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Differential Differentiation- and Survival and Invasion-related T-/H-cadherin (*CDH13*) Computational Downsteam Network from No-Tumor Hepatitis/Cirrhosis (HBV or HCV infection) to Human Hepatocellular Carcinoma (HCC) Malignant Transformation

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Abstract

We constructed and analyzed the low- and high-expression (fold change ≥2) different-activated and -inhibited T-/H-cadherin (*CDH13*) downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation in GEO data set by integration of gene regulatory network inference method based on linear programming and decomposition procedure with GO database. Our results show that the low-expression *CDH13* downstream network has the multi-activated and -inhibited molecular pattern in no-tumor hepatitis/cirrhosis, whereas high-expression *CDH13* downstream mainly somewhat inhibited molecular connections but significant reduced network (fold ≥2) in HCC. We suppose that the low-expression *CDH13* downstream network mainly activates cell differentiation cell adhesion, but inhibits nuclear chromosome, mitosis in no-tumor hepatitis/cirrhosis, whereas the high-expression *CDH13* downstream network activates Rab-protein geranylgeranyltransferase activity, protein modification, but inhibits modification-dependent protein catabolism and nucleotide binding in HCC. We put forward hypothesis that low-expression *CDH13* activates cadherin binding, homophilic cell adhesion, negative regulation of cell adhesion, positive regulation of calcium-mediated signaling, calcium-dependent cell-cell adhesion, positive regulation of cell-matrix adhesion, low density lipoprotein mediated signaling and inhibits regulation of endothelial cell proliferation, positive regulation of smooth muscle cell proliferation, keratinocyte proliferation, as a result of inducing differentiation in no-tumor hepatitis/cirrhosis, whereas high-expression *CDH13* activates positive regulation of survival gene product activity, protein homodimerization activity, Rho protein signal transduction, Rac protein signal transduction, positive regulation of cell migration, sprouting angiogenesis, positive regulation of positive chemotaxis, epidermal growth factor receptor signaling pathway, endothelial cell migration, lamellipodium biogenesis, and inhibits regulation of endocytosis, caveola, as a result of inducing survival and invasion in HCC. Our inferences are consistent with different-activated and -inhibited *CDH13* downstream network, GO database and literatures, respectively.

Keywords: T-/H-cadherin *(CDH13)* computational network; no-tumor hepatitis/cirrhosis; HCC; malignant transformation; differentiation; survival and invasion

Introduction

T-/H-cadherin (*CDH13*) is our identified significant higher expression gene (fold change ≥2) in Human Hepatocellular Carcinoma (HCC) compared with no-tumor hepatitis/cirrhosis (HBV or HCV infection) from GEO data set GSE10140-10141 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10140,http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10141). Malignant transformation of no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC is associated with inflammation, proliferation and invasion. Such as some references as follows: oval cells are liver stem cells involved in liver regeneration following liver damage, oval cells develop and proliferate in a model of experimental liver fibrosis [1]; High rates of hepatocellular carcinoma in cirrhotic patients with high liver cell proliferative activity [2]; Hepatocyte proliferation and risk of hepatocellular carcinoma in cirrhotic patients [3]; A cytokine cascade including IL-6 may participate in hepatic stellate cell proliferation in Liver Cirrhosis (LC) patients [4]; Hepatocyte proliferative activity in human liver cirrhosis [5]; A high hepatocyte proliferation rate is a major risk factor for hepatocellular carcinoma development in the cirrhotic liver [6]; Greater proliferative activity in the epithelial cells of inflamed odontogenic keratocysts is associated with the disruption of the typical structure of odontogenic keratocyst linings [7]; Acute inflammation of the proliferative zone of gastric mucosa in Helicobacter pylori gastritis [8]; Malignant transformation of proliferative verrucous leukoplakia to oral squamous cell carcinoma [9]; Coincidental acquisition of growth autonomy and metastatic potential during the malignant transformation of factor-dependent CCL39 lung fibroblasts [10]; Eyelid metastasis from mediastinal teratoma with malignant transformation [11]; Malignant transformation of an abdominal inflammatory myofibroblastic tumor with distant metastases in a child [12]; Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence [13]; Role of Pin1 in UVA-induced cell proliferation and malignant transformation in epidermal cells [14]; Increased p53 expression in the malignant transformation of Barrett's esophagus is accompanied by an upward shift of the proliferative compartment [15];

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Expression of proliferating cell nuclear antigen (PCNA) in proliferative phase functions and malignant transformation of melanocytes [16]; Cell proliferation, apoptosis, and apoptosis inhibition in malignant transformation of sinonasal inverted papilloma [17]; Malignant transformation of recurrent meningioma with pulmonary metastases [18]; Proliferative verrucous leukoplakia and malignant transformation [19]; Malignant transformation of a benign enchondroma of the hand to secondary chondrosarcoma with isolated pulmonary metastasis [20]; Proliferative potential and malignant transformation of ganglioglioma by MIB-1 and p53 staining [21]; Intramedullary spinal cord metastasis following spontaneous malignant transformation from giant cell tumor of bone 16 years after pulmonary metastasis [22]. And also, different molecular concentrations are responsible for different functions; the same molecule together with different molecules will have different functions. Yet the distinct low- and high-expression *CDH13* downstream networks from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation remain to be elucidated.

Hepatocellular carcinoma as the most common primary malignancy of the liver accounts for as many as one million deaths annually worldwide [23]. So to develop novel drugs in HCC has become a challenge for biologists. And also, the mechanisms that shut off a signal are as important as the mechanisms that turn it on. Here, we constructed the low- and high-expression activated and inhibited *CDH13* downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation in GEO data set by gene regulatory network inference method based on linear programming and decomposition procedure.

In this study, we are planning to identify significant high-expression molecules of HCC by gene selection algorithms, establish and compute *CDH13* downstream network between low- and high-expression *CDH13* downstream network of no-tumor hepatitis/cirrhosis (HBV or HCV infection) and HCC by GRNInfer, interpret *CDH13* by molecule annotation system, put forward hypothesis and also find different evidences from literatures to support our inferences.

Materials and Methods

Microarray data

We used microarrays containing 6,144 genes from 25 no-tumor hepatitis/cirrhotic tissues and 25 HCC patients in GEO data set GSE10140-10141(http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10140,http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10141). We preprocessed raw microarray data as log2.

Gene selection algorithms

Potential HCC molecular markers were identified using Significant Analysis of Microarrays (SAM) (http://wwwstat.stanford.edu/~tibs/SAM/) [24]. We normalized data by log2, selected two classes unpaired and minimum fold change ≥2 and chose the significant higher expression value genes of HCC compared with that of no-tumor hepatitis/cirrhosis 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.

Unsupervised Clustering

Significant higher expression genes from no-tumor hepatitis/cirrhosis versus HCC were done by cluster 3.0 (http://bonsai.ims.tokyo.ac.jp/~mdehoon/software/cluster). The steps were as follows: *Step 1* loading and filtering 100% data, *Step 2* normalizing log transform data for adjusting data, *Step 3* choosing gene culster and array cluster; *Step 4* choosing average linkage of hierarchical clustering, *Step 5* doing TreeView.

Network establishment of candidate genes

CDH13 downstream network was constructed using 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 networks [25]. The method theoretically ensures the derivation of the most consistent network structure with respect to all of the data sets, thereby not only significantly alleviating the problem of data scarcity but also remarkably improving the reconstruction reliability [25]. The following Eq.1 represents all of the possible networks for the same data set.

$$J = (X' - A)U\Lambda^{-1}V^T + YV^T = \hat{J} + YV^T \quad (1)$$

where $J = (J_{ij})_{n\times n} = \partial f(x)/\partial x$ is an n×n Jacobian matrix or connectivity matrix, $X= (x(t_1),\ldots,x(t_m))$, $A=(a(t_1),\ldots,a(t_m)$ *and* $X'=(x'(t_1),\ldots,x'(t_m))$ are all $n\times m$ matrices with $x'_i(t_j)=[x_i(t_j+1)-x_i(t_j)]/[t_{j+1}-t_j]$ *for* $i=1,\ldots,n;\ j=1,\ldots,m$. $X(t)=(x_1(t),\ldots,x_n(t))^T\in R_n$, $a=(a_1,\ldots,a_n)^T\in R_n$, $x_i(t)$ is the expression level (mRNA concentrations) of gene i at time instance t. $y=(y_{ij})$ is an $n\times n$ matrix, where y_{ij} is zero if $ej\neq 0$ and is otherwise an arbitrary scalar coefficient. $\wedge^{-1}=diag\ (1/e_i)$ and $1/e$ is set to be zero if $e_i=0$. U is a unitary $m\times n$ matrix of left eigenvectors, $\wedge=diag\ (e_1,\ldots,e_n)$ is a diagonal $n\times n$ matrix containing the n eigenvalues and V^T is the transpose of a unitary $n\times n$ matrix of right eigenvectors [25]. We established *CDH13* network 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 different edge density.

In order to candidate *CDH13* downstream network, we first setup the total network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation, respectively, by using GRNInfer as follows: *Step 1* format microarray data set into desired datafile, *Step 2* open the datafile by clicking the "open" button and browsing the datafile location, *Step 3* choose proper parameters. There are two parameters you can choose to provide by altering the default value: (i) *Lambda:* This parameter is used in the inferring algorithm to adjust the spars structure of the network. The default value is 0.0. (ii) *Threshold*: This parameter is used in the control output file GRN.dot, which can be visualized by the Neato tool of software Graphviz. 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 1.0*e*-6. *Step 4* computing by clicking the "Infer" button when the datafile and parameters are ready. *Step 5* checking the results.

Molecule annotation system

Molecule Annotation System, MAS (CapitalBio Corporation, Beijing, China; http://bioinfo.capitalbio.com/mas3/) is a Web-based software toolkit for a whole data mining and function annotation solution to extract and analyze biological molecules relationships from public databases. MAS uses relational database of biological networks created from millions of individually modeled relationships between genes, proteins, complexes, cells, disease and tissues. MAS allows a view on your data, integrated in biological networks according to different kinds of biological context. This unique feature results from

multiple lines of evidences that are integrated in MASCORE. MAS helps to understand the relationship of gene expression data through the given molecular symbols list and provides thorough, unbiased and visible results. The primary databases of MAS integrated various well-known biological resources, such as Gene Ontology (http://www.geneontology.org), KEGG (http://www.genome.jp/kegg/), BioCarta (http://www.biocarta.com/), GenMapp (http://www.genmapp.org/), HPRD (http://www.hprd.org/), MINT (http://mint.bio.uniroma2.it/mint/Welcome.do), BIND (http://www.blueprint.org/), Intact (http://www.ebi.ac.uk/intact/), UniGene (www.ncbi.nlm.nih.gov/UniGen), OMIM (http://www.ncbi.nlm.nih. gov/entrez/query.fcgi?db=OMIM) and disease ((http://bioinfo.capitalbio.com/mas3/). MAS offers various query entries and graphics result. The system represents an alternative approach to mining biological signification for high-throughput array data.

The same used algorithm is *P, Q* value in GO and pathway of module. In the actual analysis, we need further screening process for a certain *P* value threshold to obtain the significance of GO/pathway of the false positive rate, namely, False Discovery Rate (FDR). So it is more convenient to give a *Q* value for each *P* value to reflect the FDR as *P* value threshold. The *Q* threshold value is supposed to be 0.05. If it is less than 0.05, then it means the *P* value as the significance level of the false positive rate is lower. The *P* value is smaller indicating that protein (or gene) in the GO (or pathway) is more significantly enriched.

The algorithm used to calculate the *P* value in GO and pathway of module is hypergeometric distribution.

$$p = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

Where *N* refers to the number of genes (genome-wide gene universe) of a species included in the MAS library, *M* (the intersection of *M* and *n*) is the number of genes contained in the specific pathway. p0=*m/M,* p1=*n/N*. *P* value is the probability value of the false negative null hypothesis H0: p0 = p1. When *P* value is less than 0.05, it means differentially expressing genes were significantly enriched in this pathway.

Results

Identification of significant high-expression molecules of HCC by Gene Selection algorithms

We obtained 225 significant high-expression molecules (fold change ≥2) from 6,144 genes of 25 HCC vs 25 no-tumor hepatitis/cirrhosis in the same GEO data set GSE10140-10141 by using significant analysis of microarrays (SAM) (http://wwwstat.stanford.edu/~tibs/SAM/) [24]. We normalized data by log2, selected two classes unpaired and minimum fold change ≥2 and chose the significant higher expression value genes of HCC compared with that of no-tumor hepatitis/cirrhosis

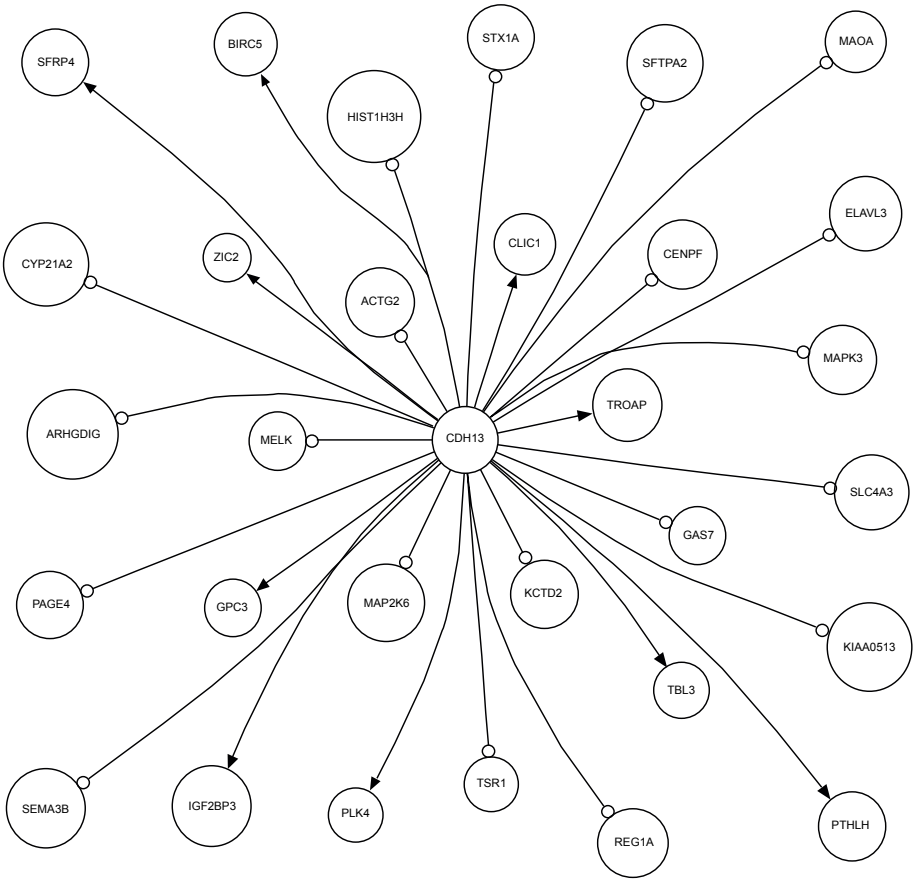

Figure 1: Activated and inhibited downstream network of low-expression *CDH13* in no-tumor hepatitis/cirrhosis by GRNInfer. Arrowhead represents activation relationship and empty cycle represents inhibition relationship.

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. *CDH13* relative fold changes in expression of HCC were compared with that of no-tumor hepatitis/cirrhosis, as shown in Table 2.

Establishment and Molecular Numbers Computation of *CDH13* Downstream Network in No-tumor Hepatitis/cirrhotic Tissues (HBV or HCV infection) and HCC by GRNInfer

First, we setup total low- and high-expression *CDH13* downstream network from our constructed total network from no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) to HCC malignant transformation by GRNInfer separately. Second, we identified *CDH13* activation and inhibition downstream network in no-tumor hepatitis/cirrhosis (HBV or HCV infection) and HCC. Third, we further extracted the different novel molecules between low- and high-expression *CDH13* downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation. Our results show *CDH13*-activated *SFRP4, TROAP, BIRC5, PLK4, IGF2BP3, CLIC1, ZIC2, GPC3, PTHLH, TBL3* and *CDH13*-inhibited *HIST1H3H, CENPF, SLC4A3, MELK, SEMA3B, KIAA0513, ARHGDIG, ACTG2, CYP21A2, SFTPA2, KCTD2, MAPK3, ELAVL3, REG1A, MAP2K6, STX1A, MAOA, GAS7, PAGE4, TSR1* in no-tumor hepatitis/cirrhosis; whereas *CDH13*-activated *RABGGTA* and *CDH13*-inhibited *RBCK1, RBM34, LTBP2, SORT1, ST6GALNAC, TPSD1, WDR1, CBX5, KATNB1, NINJ2, DMN, LOX* in HCC, as shown in Figure 1 and 2. Fourth, we computed different activation and inhibition numbers of low- and high-expression *CDH13* downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation. Our results show that the low-expression *CDH13* downstream network has #9 molecular pattern in no-tumor hepatitis/cirrhosis, whereas high-expression *CDH13* downstream #10 in HCC, as shown in Table 1.

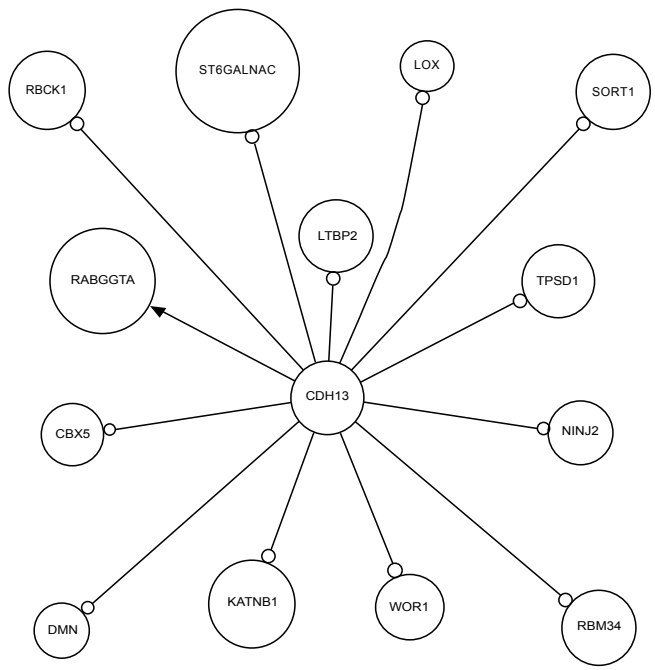

Figure 2: Activated and inhibited downstream network of high-expression *CDH13* in HCC by GRNInfer. Arrowhead represents activation relationship and empty cycle represents inhibition relationship.

Gene	con(act)	con(inh)	exp(act)	exp(inh)	con（act）/ ex（act）	con（inh）/ ex（inh）
CDH13	10	20	1	12	10	1.7

Table 1: Activated and inhibited molecular numbers and fold between low- and high-expression *CDH13* downstream network between HCC and no-tumor hepatitis/cirrhosis. Con represents no-tumor hepatitis/cirrhosis; ex represents HCC; act represents activation relationship; inh represents inhibition.

Interpretation of *CDH13* by Molecule Annotation System

We interpreted *CDH13* cellular component, molecular function, biological process and pathway, etc by using Gene Ontology, KEGG, BioCarta, GenMapp, as shown in Table 2.

Discussion

Our aim is to construct, interpret and verify novel different low- and high-expression *CDH13* downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation. We have already constructed some novel molecular networks from different databases and analyzed significance of our work presented in our articles [26-33]. Such as, we inferred *BIRC5* cell cycle module more mitosis but less complex-dependent proteasomal ubiquitin-dependent protein catabolism, as a result of increasing cell division and cell numbers in no-tumor hepatitis/cirrhosis; more protein amino acid autophosphorylation but less negative regulation of ubiquitin ligase activity during mitotic cell cycle, as a result of increasing growth and cell volume in HCC [34]. In this study, we identified significant high-expression molecules of HCC by gene selection algorithms, and constructed the low- and high-expression different-activated and -inhibited *CDH13* downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation in GEO data set by gene regulatory network inference method based on linear programming and decomposition procedure. We further computed different molecular numbers of *CDH13* downstream network, interpreted *CDH13* by molecule annotation system. We put forward hypothesis and also found different evidences from literatures to support our inferences.

To verify whether our identified genes could separate two samples groups (no-tumor hepatitis/cirrhosis versus HCC), we did average linkage of hierarchical clustering. Our results show that the most of control group (no-tumor hepatitis/cirrhosis) clustered together and the most of experiment group (HCC) as well. Two groups were observed red colors reflecting all the upregulation including *CDH13* and no missing data in both no-tumor hepatitis/cirrhosis (HBV or HCV infection) and HCC (figure already used before by us).

Why did we choose *CDH13* downstream network? *CDH13* upstream network includes many other molecules and functions. And also, *CDH13* upstream molecules effect on *CDH13*, whereas *CDH13*can not effect on its upstream molecules. *CDH13* downstream network focuses on *CDH13* functions itself. Therefore, we chose *CDH13* downstream network.

Low-expression *CDH3* dDownstrem nNetwok aAnalysis n no-tTumr hHepatits/cCirrhosis

We identified and computed the different novel molecules and numbers of low-expression *CDH13* downsteam network in no-tumor hepatitis/cirrhosis (HBV or HCV infection) compared with HCC. Our results show *CDH13*-activated *SFRP4, TROAP, BIRC5,*

PLK4, IGF2BP3, CLIC1, ZIC2, GPC3, PTHLH, TBL3 and *CDH13*-inhibited *HIST1H3H, CENPF, SLC4A3, MELK, SEMA3B, KIAA0513, ARHGDIG, ACTG2, CYP21A2, SFTPA2, KCTD2, MAPK3, ELAVL3, REG1A, MAP2K6, STX1A, MAOA, GAS7, PAGE4, TSR1* in no-tumor hepatitis/cirrhosis (Fiure.1). The low-expression *CDH13* downstream network has #9 molecular pattern in no-tumor hepatitis/cirrhosis (Table1). We analyzed several different-activated and -inhibited molecules of cellular component, molecular function, and biological process in low-expression *CDH13* network of no-tumor hepatitis/cirrhosis by GO database. Such as, activated *SFRP4* is relevant to extracellular region, extracellular space, protein binding, signal transduction, embryo implantation, Wnt receptor signaling pathway, cell differentiation (GO database); Activated *TROAP* is concerned with cytoplasm, protein binding, cell adhesion (GO database); Inhibited *HIST1H3H* is involved in nucleosome, nucleus, chromosome, DNA binding, protein binding, nucleosome assembly (GO database); Inhibited *CENPF* contains chromatin, spindle pole, outer kinetochore of condensed chromosome, nucleus, nuclear envelope, chromosome, cytoplasm, nuclear matrix, chromatin binding, protein C-terminus binding, transcription factor binding, protein homodimerization activity, dynein binding, protein heterodimerization activity; G2 phase of mitotic cell cycle, mitosis, mitotic spindle checkpoint, cell proliferation, regulation of striated muscle development, negative regulation of transcription, response to drug, cell division, metaphase plate congression, kinetochore assembly (GO database). We suppose that the low-expression *CDH13* downstream network mainly activates cell differentiation, cell adhesion, but inhibits nuclear chromosome, mitosis, as a result of inducing differentiation in no-tumor hepatitis/cirrhosis

High-expression *CDH3* dDownstrem nNetwok aAnalysis in HCC

We identified and computed the different novel molecules and numbers of high-expression *CDH13* downstream network in HCC compared with no-tumor hepatitis/cirrhosis. Our results show *CDH13*-activated *RABGGTA*, and *CDH13*-inhibited *RBCK1, RBM34, LTBP2, SORT1, ST6GALNAC, TPSD1, WDR1, CBX5, KATNB1, NINJ2, DMN, LOX* in HCC (Figre .2). The high-expression *CDH13* downstream has #10 in HCC (Table1). We analyzed serveral different-activated and -inhibited molecules of cellular component, molecular function, and biological process of high-expression *CDH13* network in HCC by GO database. Such as, activated *RABGGTA* is relevant to Rab-protein geranylgeranyltransferase activity, protein binding, zinc ion binding, protein prenyltransferase activity, transferase activity,

Proteins	Fold（HCC versus hepatitis/ cirrhosis）by SAM	Cellular Component	Molecular Function	Biological Process	KEGG	GenMAPP	BioCarta
CDH13	2.9	extracellular space, cytoplasm, plasma membrane, caveola, external side of plasma membrane, anchored to membrane, neuron projection	calcium ion binding, low-density lipoprotein binding, protein homodimerization activity, cadherin binding, adiponectin binding	positive regulation of endothelial cell proliferation, positive regulation of cell-matrix adhesion, sprouting angiogenesis, homophilic cell adhesion, negative regulation of cell adhesion, Rho protein signal transduction, negative regulation of cell proliferation, calcium-dependent cell-cell adhesion, Rac protein signal transduction, lamellipodium biogenesis, regulation of endocytosis, positive regulation of cell migration, regulation of epidermal growth factor receptor signaling pathway, endothelial cell migration, keratinocyte proliferation, positive regulation of survival gene product activity, positive regulation of smooth muscle cell proliferation, positive regulation of calcium-mediated signaling, positive regulation of positive chemotaxis, localization within membrane, low density lipoprotein mediated signaling	none	cell-cell adhesion	none

Table 2: *CDH13* relative fold changes in expression of HCC versus no-tumor hepatitis/cirrhosis by SAM and interpretation by molecule annotation system.

metal ion binding, protein modification, visual perception, protein geranylgeranylation, protein amino acid prenylation (GO database); Inhibited *RBCK1* is involved in intracellular, protein binding, zinc ion binding, metal ion binding, protein modification, modification-dependent protein catabolism, interspecies interaction between organisms (GO database); Inhibited *RBM34* contains nucleus, nucleotide binding, RNA binding (GO database). We suppose that the high-expression *CDH13* downstream network mainly activates Rab-protein geranylgeranyltransferase activity, protein modification, but inhibits modification-dependent protein catabolism, nucleotide binding, as a result of inducing survival and invasion in HC.

CDH1 GO aAnalysis beteennNo-tTmor hHepattis/cCirrhosis and HCC

We analyzed *CDH13* cellular component, molecular function, and biological process by GO database. *CDH13* is related to extracellular space, cytoplasm, plasma membrane, caveola, external side of plasma membrane, anchored to membrane, neuron projection, calcium ion binding, low-density lipoprotein binding, protein homodimerization activity, cadherin binding, adiponectin binding, positive regulation of endothelial cell proliferation, positive regulation of cell-matrix adhesion, sprouting angiogenesis, homophilic cell adhesion, negative regulation of cell adhesion, Rho protein signal transduction, negative regulation of cell proliferation, calcium-dependent cell-cell adhesion, Rac protein signal transduction, lamellipodium biogenesis, regulation of endocytosis, positive regulation of cell migration, regulation of epidermal growth factor receptor signaling pathway, endothelial cell migration, keratinocyte proliferation, positive regulation of survival gene product activity, positive regulation of smooth muscle cell proliferation, positive regulation of calcium-mediated signaling, positive regulation of positive chemotaxis, localization within membrane, low density lipoprotein mediated signaling (GO database) (Table2). We put forward hypothesis that low-expression *CDH13* activates cadherin binding, homophilic cell adhesion, negative regulation of cell adhesion, positive regulation of calcium-mediated signaling, calcium-dependent cell-cell adhesion, positive regulation of cell-matrix adhesion, low density lipoprotein mediated signaling and inhibits regulation of endothelial cell proliferation, positive regulation of smooth muscle cell proliferation, keratinocyte proliferation, as a result of inducing differentiation in no-tumor hepatitis/cirrhosis, whereas high-expression *CDH13* activates positive regulation of survival gene product activity, protein homodimerization activity, Rho protein signal transduction, Rac protein signal transduction, positive regulation of cell migration, sprouting angiogenesis, positive regulation of positive chemotaxis, epidermal growth factor receptor signaling pathway, endothelial cell migration, lamellipodium biogenesis, and inhibits regulation of endocytosis, caveola in HCC, as a result of inducing survival and invasion in HCC. We found evidence from literatures to support this inference. Such as T-/H-cadherin absence of a transmembrane region and a cytoplasmic domain was a new marker for the differentiation of the podocytes and the formation of the glomerular capillary network [35]; H-cadherin, T-cadherin promoter region is specifically methylated in poorly differentiated colorectal cancers [36]; H-cadherin gene expression can be used as an indicator for invasion in both ER-positive and -negative breast tumors [37]; Cadherin 13 (CDH13, T-cadherin, H-cadherin) overexpression in endothelial cells promotes their proliferation and migration, and has a pro-survival effect [38]; The tumor-specific downregulation of expression and methylation of of H-cadherin (CDH13) may be involved in the development and invasive growth of pituitary adenomas [39]. Therefore, our inference is consistent with different-activated and -inhibited *CDH13* downstream network, GO database and literatures, respectivey.

Conclusions

In summary, we constructed and analyzed the low- and high-expression (fold change ≥2) different -activated and -inhibited *CDH13* downstream network from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation in GEO data set by integration of gene regulatory network inference method based on linear programming and decomposition procedure with GO database. Our results show that the low-expression *CDH13* downstream network has the multi-activated and -inhibited molecular pattern in no-tumor hepatitis/cirrhosis, whereas high-expression *CDH13* downstream mainly somewhat inhibited molecular connections but significant reduced network (fold ≥2) in HCC. We suppose that the low-expression *CDH13* downstream network mainly activates cell differentiation, cell adhesion, but inhibits nuclear chromosome, mitosis in no-tumor hepatitis/cirrhosis, whereas the high-expression *CDH13* downstream network activates Rab-protein geranylgeranyltransferase activity, protein modification, but inhibits modification-dependent protein catabolism and nucleotide binding in HCC. We put forward hypothesis that low-expression *CDH13* activates cadherin binding, homophilic cell adhesion, negative regulation of cell adhesion, positive regulation of calcium-mediated signaling, calcium-dependent cell-cell adhesion, positive regulation of cell-matrix adhesion, low density lipoprotein mediated signaling and inhibits regulation of endothelial cell proliferation, positive regulation of smooth muscle cell proliferation, keratinocyte proliferation, as a result of inducing differentiation in no-tumor hepatitis/cirrhosis, whereas high-expression *CDH13* activates positive regulation of survival gene product activity, protein homodimerization activity, Rho protein signal transduction, Rac protein signal transduction, positive regulation of cell migration, sprouting angiogenesis, positive regulation of positive chemotaxis, epidermal growth factor receptor signaling pathway, endothelial cell migration, lamellipodium biogenesis, and inhibits regulation of endocytosis, caveola, as a result of inducing survival and invasion in HCC. Our inferences are consistent with different-activated and -inhibited *CDH13* downstream network, GO database and literatures, respectively. Therefore, it is very useful to identify low- and high-expression *CDH13* downstream network for the understanding of molecular mechanism from no-tumor hepatitis/cirrhosis (HBV or HCV infection) to HCC malignant transformation.

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