Abstract

We constructed the high-expression ALK activated transport and signal network in human hepatocellular carcinoma (HCC) compared with low-expression (fold change ≥2) no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) in GEO data set, by using integration of gene regulatory activated and inhibited network inference method with gene ontology (GO) analysis. Our result showed that ALK transport and signal upstream network ECT2, FOLR1, GNAZ, GRM1, ITGA2, LEF1, NRS5A1, PTHLH, RIMS3, SORT1, SOX2 activated ALK, and downstream ALK-activated BAP1, CAD, CDH13, CNTNAP2, GRM1, ITGA2, LAPT4B, MAP2K6, NRS5A1, STMN1 in HCC. We obtained that the different biological processes of ALK activated network consisted of folic acid transport, cell surface receptor linked signal transduction, cell-cell signaling, G-protein coupled receptor protein signaling pathway, integrin-mediated signaling pathway, intracellular signaling cascade, low density lipoprotein mediated signaling, Rac protein signal transduction, Rho protein signal transduction in HCC compared with the activated network of no-tumor hepatitis/cirrhotic tissues, as a result of inducing folic acid transport and integrin signal induced-angiogenesis in HCC. Our hypothesis was verified by the different and the same biological processes of ALK activated transport and signal network of HCC compared with the corresponding inhibited network of no-tumor hepatitis/cirrhotic tissues and HCC, respectively.

Keywords: ALK; Human hepatocellular carcinoma (HCC); Folic acid transport and integrin signal induced-angiogenesis network; Systems-theoretical analysis.

Introduction


Folic acid transport, integrin signal and lipoprotein are involved in angiogenesis. Such as, Folic-acid-mediated inhibition of human colon-cancer cell growth [1]; The Foxc2 transcription factor modulates angiogenesis via induction of integrin beta3 expression [2]; alphavbeta3 integrin and a moody integrin angiogenesis in a changing environment [3]; Active tumor targeting of nonmaterial’s using folic acid, integrin receptors and transferring [4]; Role of tetraspanin CD151-alphav/alph6 integrin complex in angiogenesis [5]; Integrin affinity modulation in angiogenesis [6]; RECK function of ss1-integrin-dependent in physiologic and tumor angiogenesis [7]; Activation of Ras/MAP kinase is required in high density lipoprotein-induced angiogenesis in human coronary artery endothelial cells [8]; Collateral formation Impairment in lipoprotein(a) transgenic mice therapeutic angiogenesis induced by human hepatocyte growth factor gene [9]; Homocysteine and folic acid effects on angiogenesis and VEGF expression during chicken vascular development [10]; Pharmacological inhibition of integrin alphavbeta3 aggravates experimental liver fibrosis and inhibits hepatic angiogenesis [11]; Lipoprotein contributes to angiogenesis on the chick embryo chorioallantoic membrane [12]; Interaction of alphavbeta1 integrin with thrombospondin-1 promotes angiogenesis [13]; Reconstituted high-density lipoprotein enhances differentiation of endothelial progenitor cells and stimulates ischemia-induced angiogenesis [14]; Angiogenesis requires Beta1 integrin expression on endothelial cells but not vasculogenesis [15]; Integrin alpha9beta1 directly binds to vascular endothelial growth factor (VEGF)-A and induces VEGF-A-induced angiogenesis [16]; A novel mediator of ovarian angiogenesis follicular fluid high density lipoprotein-associated sphingosine 1-phosphate [17]; Integrin-linked kinase modulates melanoma angiogenesis by activating NF-kappaB/interleukin-6 signaling pathway [18]; Relationship between oxidized lipoprotein, human coronary atherosclerotic plaque stabilization and angiogenesis [19]; Possible roles for folic acid in the modulation of trophoblast invasion and placental development in normal early human pregnancy [20]; Alpha(5)beta(1) integrin ligand PHSRN contributes invasion and alpha(5) mRNA in endothelial cells to stimulate angiogenesis [21]. Yet the distinct high-expression ALK folic acid transport and integrin signal induced-angiogenesis network in HCC remains to be elucidated. Here we constructed the high-expression ALK activated transport and signal network in HCC from GEO data set by gene regulatory network inference method based on linear programming and decomposition procedure.

In this study, we constructed ALK up- and down-stream activated and inhibited transport and signal network in no-tumor hepatitis/cirrhotic tissues and HCC. The biological process and data analysis of...
the low- and high-expression ALK transport and signal network was done in no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) and HCC by GO database. By comparison with the same and different gene ontology (GO) of ALK activated and inhibited transport and signal network between no-tumor hepatitis/cirrhotic tissues and HCC, we put forward hypothesis of ALK activated transport and signal network of inducing folic acid transport and integrin signal induced-angiogenesis in HCC.

**Materials and Methods**


Significant expressed genes of ALK transport and signal network were identified using significant analysis of microarrays (SAM) (http://wwwstat.stanford.edu/~tibs/SAM/) [22]. We selected two classes unpaired and minimum fold change ≥2 and chose the significant highly expressed value genes of HCC compared with that of no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) under the false-discovery rate and q-value were 0%. The q-value is like the well-known P-value, but adapted to multiple-testing situations.

ALK transport and signal network was constructed based on GRNInfer and GVedit tools (http://www.graphviz.org/About.php). GRNInfer is a novel mathematic method called GNR (Gene Network Reconstruction tool) based on linear programming and a decomposition procedure for inferring gene network [23]. We established ALK activated network of HCC based on the fold change ≥2 distinguished genes and selected parameters as lambda 0.0 because we used one data set. Lambda was a positive parameter which balanced the matching and sparsity terms in the objective function. Using different thresholds, we could predict various networks with the different edge density. The threshold parameters make the edge whose strength of link is smaller than threshold not shown in the network graph. The smaller this parameter, the more edges in the network graph. We selected threshold 1.0e-7.


**Results**

We constructed ALK up- and down-stream activated and inhibited transport and signal network in no-tumor hepatitis/cirrhotic tissues and HCC from our total network of 225 significant high-expression molecules (fold change ≥2) from 6,144 genes of 25 HCC compared with 25 no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) by GRNInfer, respectively.

We extracted the biological process of GO terms and did numbers data analysis of the different biological processes of ALK activated transport and signal network in HCC compared with activated network of no-tumor hepatitis/cirrhotic tissues, the same biological processes of ALK activated transport and signal network in HCC compared with

![Figure 1: ALK upstream activated transport and signal network in HCC by GRNInfer. Arrowhead represents activation relationship.](image1.png)

![Figure 2: ALK downstream activated transport and signal network in HCC by GRNInfer. Arrowhead represents activation relationship.](image2.png)
inhibited network of no-tumor hepatitis/cirrhotic tissues, as shown in Table 1. GO terms and numbers data was analyzed the different biological processes of ALK activated compared with inhibited transport and signal network in HCC, as shown in Table 2.

ALK activated transport and signal network was constructed in HCC. Our result showed that upstream ECT2, FOLR1, GNAZ, GRM1, ITGA2, LEF1, NR5A1, PTHLH, RIMS, SORT1, SOX2 activated ALK, and the downstream ALK-activated BAP1, CAD, CDH13, CNTNAP2, GRM1, ITGA2, LAPTM4B, MAP2K6, NR5A1, STMN1 in HCC, as shown in Figure 1 and Figure 2.

Discussion

Our aim is to construct, interpret, verify and predict the function of novel high-expression ALK folic acid transport and integrin signal induced-angiogenesis network in HCC. We have already constructed and analyzed some novel molecular network from different databases presented in our articles [24-37]. In this study, we constructed ALK up- and down-stream activated and inhibited transport and signal network in no-tumor hepatitis/cirrhotic tissues and HCC. The biological process and data analysis of the low- and high-expression ALK transport and signal network was done in no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) and HCC by GO database. By comparison with the same and different gene ontology (GO) of ALK activated and inhibited transport and signal network between no-tumor hepatitis/cirrhotic tissues and HCC, we obtained that the different biological processes of ALK activated network consisted of folic acid transport, cell surface receptor linked signal transduction, cell-cell signaling, G-protein coupled receptor protein signaling pathway, integrin-mediated signaling pathway, intracellular signaling cascade, low density lipoprotein mediated signaling, Rac protein signal transduction, Rho protein signal transduction in HCC compared with inhibited network of no-tumor hepatitis/cirrhotic tissues, as a result of inducing folic acid transport and integrin signal induced-angiogenesis in HCC.

We extracted the biological process of GO terms and did numbers data analysis of the different biological processes of ALK activated transport and signal network in HCC compared with activated network of no-tumor hepatitis/cirrhotic tissues, the same biological processes of ALK activated transport and signal network in HCC compared with inhibited network of no-tumor hepatitis/cirrhotic tissues (Table 1 and Table 2). We constructed the high-expression ALK activated transport and signal network in human hepatocellular carcinoma (HCC) compared with low-expression (fold change ≥2) no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) in GEO data set using integration of gene regulatory network inference method. Our result showed that ALK transport and signal upstream network ECT2, FOLR1, GNAZ, GRM1, ITGA2, LEF1, NR5A1, PTHLH, RIMS3, SORT1, SOX2 activated ALK, and downstream ALK-activated BAP1, CAD, CDH13, CNTNAP2, GRM1, ITGA2, LAPTM4B, MAP2K6, NR5A1, STMN1 (Figure 1 and Figure 2) in HCC.

By further comparison with the same and different gene ontology (GO) of ALK activated and inhibited transport and signal network between no-tumor hepatitis/cirrhotic tissues and HCC, we obtained that the different biological processes of ALK activated network consisted of folic acid transport, cell surface receptor linked signal transduction, cell-cell signaling, G-protein coupled receptor protein signaling pathway, integrin-mediated signaling pathway, intracellular signaling cascade, low density lipoprotein mediated signaling, Rac protein signal transduction, Rho protein signal transduction in HCC compared with activated network of no-tumor hepatitis/cirrhotic tissues, as a result of inducing folic acid transport and integrin signal induced-angiogenesis in HCC.

The same biological processes of ALK activated network included transport, cell surface receptor linked signal transduction, cell-cell signaling, G-protein signaling, signal transduction in HCC compared with inhibited network of no-tumor hepatitis/cirrhotic tissues. It is consistent with the different biological processes of ALK activated transport and signal network of HCC compared with activated network of no-tumor hepatitis/cirrhotic tissues. The different biological processes of ALK activated network consisted of endosome to lysosome transport, endosome transport via multivesicular body sorting pathway, folic acid transport, Golgi to

Table 1: GO Terms and numbers data analysis of the different biological processes of ALK activated transport and signal network of no-tumor hepatitis/cirrhotic tissues, the same biological processes of ALK activated transport and signal network of HCC compared with inhibited network of no-tumor hepatitis/cirrhotic tissues.

<table>
<thead>
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<th>Terms</th>
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<tr>
<td>folic acid transport</td>
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<td>Rho protein signal transduction</td>
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<td>cell-cell signaling</td>
<td>3</td>
<td>low density lipoprotein mediated signaling</td>
<td>1</td>
</tr>
<tr>
<td>integrin-mediated signaling pathway</td>
<td>2</td>
<td>cell surface receptor linked signal transduction</td>
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<td>intracellular signaling cascade</td>
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<td>G-protein coupled receptor protein signaling pathway</td>
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<td>Rac protein signal transduction</td>
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The Same ALK Activated Transport and Signal Network of HCC compared with Inhibited Network of No-tumor Hepatitis/cirrhotic Tissues

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<td>transport</td>
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<tr>
<td>G-protein signaling</td>
<td>1</td>
<td>signal transduction</td>
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<tr>
<td>cell-cell signaling</td>
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Table 2: GO Terms and numbers data analysis of the different biological processes of ALK activated transport and signal network of HCC compared with inhibited network of no-tumor hepatitis/cirrhotic tissues.

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<th>Terms</th>
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<tr>
<td>endosome to lysosome transport</td>
<td>1</td>
<td>intracellular signaling cascade</td>
<td>3</td>
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<tr>
<td>folic acid transport</td>
<td>1</td>
<td>integrin-mediated signaling pathway</td>
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<td>Golgi to endosome transport</td>
<td>1</td>
<td>Rac protein signal transduction</td>
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<tr>
<td>plasma membrane to endosome transport</td>
<td>1</td>
<td>Rho protein signal transduction</td>
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<tr>
<td>endosome transport via multivesicular body sorting pathway</td>
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<td>low density lipoprotein mediated signaling</td>
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<tr>
<td>cell surface receptor linked signal transduction</td>
<td>1</td>
<td>induction of apoptosis by extracellular signals</td>
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endosome transport, plasma membrane to endosome transport, cell surface receptor linked signal transduction, induction of apoptosis by extracellular signals, integrin-mediated signaling pathway, intracellular signaling cascade, low density lipoprotein mediated signaling, Rac protein signal transduction, Rho protein signal transduction in HCC compared with inhibited network of HCC. It is consistent with the different biological processes of ALK activated transport and signal network of HCC compared with activated network of no-tumor hepatitis/cirrhotic tissues, the same biological processes of ALK activated transport and signal network of HCC compared with inhibited network of no-tumor hepatitis/cirrhotic tissues, respectively.

Therefore, our ALK activated folic acid transport and integrin signal induced-angiogenesis hypothesis was verified by the different and the same biological processes of ALK activated transport and signal network of HCC compared with the corresponding inhibited network of no-tumor hepatitis/cirrhotic tissues and HCC, respectively.

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References


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