RESEARCH ARTICLE

Pathway Crosstalk Analysis Based on Protein-protein Network Analysis in Ovarian Cancer

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Abstract

Ovarian cancer is the fifth leading cause of cancer death in women aged 35 to 74 years. Although there are several popular hypothesis of ovarian cancer pathogenesis, the genetic mechanisms are far from being clear. Recently, systems biology approaches such as network-based methods have been successfully applied to elucidate the mechanisms of diseases. In this study, we constructed a crosstalk network among ovarian cancer related pathways by integrating protein-protein interactions and KEGG pathway information. Several significant pathways were identified to crosstalk with each other in ovarian cancer, such as the chemokine, Notch, Wnt and NOD-like receptor signaling pathways. Results from these studies will provide the groundwork for a combination therapy approach targeting multiple pathways which will likely be more effective than targeting one pathway alone.

Keywords: Ovarian cancer - protein-protein network - pathway crosstalk

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Introduction

Ovarian cancer is the fifth leading cause of cancer death in women aged 35 to 74 years (Torpyet al., 2011). The survival rate of ovarian cancer still remains lower than other types of gynecological cancer, with an overall 5-year mortality of 70%, although considerable progress achieved in the management of gynecological cancers (Colomiere et al., 2009). This lower survival rate is partly result from that ovarian cancer is usually found at an advanced stage. Epithelial ovarian cancer is the most common type of ovarian cancer, which make up 85-90% of all human ovarian malignant tumors and arise from the surface (epithelium), or serosa, of the ovary perhaps (Torpy and Burke, 2011).

Currently, there are several popular hypothesis of ovarian cancer pathogenesis (Berchuck et al., 1994; Nanjundan et al., 2008; Shih Ie and Davidson, 2009; Merritt and Cramer, 2011), however, the genetic mechanisms of ovarian cancer are far from being clear. The most common genetic alterations seen among ovarian cancer are KRAS, BRAF, ERBB2, PTEN, β-catenin, TP53 mutations (Obata and Hoshiai, 2000; Saito et al., 2000;Singer et al., 2002; Singer et al., 2003; Shih Ie and Kurman, 2004; Sieben et al., 2004; Singer et al., 2005; Oliva et al., 2006). Oncogenic mutations in KRAS, BRAF and ERBB2 result in constitutive activation of the mitogen activated protein kinase (MAPK) signal transduction pathway which plays a critical role in the transmission of growth signals into the nucleus and contributes to neoplastic transformation. The PTEN mutations typically result in constitutive PI3K signaling. Wnt and TGF-β signaling pathways are also of potential importance for ovarian cancer pathogenesis, based on the presence of β-catenin mutations. However, these investigations rarely consider the potential relationships among these pathways. The disease complexity is coming from not only the cooperations of proteins in the form of pathways but also the interactions of pathways, i.e., the crosstalks of these pathways (Francesconi et al., 2008; Li et al., 2008).

Recently, systems biology approaches such as network-based methods have been successfully applied to elucidate the mechanism of diseases (Ideker and Sharan, 2008; Zhao et al., 2008). From the systematic perspective, analysis of ovarian cancer related bio-molecular interaction networks will improve the understanding of the complexity of molecular pathways underlying ovarian cancer and will help to uncover the dynamic processes of disease progression.

In this study, we propose a network-based analysis for the crosstalks among ovarian cancer related pathways by integrating protein-protein interactions and gene expression profiles. The availability and integration of high-throughput gene expression data and the genome-wide protein-protein interaction may shed new lights on ovarian cancer study.

Materials and Methods

Data sources

Affymetrix microarray data

We extracted the gene expression profile GSE14407 (Bowenet al., 2009) data of ovarian cancer from NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) database which

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is based on the Affymetrix Human Genome U133 plus 2.0 Array. Total 24 chips were available for further analysis. Twelve healthy ovarian surface epithelia samples (OSE) were compared to twelve laser capture microdissected serous ovarian cancer epithelia samples (CEPI) via Affymetrix 3' expression array. Normal ovarian surface epithelial cells were collected from ovaries at time of surgery using a Cytobrush Plus. Tumor tissues were surgically removed and immediately (<1 minute) placed in cryotubes and snap frozen in liquid nitrogen (Turashviliet al., 2007).

Protein-Protein Interaction (PPI) data

The Human Protein Reference Database (HPRD) (Keshava Prasad et al., 2009) is a protein database accessible through the internet. The Biological General Repository for Interaction Datasets (BioGRID) (Stark et al., 2011) is a curated biological database of protein-protein and genetic interactions. Total 326119 unique PPI pairs were collected in which 39240 pairs are from HPRD and 379426 pairs are from BioGRID. We constructed an ensemble protein-protein interaction network by integrating the PPI data collected from the two above PPI databases in human.

Expression profile analysis

The Affy library based on R (Team, 2011) was used to perform data preprocessing. Raw expression levels were normalized using the RMA summarization algorithm. This normalization method is a mathematical technique used to obtain variance stabilization and reduce discrepancies in hybridization patterns that might result from variables in target amplification, hybridization conditions, staining, or probe array lots. The expression profiles were selected after the data preprocessing

Significance analysis of pathway under PPI data

To determine the co-expressed significance of a gene pair in disease cases, we used the PCC test to calculate the p-value.

Map those p-values to the nodes and edges in the PPI network. The following formula is used to define a function as the combination of statistical significance of an interaction by a scoring scheme. The detailed description could be seen in Liu et al (Liu et al., 2010).

$$S(e) = f(diff(x), corr(x, y), diff(y))$$

$$= -2\sum_{i=1}^{k} \log_e(p_i)$$

The diff(x) and diff(y) are differential expression assessments of gene x and gene y, respectively. corr (x,y) represents their correlation between gene x and gene y. f is a general data integration method that can handle multiple data sources differing in statistical power. Where k = 3, p1 and p2 are the p-values of differential expression of two nodes, p3 is the p-value of their co-expression.

To define the significance of a pathway P, Sp, we summarize all the scores of edges S(e) of every pathway.

$$Sp = \sum_{e \in p} S(e)$$

To estimate a p-value for significance of this pathway, we iteratively compute similar scores 105 times on randomly generated pathways of the same size as that of pathway P. The frequency of scores that are larger than Sp is used as the significant p-value of pathway P. We considered the pathway with the p-value <0.05 as the significant pathway.

Pathway crosstalk analysis based on the GO enrichment analysis

Analysis of crosstalk of relationships among pathways is then investigated, especially that with overlapping GO-ID of two significant pathway analysis results.

The functional enrichment among proteins in one pathway is defined as: $P = 1 - \sum_{i=0}^{k-1} \frac{\binom{f}{i}\binom{n-f}{m-i}}{\binom{n}{m}}$

where n is the number of nodes in the network, f is the number of proteins annotated with a particular GO function, m is the number of proteins involved in the pathway and k is the frequency of the GO-ID. We identified the GO function enrichment of the pathways respectively with the p-value <0.05.

Results

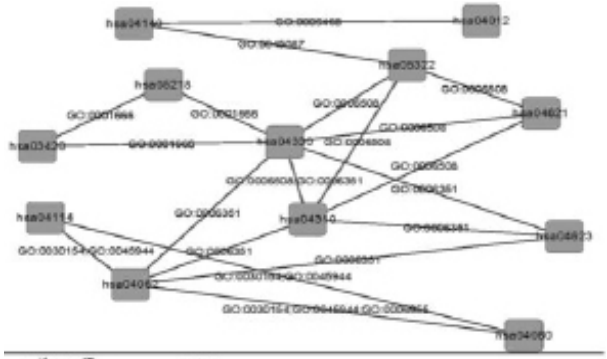
Significance analysis of pathway under PPI data

To get expression profile of ovarian cancer epithelia (CEPI), we obtained publicly available microarray data sets GSE14407 from GEO. The expression profiles were selected after the data preprocessing.

Pathway can provide an alternative way to relax the significance threshold applied to single genes and may lead to a better biological interpretation (Francesconi, Remondini, 2008). To identify the relevant pathways changed in ovarian cancer, we used a statistical approach

Table 1. The Significant Pathways (Only List Pathways with P-value Less Than 0.05)

pathway ID	name	p-value
hsa04140	Regulation of autophagy	0
hsa00785	Lipoic acid metabolism	0
hsa00604	Glycosphingolipid biosynthesis - ganglio series	0
hsa05211	Renal cell carcinoma	0
hsa04330	Notch signaling pathway	0
hsa05219	Bladder cancer	0
hsa00480	Glutathione metabolism	0
hsa05322	Systemic lupus erythematosus	0
hsa04012	ErbB signaling pathway	1.00E-05
hsa04062	Chemokine signaling pathway	2.00E-05
hsa04060	Cytokine-cytokine receptor interaction	3.00E-05
hsa03420	Nucleotide excision repair	6.00E-05
hsa00534	Glycosaminoglycan biosynthesis - heparan sulfate	0.00163
hsa04114	Oocyte meiosis	0.00354
hsa04310	Wnt signaling pathway	0.00658
hsa05218	Melanoma	0.00696
hsa00603	Glycosphingolipid biosynthesis - globo series	0.0156
hsa00460	Cyanoamino acid metabolism	0.01827
hsa00900	Terpenoid backbone biosynthesis	0.02236
hsa04920	Adipocytokine signaling pathway	0.02788
hsa04623	Cytosolic DNA-sensing pathway	0.03583
hsa04621	NOD-like receptor signaling pathway	0.03584
hsa03030	DNA replication	0.04396



pathway ID	name
hsa04062	Chemokine signaling pathway
hsa05218	Melanoma
hsa04330	Notch signaling pathway
hsa04060	Cytokine-cytokine receptor interaction
hsa04310	Wnt signaling pathway
hsa05322	Systemic lupus erythematosus
hsa04621	NOD-like receptor signaling pathway
hsa04114	Oocyte meiosis
hsa04140	Regulation of autophagy
hsa03420	Nucleotide excision repair
hsa04623	Cytosolic DNA-sensing pathway
hsa04012	ErbB signaling pathway

GO ID	name
GO:0006351	transcription, DNA-dependent
GO:0001666	response to hypoxia
GO:0030154	cell differentiation
GO:0045944	positive regulation of transcription from RNA polymerase II promoter
GO:0006955	immune response
GO:0006508	proteolysis
GO:0045087	innate immune response
GO:0006468	protein phosphorylation

Figure 1. Crosstalk Between Significant Pathways. The lines indicate that the two pathways are connected with the same GO-ID. The gray nodes stand for the significant pathways

on pathway level. The significance analysis of pathway is based on the protein-protein interaction database. The impact analysis method yielded many significant pathways containing regulation of autophagy, lipoic acid metabolism, glycosphingolipid biosynthesis-ganglio series, renal cell carcinoma, Notch signaling pathway and so on (Table 1). Table 1 shows significant pathways with p-value less than 0.05.

Crosstalk of GO relationships among pathways

We considered the pathway crosstalk between significant pathways detected by the overlapping GO-IDs. For detailed analysis of the crosstalk between pathways, we used the hypergeometric test to find the significant GO-ID in each pathway with the p-value less than 0.05, respectively. We found that these 12 pathways crosstalk with each other (Figure 1). The results of the top five GO-ID in part of the pathways are used to construct the connection among pathways. From the significant GO enrichments, we know the crosstalk of GO biological processes during the transcription and immune response among the pathways. In figure 1, We found that the Melanoma (hsa05218), Nucleotide excision repair (hsa03420) and Notch signaling pathway (hsa04330) crosstalk with the same GO ID response to hypoxia (GO:0001666). Notch signaling pathway (hsa04330) and Wnt signaling pathway (hsa04310) acted as the hub nodes in the figure crosstalk with the transcription, DNA-dependent (GO:0006351) and proteolysis (GO:0006508).

Discussion

In this work, we constructed a crosstalk network among ovarian cancer related pathways by integrating protein-protein ensemble interactions and KEGG pathways information. The crosstalk of pathways underlying their interaction significance was detected by corresponding gene transciptome information in ovarian cancer. Some of the identified crosstalks between the pathways are consistent with our knowledge for ovarian cancer. Some of them provide valuable alternatives for the mechanism of ovarian cancer, especially from the pathway relationship perspective. The interaction of these pathways provides more insights for the ovarian cancer progression. Besides, we also identified the biological processes enrichments underlying these interacted pathways. The GO functional linkages of these pathways provided more implications for their crosstalk.

In our network, several significant pathways were identified crosstalk with each other in ovarian cancer, such as chemokine signaling pathway, Notch signaling pathway, Wnt signaling pathway, NOD-like receptor signaling pathway and so on. Especially, hsa04330 (Notch signaling pathway), hsa04310 (Wnt signaling pathway) and has04062 (chemokine signaling pathway) are hub nodes which suggesting these pathways play an important role in the development of ovarian cancer.

Notch signaling pathways are known to regulate many cellular processes, including cell proliferation, apoptosis, migration, invasion, and angiogenesis, and is one of the most important signaling pathway during normal development (Wang et al., 2010). Notch signaling pathway has been associated with human cancers and proposed as potential therapeutic target for several types of cancer, including ovarian cancer (Rose, 2009; Rose et al., 2010). Classical activation of Notch signaling is triggered by ligation of Notch receptors and ligands which leads to proteolytic cleavage of Notch and the release of the Notch intracellular domain (NICD). NICD subsequently translocates to the nucleus and binds to the DNA-binding protein Rbp-j. This interaction results in assembly of a transcriptional activation complex that drives the expression of Notch target genes (Bray, 2006).

Chemokines are soluble factors shown to play important roles in regulating immune cell recruitment during inflammatory responses and defense against foreign pathogens (Zabel et al., 2006; De Paepe et al., 2008). It is clear that chemokines not only regulate cellular migration of immune cells, but also the migration, proliferation and survival signals in multiple cell types. New evidence shows that the chemokine signaling plays crucial functions in the cancer progression and indicate complex and diverse functions in the tumor microenvironment (Hembruff and Cheng, 2009). Especially, the imbalanced or aberrant expression of CXCL12 and its receptor CXCR4 strongly affects ovarian cancer cell proliferation, recruitment of immunosuppressive cells (Barbieri et al., 2010).

The Wnt signaling pathway participates in many physiologic events including cell fate specification, control of proliferation, and migration. Wnt signaling plays a key role in the embryonic development of the ovary. β-catenin which encoded by CTNNB1 gene, is the key effector in Wnt signaling pathway. Previous study demonstrates that mutations in the CTNNB1 gene are mostly found in

ovarian cancer (Gatcliffe et al., 2008).

The Wnt signaling has been reported to crosstalk with the Notch signaling pathway in several malignant cells. The Notch pathway represses Wnt signaling pathway during development and homeostasis by associating with and regulating the transcription of β-catenin (Hayward et al., 2005). Likewise, stimulation of the Wnt signaling pathway can antagonize Notch signaling through Disheveled (Axelrod et al., 1996). Evidence suggests that the two pathways cooperated in accelerating tumor initiation and progression. In hepatoblastomas, mutation in the CTNNB1 gene is commonly found in the presence of increased expression of the Notch ligand D1k1 (Adesina et al., 2009). Increased Wnt expression in breast epithelial cells triggers an oncogenic conversion through Notch activation (Ayyanan et al., 2006). The cooperation of Notch signaling pathway and Wnt signaling pathway is also seen in other tumors, such as colorectal tumors (van Es et al., 2005), prostate cancer (Moreno, 2010) and so on.

Additionally, it has been demonstrated that Wnt signaling is involved in chemokine signaling, regulating directional movement of benign and malignant cells. There is no direct evidence, to our knowledge, of the crosstalk between Notch signaling pathway and chemokine signaling pathway, though our analysis indicated their relationship. NF-$\varkappa\beta$ is an important regulator of the expression of several chemokines, and Notch-initiated signaling may thereby affect chemotaxis and cell trafficking (Bruserudet al., 2007; Bruserud and Kittang, 2010). Therefore, we suggested an indirect manner was present between them mediated by NF-$\varkappa\beta$ signaling pathway.

In this study, we identified the crosstalk among ovarian cancer related pathways and provided detailed analysis of the hub interactions in the crosstalk network. We also identified the biological processes enrichments underlying these interacted pathways. Many signaling pathways which play crucial roles in ovarian cancer were linked by our method. Results from these studies will provide the groundwork for a combination therapy approach targeting multiple pathways which will likely be more effective than targeting one pathway alone. In addition, our work showed that comprehensive and system-wide analysis provides complements for the traditional component-based approaches.

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