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Identification of Key Proteins in different stages of Hepatocellular carcinoma (HCC) compared to Normal Liver tissue with a Systems Biology approach

پژوهشکده ملی ژنتیک و زیست فناوری

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Abstract

Hepatocellular carcinoma is the most prevalent liver malignancy.in this study a mRNA microarray dataset was selected from GEO to evaluate differentially expressed genes (DEGs) between HCC and normal tissue, then biological significance of DEGs was identified using Enrichr and in order to identifying key proteins PPI network was drawn using string-dB andcytoscape.

as a result CCNA2,CDK1,TTK,SRPX and ESR1 proteins were identified as significant proteins in HCC that could be suggestions for more studies on them as therapeutic or diagnostic targets of HCC.

Keywords: Liver cancer, hepatocellular carcinoma, systems biology, biological network, bioinformatics

Introduction

Liver cancer is the sixth most common cancer worldwide, among different liver malignancies, hepatocellular carcinoma (HCC) is the most prevalent. Bad prognosis and increasing incidence rate of liver cancer leads to extensive studies on it.in this study with a holistic look to associated proteins in liver cancer in a network and utilizing bioinformatics tools to analyze them, key proteins of this disease that can be use as therapeutic or diagnostic targets, have been identified with a systems biology approach.

Materials and Methods

In this study, mRNA microarray dataset GSE101685, was downloaded from the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/) a total of 24 HCC tissues with 8 samples in each T1(a single tumor in liver, without vascular invasion),T3a(multiple large tumors),T3b(multiple large tumors with vascular invasion), stages and 8 normal control samples were defined in this dataset. for obtaining differentially expressed genes (DEGs) between HCC and normal tissue, samples analysis by statistical software R (3.6.1 https://www.r-project.org/) and packages of Bioconductor (http://www.bioconductor.org/) were applied and quality detection on microarray data was conducted by relative log expression (RLE) box plot, normalized box plot , removing unqualified samples, principal components plot (PCA) and clustering analysis diagram based on “gplots,” “ggplot2,” “affy,” “gplots,” and “pheatmap,” packages. In order to obtaining DEGs of HCC in 4 Groups with comprising expression level of intended proteins between normal tissue with each case gropus, common DEGs between case groups extracted by FunRich Version 3.1.3, then their IDs converted to gene official symbol using gprofiler(https://biit.cs.ut.ee/gprofiler/convert) the enrichment and pathway analysis For detection biological significance of DEGs including biological process, cellular component and molecular function was performed by Enrichr(https://amp.pharm.mssm.edu/Enrichr/). in order to identifying key proteins, Protein-protein interaction network was drawn using string-dB version 11.0(http://www.string-db.org/) and also constructed using cytoscape software version 3.7.1 and analyzed with cytoscape analysis tool and its plugins to determine significance of modules in HCC by *P* - values less than 0.05.and GO analysis applied by Molecular Complex Detection (MCODE), Biological Network Gene Ontology tool (BiNGO) and cluterONE also used for determining HUBs and bottleneck nodes in the network.

Results

Initial list of HCC associated proteins, was extracted from GSE101685 and after normalizing raw data and obtaining 256 DEGs genes (by fold change >2) consisting 148 down-regulated and 108 up-regulated genes in comparison with normal controls. PPI network with 256 nodes was drawn using string-dB unconnected nodes of this network were excluded and a new network with 185 nodes and 2329 edges was redrawn with average nodes degree: 25.2,average each node neighbors:28.57 ,network diameter:7 and then betweenness centrality and degree of each node was obtained, the PPI network was presented to Cytoscape analysis tool, for obtaining hub and bottleneck nodes and betweenness centrality. studying these indexes showed that CCNA1&2,CDK1, TTK, SPRX, ESR1 and CYP3A4 proteins have the most degrees and the betweenness centralities respectively. with a more precise evaluation using clusterONE plugin of cytoscape,The nodes clustered based on their combined score and the most two reliable clusters base on their P-value selected and their degree and betweenness centrality studied in their clusters, as a result in the first cluster, the results was as same as the main cluster, and in the second cluster with 14 nodes, the most degrees and the most betweenness centralities belonged to CYP3A1,CYP2E1,CYP1A1 and CYP3A4,CYP2E1 respectively. in GO enrichment analysis of DEGs as to biological process, the up-regulated DEGs enriched in cell population proliferation, epoxygenase p450 pathway and regulation of signaling and the down-regulated DEGs significantly enriched in response to apoptotic cell clearance, regulation of cell cycle and mitotic cell cycle checkpoint.

Discussion, Conclusion and Suggestions

among the presented proteins in the results, according to their frequency between the most significant proteins in the network of DEGs of mentioned GEO dataset based on the most degrees and betweenness centralities and common nodes between them, CCNA2 is supposed as the hub node of network and its well-known role in dividing somatic cells can prove its role in liver malignant neoplasia. CDK1and TTK protein has also key roles in controlling the progression of cells through cell division and tumor aggression respectively, so they can supposed significant proteins in HCC associated proteins network that could suggest them as good candidates for more studies on them as therapeutic targets. on the other hand up-regulation of SRPX and ESR1 before symptomatic cirrhosis could suggest them as a biomarker in virus related HCC that is the most significant etiology of liver malignancies.

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