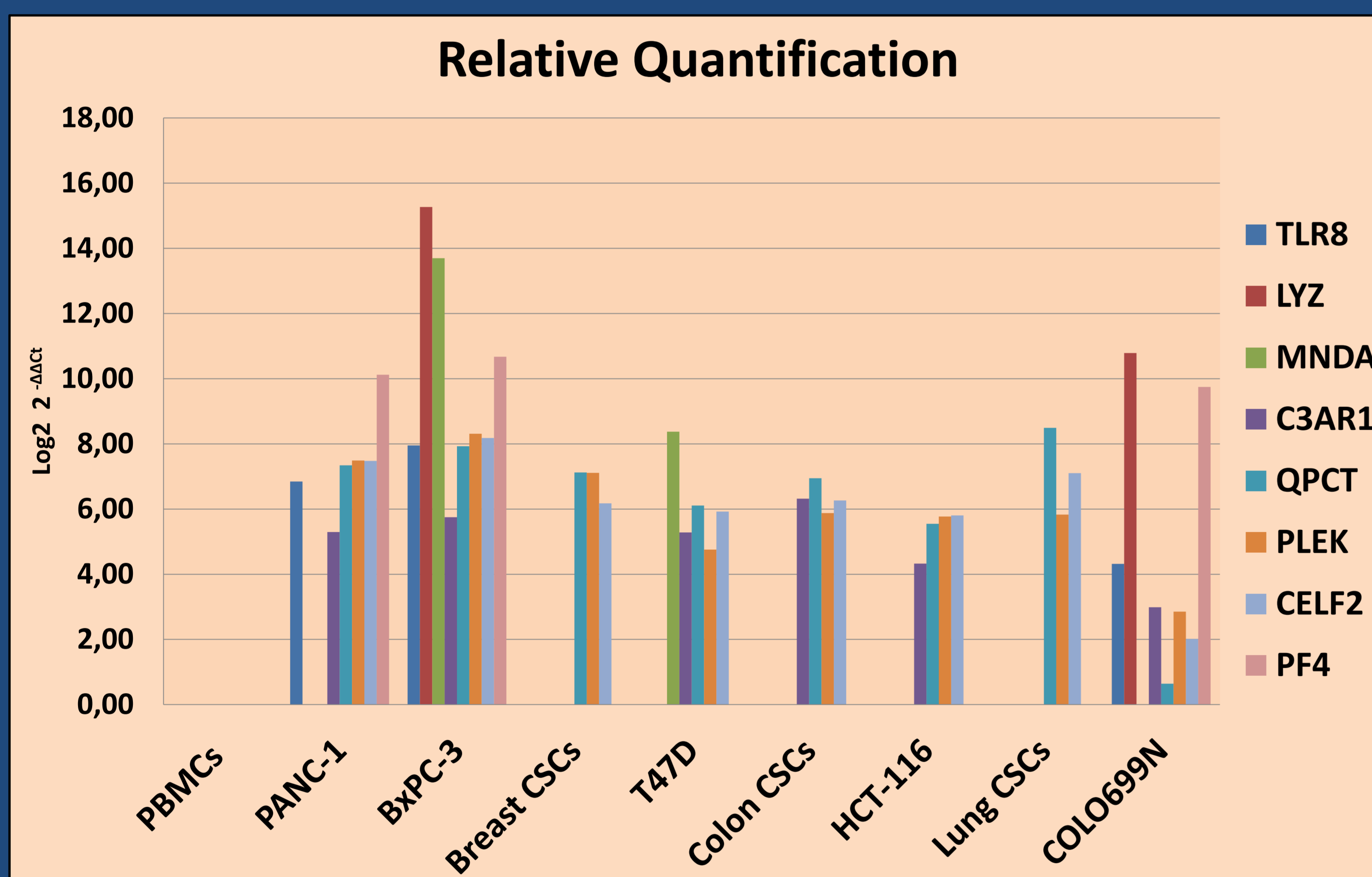


Figure 1: Relative gene expression analysis. The samples normalized according to normal PBMCs sample, by using 18srRNA as housekeeping gene. The relative analysis was performed according to Livak method.

Conclusions: According to literature and experimental data, there are many biomarkers for pancreatic cancer. PF4 and LYZ are two of them. It has been shown that among toll-like receptors, many of them have been correlated with this type of cancer. However there is no correlation with TLR8. It is remarkable that the above genes are expressed only in pancreatic and differentiated lung cancer cells, demonstrating the common features displaying both types of cancer, as well as the common therapeutic approaches. It is also noteworthy that the above genes are not classified as lung cancer biomarkers. Therefore, they might be considered not only as pancreatic cancer biomarkers, but as potential indicators of metastasis to the lung. It is essential to perform further studies in other cancer cell lines and in more samples, so as to be used clinically. However the first results are quite encouraging.

Symbol	Description	Role
TLR8	Homo sapiens toll-like receptor 8	Toll-like receptor family which plays a fundamental role in pathogen recognition and activation of innate immunity.
LYZ	Homo sapiens lysozyme	This gene encodes human lysozyme (LYZ), whose natural substrate is the bacterial cell wall peptidoglycan.
MNDA	Homo sapiens myeloid cell nuclear differentiation antigen	These genes participate in blood cell-specific responses to interferons.
C3AR1	Homo sapiens complement component 3a receptor 1	The C3a receptor is a G protein-coupled receptor protein involved in the complement system
QPCT	Homo sapiens glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	This gene encodes human pituitary glutaminyl cyclase, which is responsible for the presence of pyroglutamyl residues in many neuroendocrine peptides.
PLEK	Homo sapiens pleckstrin	Pleckstrin is a protein found in platelets. Diseases associated with PLEK include aarskog-scott syndrome, and centronuclear myopathy.
CELF2	Homo sapiens CUGBP, Elav-like family member 2	Members of this protein family regulate pre-mRNA alternative splicing and may also be involved in mRNA editing, and translation.
PF4	Homo sapiens platelet factor 4 (chemokine (C-X-C motif) ligand 4)	This protein is chemotactic for numerous other cell type and also functions as an inhibitor of hematopoiesis, angiogenesis and T-cell function.
SH2D1A	Homo sapiens SH2 domain protein 1A	This gene encodes a protein that plays a major role in the bidirectional stimulation of T and B cells. This protein contains an SH2 domain and a short tail.

Table 1: Analysis of genes that were studied.

Results: The microarray experiments demonstrated 9 different genes that are over-expressed in pancreatic cancer. Among them, the TLR8, LYZ and PF4 are expressed only in pancreatic and differentiated lung cancer cell lines. The QPCT, PLEK and CELF2 are expressed in all cell lines as well as in normal samples. The MNDA gene is expressed only in primary pancreatic cancer and in T47D cell line. Furthermore, the C3AR1 is expressed in all cases apart from breast and lung CSCs. Finally, SH2D1A is not expressed in colorectal cancer, lung CSCs and in normal samples.

A/A	Gene	PANC-1	BxPC-3	Breast CSCs	T47D	Colon CSCs	HCT-116	Lung CSCs	COLO 699N
1	TLR8	+	+	-	-	-	-	-	+
2	LYZ	-	+	-	-	-	-	-	+
3	MNDA	-	+	-	+	-	-	-	-
4	C3AR1	+	+	-	+	+	+	-	+
5	QPCT	+	+	+	+	+	+	+	+
6	PLEK	+	+	+	+	+	+	+	+
7	CELF2	+	+	+	+	+	+	+	+
8	PF4	+	+	-	-	-	-	-	+
9	SH2D1A	+	+	+	+	-	-	-	+

Table 2: The presence of specific genes among different cancer cell lines. The "+" indicates the presence while the "-" shows the absence of each gene.



Selected References:

- Fiedler GM, Leichtle AB, Kase J, et al. Serum peptidome profiling revealed platelet factor 4 as a potential discriminating Peptide associated with pancreatic cancer. Clin Cancer Res. 2009; 15: 3812-9.
- Ridnour LA, Cheng RY, Switzer CH, et al. Molecular pathways: toll-like receptors in the tumor microenvironment—poor prognosis or new therapeutic opportunity. Clin Cancer Res. 2013; 19: 1340-6.

