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Genome Wide Transcriptional Analysis of Gene Expression Signatures and Pathways on Neoplastic Pancreatic Cancer

Biotechnology

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ABSTRACT

The neoplastic pancreatic cancer originated from both endocrine and exocrine organs shows cancer related deaths. There are various cell types with their complex interactions between tumor and non tumor cells. Extracellular matrix of pancreas show inflammation on tumor progression. The inflammation enhanced cancer progression and cellular transformation that leads to entry of cancer cells into the circulation and regulates the gene transcription. Our goal of this study on experimental data that supports inflammation in pancreatic cancer development and pathway may create an extracellular matrix that supports tumor formation. Human natural killer cells showkey effectors of innate immune response and there a function of cells is enhanced by cytokines and interleukin-2(Il-2) in inflammatory pathway (AKT, PI3K, NF-kB). Thus the pancreatic inflammatory signaling pathway with the target genes and proteins acts as a potential drug targets in the drug discovery against neoplastic pancreatic cancer. signaling pathway belongs to a complex system of communication that governs cellular process. any change in signaling cascade leads to change in cell function and causes many diseases

INTRODUCTION:

Pancreatic cancer (PC) is a malignant neoplasm developing from the transformed cells in the tissues of pancreas. There are a number of types in pancreatic cancer; the most prominent type of PC is adenocarcinomas. The adenocarcinomas of tumor arise within the exocrine segment of the pancreas which developing in the duct cells that convey the enzymes to the bloodstream. The minor types of PC include those arising from the islet cells or the endocrine region (also called as neuroendocrine tumors). The German physician Dr. Giovanni Battista Morgagni was found the pancreatic tumors. He explains the pancreatic cancer is most deadly disease and it is most importantly affects glandular organ in the body. The glandular organs responsible for synthesizing digestive hormones like glucagon and insulin that help maintain sugar levels in the blood stream. Pancreatic cancer greatly impairs the function of the pancreatic gland. The common symptoms of PC are pain in the abdomen region and jaundice. Thus, the yellow skin is one of the most obvious signs of pancreatic cancer. Physicians belonging to the Greece, China and Egypt recognized this and named it as a poor outcome. It doesn't appear they were aware of a cancerous growth in the pancreatic region and once examining dead bodies was culturally sanctioned for medical requirements, they could discover tumors [1]. Most instances of this disease are difficult to detect and are found only in their advanced stages and making it nearly impossible to cure completely.

In 2009 review shows pancreatic cancer is fourth leading cause of cancer deaths among men and women, being responsible for 6%of all cancer-related deaths. Pancreatic cancer is difficult to diagnose in its early stages. At the time of diagnosis, 52% of all patients have distant disease and 26% have regional spread. The relative 1yrs survival rate for pancreatic cancer is only 26%, and the overall 5-year survival is 6%. Approximately, 33,730 people are develop pancreatic cancer and 30,300 people will die from the disease in 2010 [2]. The lethal nature of pancreatic cancer stems from its propensity to rapidly disseminate to the lymphatic system and distant organs. There are various types of pancreatic cancer include 80% are adenocarcinomas of the ductal epithelium. Only 2% of tumors of the exocrine pancreas are benign. The less common histological appearances of exocrine pancreatic cancers include giant cell carcinoma, mucinous carcinoma,microglandularadenocarcinomas, adenosquamous carcinoma, acinarcystadenocarcinoma, cystadenocarcinoma, papillary cysticcarcinoma and acinar cell cystadenocarcinoma. The most common of these is primary pancreatic lymphoma [3]. The pancreatic adenocarcinomas of the exocrine pancreas are the most common type of pancreatic malignancy. It presents symptoms only when it is already in an advanced state and curative resection is no longer possible. The symptoms may be changeable, non-specific and represent a variety of possibilities besides pancreatic cancer. Characteristically, itching, dark urine, pale bowel movements and jaundice may indicate the growth of pancreatic cancer. Often tiredness, weight loss, loss of appetite and decreased energy are experienced. Pain may be present if tumor is advanced. The detailed study shows that the blood clot strongly linked with pancreatic cancer and can be the initial symptom of the disease. A rare type of pancreatic cancer begins in the cells that make insulin and other hormones it is known as islet cell cancer. The cancer cells spread (metastasizes) outside the pancreas, cancer cells are found in nearby lymph nodes and other tissues like lungs or liver. The risk factors include smoking, imbalanced food, and hereditary factors are major in pancreatic cancer. 5-10% of the cases of pancreatic cancer are believed to be caused by genetic factors. The genes will carry genetic information within each cell of human body. The molecular biology of gene mutation may have a significant impact of genetic alterations at different levels. These alteration contains oncogene mutations (most commonly, K-RAS mutations, which occur in 75% to more than 95% of pancreatic cancer tissues), [4] tumors suppressor genes alterations (mainly, p53, p16, DCC, etc.), over expression of growth factors (such as TGF alpha, EGF, TGF beta 1-3, bTGF, aFGF, etc.) and their receptors (i.e., EGF receptor, TGF beta receptor I-III, etc.) [5]. Detailed knowledge of genes involved in the growth of pancreatic adenocarcinomas might enable construction of molecular tests for earlier diagnosis [6] and identify new targets for therapy.

MATERIALS AND METHODS

RNA PREPARATION FOR MICROARRAY:

The total RNA was isolated from NK cells and determine by two-color flow cytometry with Alexa488-labeled monoclonal antibody (mAb) against CD3 and Alexa647-labeled mAb against CD56 or CD16. Six samples with a minimum RNA integrity number of 6.6 were used for microarray hybridization.

AFFYMETRIX MICROARRAY:

The total RNA from each sample was amplified and platinum-linked cyanine dye using the MicroMax ASAP RNA labeling kit according to manufacturer's protocol. The Affymetrix and other microarray core facility performed the amplification and hybridization using the Affymetrix GeneChip Human Genome U133 and additional identically replicated Human Genome Chip U133 plus 2.0 Array. Intensities of each probe set of complete

human genome U133 set and 6500 additional genes for analysis of over 47000 transcripts. All probe sets of GeneChip U133 is identically replicated on the GeneChip U133 plus 2.0 arrays. The sequences from which these probe sets were derived were selected from RefSeq, dbEST, and GenBank. The sequence clusters were created from the UniGene database and then refined by analysis and comparison with a number of other publicly available databases.

DATA ANALYSIS:

QUALITY ASSESSMENT AND DATA PREPROCESSING:

The Affymetrix CEL files were used to analyze quality on genomic probe level modelling and quality metrics provided by the Affy package of BioConductor. The three outlier array (two from the primary progressor group and one from the secondary non-progressor group) that did not cluster with other arrays in principal component analysis results were excluded from further analyses. In addition, we have independently applied the ANOVA model using two different p-values ($p < 0.05$ and $p < 0.15$) to identify dysregulated genes between three groups (normal CD8+ T cells, and Lymphoid tissue samples) and three groups (NK cells on Neoplastic PC) to focus on differences between these two groups. Hierarchical clustering was performed with the genes best characterizing normal pancreatic tissue identified by PAM. Genes of all 6 specimens with different expression profiles were grouped by standard correlation with BioConductor package. Hierarchical clustering of the genes was based on similarities of expression levels.

SIGNALING PATHWAY PREDICTION:

The pathway analysis was performed using the pathway analytical tool of the ConceptGen and GORILLA tool. In this tool, we studied the functionally enriched genes associated with pancreatic cancer based on statistical significance. We considered two significant p-values for groups of genes in a pathway that differentially regulated if either significance level less than 0.001 or at least 5 genes of a pathway are represented on the array.

RESULTS AND DISCUSSION:

FUNCTIONAL ENRICHED GENES PRESENT IN PANCREATIC CANCER SIGNALING PATHWAY:

The stimulation of NK cells, using IL2 receptors in tyrosine phosphorylation signal transduction of the IL2Rβ chain results in activation of the PI3K signaling pathway. Three genes present in PI3K catalytic subunits have highest expression levels (PIK-3CA, PIK3CD and PIK3CG), while the transcript of β-subunit showed a slower rise (PIK3CB). This increased PI3K expression shows negative regulator of Phosphatase PTEN showed progressively increased expression in the stimulated cell. PI3K activation increases cell survival and antagonizes apoptosis through AKT subtypes (AKT1 and -2) and it became upregulated by IL2. Activated AKT inhibits phosphorylation of BAD that is part of the BAD/BCLXL complex. Phosphorylated BAD cleaves from the BAD/BCLXL complex, thereby promoting cell survival. In addition, AKT activates EIF4E, through mTOR and EIF4E on protein synthesis and had increased expression of activated cells. (Fig-10 legend file) TGF-Beta signaling pathways shows regulation of gene expression based on gene to gene and protein to protein interaction. The regulatory gene TGF-BR3 interacts with non carbohydrate external ligand and it directly phosphorylates the membrane proteins CCNB2 and PARD6 PROTEINS. The protein-protein interaction of downstream regulation of PI3KR1 and PI3KR2 that leads to unknown function and uncharacter proteins synthesis in PKD1. The PKD1 inhibits the activation of SMAD receptor complexes inside the cytoplasm it dissociates the MAPK14. The MAPK14 and complex of MAPK8/MAPK9 phosphorylates SMAD complex signaling genes along with ERK dependent gene expression.

Total inhibitory reactions

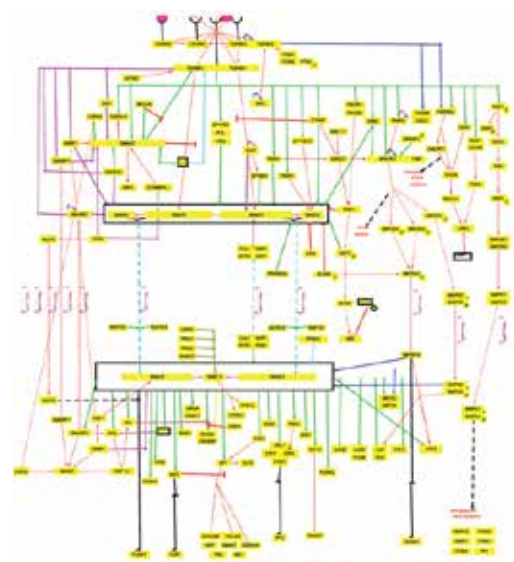
- TGFB1--| SMAD7--| TGFBR1--|inhibits the TGFB receptor in outer membrane
- Phosphorylated TRAF6 form ubiqutiation with MAP3K7 signaling proteins. It leads to inhibitory process of NF-KB signaling pathway
- Phosphorylation of SMUR1àubiqutination with RHOA to form direct inhibition to canonical signaling pathway.
- SMUR1inhibits dissolution of ligand junction on other signaling proteins then it will cause pancreatic cancer Four inhibitory signaling pathways
- NF-KB inhibitory signaling pathway • Dissolution of ligand junctions
- ERK dependent gene expression in pancreatic cell development • TGFB signaling pathways

CONCLUSION:

Our study includes gene expression profiles associated with IL2 induced increased cytotoxicity, changes in chemokines, cytokines and adhesion properties, enhanced proinflammatory and innate immune response and changes in signaling pathways in NK cells. The changes in chemo toxicity signaling and surface/adhesion profile may enable the IL2 activated NK cells to migrate and infiltrate tissues where inflammation occur and upon arrival recruit other effectors cells of the immune system.

The NK cells may be more responsive and involved in both autocrine and paracrine signaling in the local environment than previously recognized. There is good evidence indicating NF-κB activation which may play a central role in pro-survival and pro-inflammatory function in activated NK cells. The present study comprehensive analysis of pancreatic tissues in conjunction with publicly available datasets and software tools enhanced the identification of genes that may participate in disease pathogenesis, or may serve as preferred targets for diagnostic or therapeutic strategies.

1. PI3K signaling pathway predicted based on gene expression in pancreatic cancer raw data.



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