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Cyclin-dependent kinase inhibitor 3 (*CDKN3*) novel cell cycle computational network between human non-malignancy associated hepatitis/cirrhosis and hepatocellular carcinoma (HCC) transformation

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Abstract

The relationship of cyclin-dependent kinase inhibitor 3 (*CDKN3*) with tumours has previously been presented in a number of publications. However, the molecular network and interpretation of *CDKN3* through the cell cycle between non-malignancy associated hepatitis/cirrhosis and hepatocellular carcinoma (HCC) have remained to be elucidated. Here, we have constructed and analysed significant high expression gene *CDKN3* activated and inhibited cell cycle networks from 25 HCC versus 25 non-malignancy associated hepatitis/cirrhosis patients (viral infection HCV or HBV) in GEO Dataset GSE10140-10141, by combination of a gene regulatory network inference method based on linear programming, and decomposition procedure using CapitalBio MAS 3.0 software, based on integration of public databases including Gene Ontology, KEGG, BioCarta, GenMapp, Intact, UniGene, OMIM, and others. Comparing the same and differently activated and inhibited *CDKN3* networks with GO analysis, between non-malignancy associated hepatitis/cirrhosis and HCC, our results suggest a *CDKN3* cell cycle network (i) with stronger DNA replication and with weaker ubiquitin-dependent protein catabolism as common characteristics in both non-malignancy associated hepatitis/cirrhosis and HCC; (ii) with more cell division and weaker mitotic G2 checkpoint in non-malignancy associated hepatitis/cirrhosis; (iii) with stronger cell cycle and weaker cytokinesis, as a result forming multinucleate cells in HCC. Thus, it is useful to identify *CDKN3* cell cycle networks for comprehension of molecular mechanism between non-malignancy associated hepatitis/cirrhosis and HCC transformation.

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Introduction

Cyclin-dependent kinase inhibitor 3 (*CDKN3*) is one of the genes our group has identified as highly expressed in 25 HCC versus 25 non-malignancy associated hepatitis/cirrhosis cases. We data-mined *CDKN3* roles in cell components, molecular functions, biological processes, KEGG, GenMAPP, BioCarta and disease, from CapitalBio MAS 3.0 software (CapitalBio Corporation, Beijing, China). CDKN3 cell component is localized in perinuclear regions, molecular function is comprised of protein serine/threonine phosphatase activity, protein tyrosine phosphatase activity, protein binding, protein tyrosine • serine • threonine phosphatase activity and hydrolase activity, biological process includes regulation of cyclin-dependent protein kinase activity, G1/S transition after mitosis in the cell cycle, cell cycle in general, cell cycle arrest, negative regulation of cell proliferation, dephosphorylation (GO (http://www.geneontology.org)). CDKN3 is relative to hs_1-tissue-endocrine_and_cns, regulation of protein kinase activity, regulation of transferase activity, protein amino acid dephosphorylation, negative regulation of cell proliferation and regulation of cell proliferation (GenMAPP (http://www.genmapp.org/)). Studies into *CDKN3* relationships in tumours has already been presented in a number of papers (1–13). However, distinct molecular networks and interpretation concerning *CDKN3* in the cell

cycle during non-malignancy associated hepatitis/cirrhosis and hepatocellular carcinoma (HCC) transformation have remained to be elucidated.

HCC is one of the most common causes of cancer-related death the world over. Thus, to develop novel drugs for treatment of HCC has become a challenge for biologists. Here, we have constructed and analysed significantly higher expression of the gene *CDKN3* activated and inhibited in cell cycle networks from 25 HCC versus 25 non-malignancy associated hepatitis/cirrhosis patients (viral infection HCV or HBV) in GEO Dataset GSE10140-10141, by combination of gene regulatory network inference methods based on linear programming and decomposition procedures, with the CapitalBio MAS 3.0 software, based on integration of public databases including Gene Ontology, KEGG, BioCarta, GenMapp, Intact, UniGene, OMIM, and more.

Mechanisms that inhibit a signal are as important as mechanisms that initiate it. The GRNInfer tool (14) identifies molecular activation and inhibition relationships based on a novel mathematic method called Gene Network Reconstruction tool (GNR), using linear programming and a decomposition procedure for inferring gene networks. The method theoretically ensures derivation of the most consistent network structures with respect to all the datasets, thereby not only significantly alleviating the problem of data scarcity but also remarkably improving reconstruction reliability.

Here, we have compared the same and different, activated and inhibited, *CDKN3* networks with GO analysis, between non-malignancy associated hepatitis/cirrhosis and HCC. Our results suggest *CDKN3* cell cycle network (i) with stronger DNA replication and weaker ubiquitin-dependent protein catabolism, as common characteristics in both non-malignancy associated hepatitis/cirrhosis and HCC; (ii) with the stronger cell division and the weaker G2 phase checkpoint in non-malignancy associated hepatitis/cirrhosis; (iii) with stronger ell cycle and the weaker cytokinesis, as a result forming multinucleate cells in HCC. Thus, it is useful to be able to identify the *CDKN3* cell cycle network for understanding molecular mechanisms between non-malignancy associated hepatitis/cirrhosis and HCC transformation.

Materials and methods

Microarray data

We used microarrays containing 6144 genes from 25 non-malignancy associated hepatitis/cirrhosis and 25 HCC patients, in the same GEO Dataset 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) (15) and we pre-processed raw microarray data as log 2.

Gene selection algorithms

Potential HCC molecular markers were identified using significant analysis of microarrays (SAM), which is a statistical technique for finding significant genes in a set of microarray experiments. Input to SAM comprised gene expression measurements from a set of microarray experiments, as well as a response variable from each experiment. The response variable might be a grouping such as untreated, treated, groups and so on. SAM computes a statistic d_i for each gene i, and measures strength of relationship between gene expression and response variable. It uses repeated permutations of data to determine whether expression of any genes is significantly related to the response. Cut-off for significance is determined by a tuning parameter delta (chosen by the user) based on the false positive rate. We normalized data by log 2, selected two classes, paired and minimum fold change ≥2, and chose significant (high expression genes of HCC compared to non-malignancy associated hepatitis/cirrhosis) genes under the false-discovery rate and q-value being 0%. q-value [invented by John Storey (16)] is similar to the well-known *P*-value, but adapted to multiple-testing situations.

Molecule annotation system

Molecule Annotation System (MAS) is a web-based software toolkit for whole data mining and function annotation solution, to extract & analyse biological molecule relationships from public database. MAS uses relational databases of biological networks created from millions of individually modelled relationships between genes, proteins, complexes, cells, disease and tissues. MAS allows view on our data, integrated in biological networks according to different kinds of biological context. This unique feature results from multiple lines of evidence, integrated in MASCORE. MAS helps comprehension of relationships of gene expression data through the given molecular symbols list, and provides thorough, unbiased and visible results.

Primary databases of MAS integrated various well-known biological resources including Genbank, EMBL, SwissProt, Gene Ontology, KEGG, BioCarta, GenMapp, mirBase, EPD, HPRD, MIND, BIND, Intact, TRANSFAC, UniGene, dbSNP, OMIM, InterPro, HUGO, MGI and RGD. MAS offers various query entries and graphical results. The system represents an alternative approach to mining biological signification for high-throughput array data (17).

Network establishment of candidate genes

The entire network was constructed using GRNInfer (42) and GVedit tools (http://www.graphviz.org/About.php). GRNInfer is a novel mathematical method called GNR based on linear programming and a decomposition procedure for inferring gene networks. The method theoretically ensures derivation of the most consistent network structure with respect to all of the datasets, thereby not only significantly alleviating the problem of data scarcity but also remarkably improving reconstruction reliability. Equation (1) represents all of the possible networks for the same dataset.

$$J = (\mathbf{X}' - A)\mathbf{U}\Lambda^{-1}\mathbf{V}^{\mathrm{T}} + Y\mathbf{V}^{\mathrm{T}} = \hat{J} + Y\mathbf{V}^{\mathrm{T}} \qquad (1)$$

where $J = (J_{ij})_{n\times n} = \partial f(x)/\partial x$ is an $n \times n$ Jacobian matrix or connectivity matrix, $\mathbf{X} = (x(t_1), \ldots, x(t_m))$, $A = (a(t_1), \ldots, a(t_m)$ and $\mathbf{X}' = (x'(t_1), \ldots, x'(t_m))$ 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 $i = 1, \ldots, n$; $j = 1, \ldots, m$. $\mathbf{X}(t) = (x_1(t), \ldots, x_n(t))^{\mathrm{T}} \in \mathbf{R}_n$, $a = (a_1, \ldots, a_n)^{\mathrm{T}} \in \mathbf{R}_n$, $x_i(t)$ 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 $e_j \neq 0$ and is otherwise an arbitrary scalar coefficient. $\wedge^{-1} = \mathrm{diag}\,(1/e_i)$ and $1/e$ is set to be zero if $e_i = 0$. $\mathbf{U}$ is a unitary $m \times n$ matrix of left eigenvectors, $\wedge = \mathrm{diag}\,(e_1, \ldots, e_n)$ is a diagonal $n \times n$ matrix containing the n eigenvalues and $\mathbf{V}^{\mathrm{T}}$ is the transpose of a unitary $n \times n$ matrix of right eigenvectors. We established network based on the fold change ≥2 distinguished genes and selected parameters as lambda 0.0 because we used one dataset and tried several thresholds 1, 0.5, 0.1, 0.001, 0.0001, 0.000001, 0.0000001, 0.00000001, 0.000000001. 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.

Results

Identification of HCC novel molecular markers

We normalized data by log 2, selected two classes paired and minimum fold change ≥2, and chose the significant (high expression genes of HCC compared non-malignancy associated hepatitis/cirrhosis) genes under false-discovery rate and q-value being 0%. We obtained 225 significant high expression molecules (fold change ≥2) from 6144 genes of 25 HCC versus 25 non-malignancy associated hepatitis/cirrhosis in the same GEO Dataset GSE10140-10141 containing *NEK2, NUSAP1, CAD, DLGAP5, LCN2, SFRP4, RRM2, HIST1H3H, TAGLN2, MYBL2, TK1, PRCC, E2F1, ACTN2,* etc. as shown in Table 1.

Candidate novel activated and inhibited genes of CDKN3 upstream and downstream network in human non-malignancy associated hepatitis/cirrhosis and HCC by GRNInfer

We assayed GRNInfer at several thresholds 1, 0.5, 0.1, 0.001, 0.0001, 0.000001, 0.0000001, 0.00000001, 0.000000001 and finally, selected threshold 0.000000001 as its results covered *CDKN3* pathway by CapitalBio MAS 3.0 software, from the published data. We identified candidate genes of *CDKN3* upstream and downstream networks from our constructed total network between non-malignancy associated hepatitis/cirrhosis and HCC by GRNInfer separately, as shown in Table 2.

Discussion

Our aim here was to compare and analyse novel *CDKN3* cell cycle networks between non-malignancy associated hepatitis/cirrhosis and HCC transformation for potential novel markers for prognosis and therapy of HCC. On the basis of our previous published novel molecular network constructions and functional comparison from different databases presented in our papers (18–27), we constructed and analysed significant higher expression gene *CDKN3* activated and inhibited cell cycle network from 25 HCC versus 25 non-malignancy associated hepatitis/cirrhosis patients (viral infection HCV or HBV) in GEO Dataset GSE10140-10141 by a combination of gene regulatory network inference methods based on linear programming and decomposition procedure with the CapitalBio MAS 3.0 software, based on integration of public databases including Gene Ontology, KEGG, BioCarta, GenMapp, Intact, UniGene, OMIM, and more. We identified some same and other different novel activated and inhibited upstream and downstream genes of *CDKN3*'s cell cycle module between non-malignancy associated hepatitis/cirrhosis and HCC, on the condition that our *CDKN3* network covered *CDKN3* pathways and matched cell cycle enrichment analysis by the CapitalBio MAS 3.0 software, from published data.

To confirm our prediction for covering published data, we setup *CDKN3* interaction and pathways in HCC using CapitalBio MAS 3.0 software for standard comparison with our *CDKN3* network. We identified *CDKN3* interaction and pathways from our total established network by inputting 225 significant high expression genes (fold change ≥2) to the CapitalBio MAS 3.0 software based on integration of public databases including Gene Ontology, KEGG, BioCarta, GenMapp, Intact, UniGene, OMIM, and more. *CDKN3* interaction molecules and genes included *CDC2, CDK3, CDK2, CEBPA, CDC25A, CDKN3, MS4A3, CDC28_YEAST. CDKN3* pathway

Table 1. Abbreviations of 225 significant high expression genes (fold≥2) of HCC compared with human non-malignancy associated hepatitis/cirrhosis

Gene	Gene	Gene	Gene	Gene	Gene	Gene	Gene	Gene	Gene
ACTG2	CAMK1	CHST1	DKK1	GJA5	KCNQ3	MAN2A1	MYBL2	PAGE4	RBCK1
ACTN2	CBX5	CIAO1	DLGAP5	GML	KCTD2	MAOA	MYCN	PCOLCE2	RBM34
ADAMDEC1	CCL20	CKS1B	E2F1	GNAZ	KIAA0101	MAP2	MYH6	PHLDA2	REG1A
AFP	CCNA2	CLIC1	ECT2	GNG10	KIAA0513	MAP2K6	MYOM1	PIGC	REG3A
AKR1B10	CCNB1	CNTNAP2	EFNA1	GPC3	KIAA0859	MAP4K4	NAT9	PLA2G1B	RFC4
ALDH3A1	CCNB2	CORO2A	EIF1AX	GPSM2	KLHL35	MAPK3	NCAPH	PLK4	RIMS3
ALK	CCNE2	CPD	ELAVL3	GRM1	KLRC3	MAPT	NEK2	POLD1	RNF185
AMELY	CD34	CRYGA	ENAH	HIST1H2AD	KPNA2	MCM2	NFKBIB	PPP1R12B	RNF2
ARHGDIG	CDC2	CSPG4	EPHA4	HIST1H2AG	LAPTM4B	MCM4	NINJ2	PRCC	ROBO1
B4GALNT2	CDC20	CST6	ESM1	HIST1H2BJ	LCN2	MCM7	NKX2-5	PRKCG	RRM2
BAP1	CDC6	CSTB	ESPL1	HIST1H3H	LEF1	MDK	NOTCH3	PRKG2	RRP1B
BCAT1	CDH13	CSTF2	EYA1	HMGB2	LGALS3	MELK	NOTCH4	PROK1	S100P
BIRC5	CDKN2C	CTHRC1	F13A1	HOMER2	LLGL2	METAP2	NQO1	PRSS1	SBF1
BLVRA	CDKN3	CYP17A1	FGF9	HOXA5	LOX	MKRN3	NR5A1	PSMC3IP	SCGB1D2
BRCA1	CEBPA	CYP21A2	FKBP1B	HOXD4	LTBP2	MMP11	NRXN3	PTH2R	SCML2
BUB1B	CELSR2	CYP27B1	FOLR1	IGF2BP3	LTBP3	MMP9	NTN1	PTHLH	SEMA3B
C4orf8	CENPF	CYP51A1	FOXM1	IRF5	LYPD3	MRPL49	NUP62	PTTG1	SERPINB2
C9orf127	CHAF1A	DDX10	GALK1	ISG20	MAGI1	MS4A1	NUSAP1	PVRL2	SFRP4
CAD	CHL1	DDX11	GAS7	ITGA2	ZNF43	MS4A2	OCRL	RAB3B	SFTPA2B
ZIC2	CHRNA4	DFFB	GDPD5	KATNB1	ZWINT	MUTYH	ORC1L	RABGGTA	SLC16A3
ORC6L	TPSD1	TRAF2	TRIP13	TSHB	TSTA3	TUBG1	UNG	VDR	XRCC2
TOP2A	TPST2	TRIM26	TROAP	TSR1	TTK	UBE2C	VCAN	WDR1	YWHAE
SLC4A3	SORT1	SPINK1	SQLE	ST6GALNAC2	STX1A	SULT1C4	TAGLN2	TBL3	TK1
SLC6A12	SOX2	SPON2	SSTR5	STMN1	SULT1C2	SYN2	TANK	TCAP	TNFRSF9
TP53I11									

molecules consisted of *SBF1, EYA1, MCM2, MCM7, UBE2C, CHAF1A, CKS1B, SPINK1, MAPT, CYP21A2, REG3A, REG1A, STX1A, PRSS1, PLA2G1B, CYP17A1, TSHB, RIMS3, CCNE2, TNFRSF9, BAP1, GML, PTHLH, SSTR5, TTK, CIAO1, DDX11, BRCA1, BUB1B, CCNA2, CDC6, CDKN2C*. By comparison of similarities, we observed that our high expression molecules in HCC did not contain *CDKN3* interaction proteins, whereas they completely covered *CDKN3* pathway proteins (28).

To establish candidate novel genes concerned *CDKN3* upstream and downstream network covering *CDKN3* pathways, we assayed GRNInfer in several thresholds 1, 0.5, 0.1, 0.001, 0.0001, 0.000001, 0.0000001, 0.00000001, 0.000000001. Finally, we selected threshold 0.000000001 and set up candidate genes of the *CDKN3* network between non-malignancy associated hepatitis/cirrhosis and HCC by GRNInfer (Table 2). Our GRNInfer finding was verified covering *CDKN3* pathways using the CapitalBio MAS 3.0 software from published data.

To construct novel *CDKN3* cell cycle modules between non-malignancy associated hepatitis/cirrhosis and HCC respectively by GRNInfer, our *CDKN3* network needed to match cell cycle enrichment analysis. We identified cell cycle enrichment from our total established enrichment results by inputting 225 significant high expression genes (fold change ≥2) to the CapitalBio MAS 3.0 software based on integration of public databases including Gene Ontology, KEGG, BioCarta, GenMapp, Intact, UniGene, OMIM, and more. We data-mined molecules and genes of cell cycle enrichment including *NEK2, NUSAP1, CDKN3, DLGAP5, BIRC5, CDC20, MCM2, MCM7, CCNE2, CDC6, ZWINT, LLGL2, PTTG1, CCNA2, UBE2C, CCNB2, CDC2, CHAF1A, MAPK3, CKS1B, DDX11, CCNB1, KATNB1, CDKN2C*. On the basis of cell cycle enrichment, we predicted novel candidate activated and inhibited upstream and downstream networks of *CDKN3* cell cycle components between non-malignancy associated hepatitis/cirrhosis and HCC by GRNInfer separately, as shown in Figs 1 and 2. Study into ion binding relationship in tumour cells is presented previously in many papers (28–46). However, implications concerning *CDKN3* cell cycle networks between non-malignancy associated hepatitis/cirrhosis and HCC transformation remained to be elucidated. We further compared and interpreted candidate molecules of *CDKN3* cell cycle modules between non-malignancy associated hepatitis/cirrhosis and HCC transformation considering activation and inhibition relationships (29).

First, we identified the same novel molecules of *CDKN3* cell cycle modules in both non-malignancy associated hepatitis/cirrhosis and HCC. The same upstream *NUSAP1, CCNA2, UBE2C, MAPK3* activated *CDKN3,*

Table 2. Candidate upstream and downstream genes of *CDKN3* networks in human non-malignancy associated hepatitis/cirrhosis and HCC transformation by GRNInfer respectively. control: human non-malignancy associated hepatitis/cirrhosis, experiment: HCC patients

Control	Experiment
Upstream	
NUSAP1, DLG7, LCN2, SFRP4, RRM2, TROAP, HIST1H3H, TAGLN2, MYBL2, TK1, PRCC, E2F1, BRCA1, CENPF, SCML2, BIRC5, TOP2A, SPINK1, MDK, GNG10, FOXM1, TRAF2, SPON2, RAB3B, ECT2, KIAA0859, PIGC, TTK, CDC20, GPSM2, PLK4, TSTA3, VDR, MCM2, CIAO1, XRCC2, MELK, MAP4K4, CCNE2, AMELY, CDC6, SEMA3B, TUBG1, KIAA0513, CSTF2, TNFRSF9, IGF2BP3, NCAPH, YWHAE, SBF1, PVRL2, ORC1L, EIF1AX, ZWINT, GDPD5, GRM1, ARHGDIG, MCM4, BUB1B, ACTN2, RBCK1, DDX10, C9orf127, PTTG1, ACTG2, CLIC1, RNF185, CCNA2, RBM34, LTBP2, SORT1, ZIC2, CDH13, GPC3, ST6GALNAC, CYP21A2, CEBPA, PRKCG, HIST1H2AG, UBE2C, CCNB2, CST6, NAT9, MYH6, HOXD4, CAMK1, CNTNAP2, MUTYH, PSMC3IP, SFTPA2, ALK, CDC2, CD34, TP53I11, CHST1, NOTCH3, HMGB2, BLVRA, LGALS3, MKRN3, CHAF1A, KPNA2, ITGA2, CELSR2, SLC16A3, GNAZ, KCTD2, MAPK3, GJA5, RRP1B, BAP1, CAD, HIST1H2BJ, REG3A, ESM1, WDR1, TRIP13, GML, CKS1B, ROBO1, ORC6L, FGF9, SYN2, MYCN, AFP, CCL20, LEF1, SERPINB2, IRF5, BCAT1, PRKG2, REG1A, TPST2, S100P, NR5A1, HIST1H2AD, MMP9, PTHLH, SQLE, PHLDA2, CSPG4, CCNB1, CRYGA, STX1A, UNG, SULT1C2, MYOM1, MAOA, ZNF43, FKBP1B, HOXA5, GAS7, CYP51A1, EYA1, PRSS1, CHRNA4, KATNB1, MAN2A1, OCRL, ALDH3A1, F13A1, VCAN, STMN1, FLJ33790, EFNA1, PLA2G1B, KCNQ3, PAGE4, TCAP, CDKN2C, NQO1, CYP17A1, KIAA0101, TSHB, KLRC3, ESPL1, MRPL49, RIMS3, NKX2-5, PTHR2, NTN1, CTHRC1, TBL3, MMP11, PROK1, TSR1, ADAMDEC1, MS4A2, SCGB1D2, PCOLCE2, NUP62 NINJ2 LAPTM4B DMN RABGGTA SOX2 SSTR5 NRXN3 CDKN3	NEK2, NUSAP1, DLG7, LCN2, SFRP4, RRM2, TROAP, HIST1H3H, TAGLN2, MYBL2, TK1, PRCC, E2F1, BRCA1, CENPF, SCML2, TOP2A, SPINK1, MDK, GNG10, FOXM1, TRAF2, SPON2, RAB3B, ECT2, ENAH, SLC4A3, TTK, CDC20, GPSM2, PLK4, TSTA3, MAPT, VDR, MCM2, MELK, MAP4K4, CCNE2, MAP2, AMELY, SEMA3B, TUBG1, CSTF2, CPD, TNFRSF9, IGF2BP3, NCAPH, YWHAE, SBF1, PVRL2, EIF1AX, ZWINT, GDPD5, GRM1, ARHGDIG, BUB1B, GALK1, ACTN2, RBCK1, DDX10, C9orf127, PTTG1, ACTG2, CLIC1, RNF185, CCNA2, RBM34, LTBP2, SORT1, ZIC2, CDH13, GPC3, CYP21A2, CEBPA, PRKCG, HIST1H2AG, UBE2C, CCNB2, CST6, NAT9, MYH6, SFTPA2, ALK, CDC2, CD34, NFKBIB, TP53I11, NOTCH3, HMGB2, FOLR1, BLVRA, LGALS3, RFC4, MKRN3, CHAF1A, ITGA2, EPHA4, CELSR2, SLC16A3, GNAZ, MAPK3, TPSD1, GJA5, BAP1, CAD, HIST1H2BJ, PPP1R12B, REG3A, ESM1, WDR1, LYPD3, GML, CKS1B, ROBO1, FGF9, MYCN, ELAVL3, AFP, CCL20, CBX5, LEF1, IRF5, PRKG2, REG1A, C4orf8, TPST2, S100P, NR5A1, MAP2K6, CHL1, HIST1H2AD, MMP9, PTHLH, SQLE, PHLDA2, CSPG4, CCNB1, CRYGA, STX1A, SULT1C2, MYOM1, MAOA, ZNF43, GAS7, EYA1, PRSS1, CHRNA4, KATNB1, MAN2A1, OCRL, ALDH3A1, F13A1, STMN1, EFNA1, PLA2G1B, KCNQ3, PAGE4, TCAP, CDKN2C, NQO1, CYP17A1, KIAA0101, TSHB, KLRC3, DKK1, RIMS3, NKX2-5, PTHR2, NTN1, TBL3, MMP11, PROK1, TSR1, ADAMDEC1, PCOLCE2, NUP62, NINJ2, LAPTM4B, RABGGTA, SOX2, SSTR5, NRXN3, CDKN3
Downstream	
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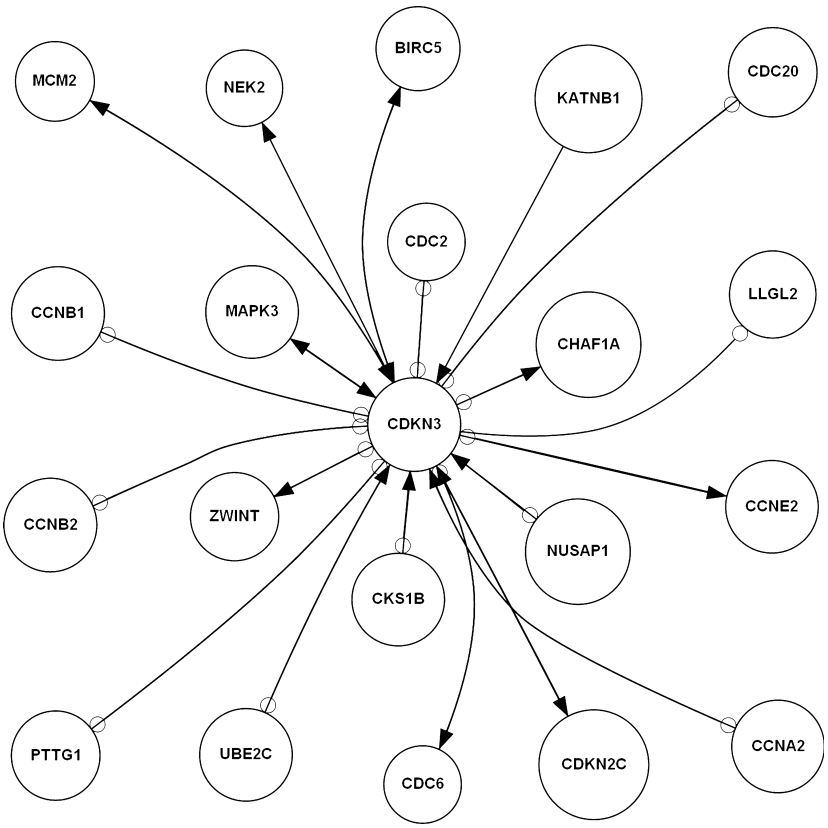

Figure 1. Candidate activated and inhibited upstream and downstream network of *CDKN3* in the cell cycle in non-malignancy associated hepatitis/cirrhosis by GRNInfer. Arrowhead represents activation relationship, empty circle represents inhibition relationship.

and *CDC20, PTTG1, CDC2* inhibited *CDKN3*. The same downstream *CDKN3* activated *MCM2, CHAF1A, MAPK3* and inhibited *CDC20, LLGL2, PTTG1, UBE2C, CCNB2, CCNB1* in both non-malignancy associated hepatitis/cirrhosis and HCC. To interpret molecular mechanisms of *CDKN3* cell cycle modules between non-malignancy associated hepatitis/cirrhosis and HCC transformation, we analysed GO of the same activated and inhibited molecules. For example, activated *MCM2* between non-malignancy associated hepatitis/cirrhosis and an HCC biological process is relevant to DNA replication, DNA unwinding during replication, DNA replication initiation, nucleosome assembly, transcription, regulation of transcription, DNA-dependency and cell cycle progression. Inhibited *CDC20* between non-malignancy associated hepatitis/cirrhosis and HCC biological processes is involved in ubiquitin-dependent protein catabolism, cell cycle progression, mitosis, anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolism, cell division, negative regulation of ubiquitin ligase activity during mitotic cell cycle and positive regulation of ubiquitin ligase activity during mitosis of the cell cycle. Compared to the same and different activated and inhibited *CDKN3* networks with GO analysis between non-malignancy associated hepatitis/cirrhosis and HCC, our results suggested that the same molecular network of *CDKN3* cell cycle modules maybe stronger DNA replication and weaker ubiquitin-dependent protein catabolism, as common characteristics in both non-malignancy associated hepatitis/cirrhosis and HCC.

Secondly, we identified the different novel molecules of *CDKN3* cell cycle modules between non-malignancy associated hepatitis/cirrhosis compared to HCC. The different upstream *BIRC5, MCM2, CKS1B, KATNB1, CDKN2C* activated *CDKN3,* and *CCNE2, CDC6, ZWINT, CCNB2, CHAF1A, CCNB1* inhibited *CDKN3;* different downstream *CDKN3* activated *NEK2, BIRC5, CCNE2, CDC6, ZWINT, CDKN2C* and inhibited *NUSAP1, CCNA2, CDC2, CKS1B* in non-malignancy associated hepatitis/cirrhosis. To further interpret molecular mechanisms of *CDKN3* in cell cycle modules of non-malignancy associated hepatitis/cirrhosis, we analysed GO of the different activated and inhibited molecules from non-malignancy associated hepatitis/cirrhosis. For example, activated *NEK2* in the non-malignancy associated hepatitis/cirrhosis biological process is relevant to cell

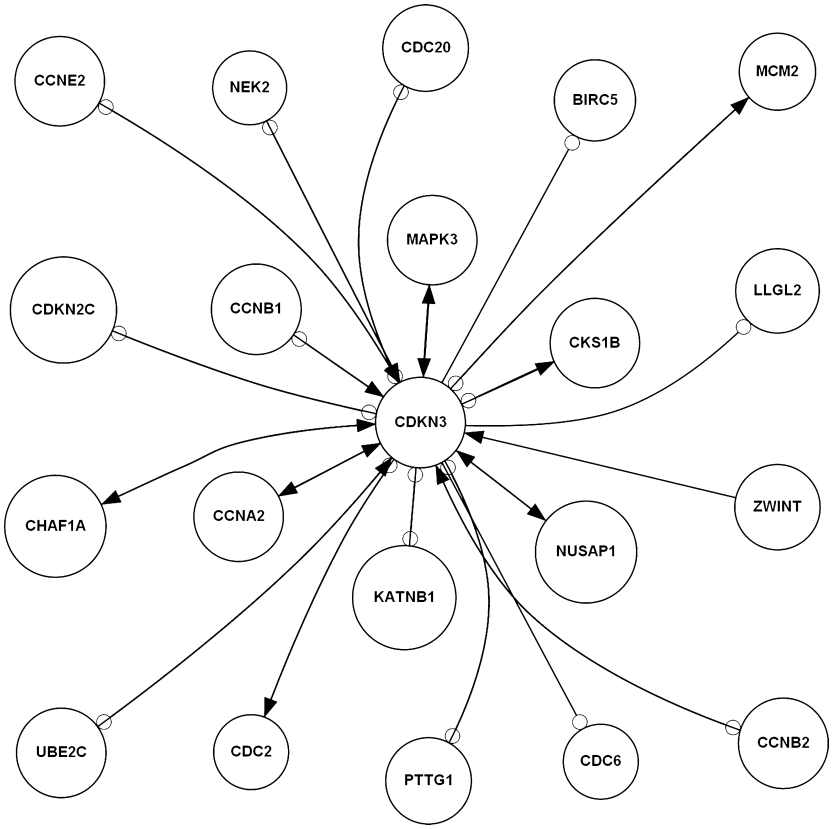

Figure 2. Candidate activated and inhibited upstream and downstream network of *CDKN3* in the cell cycle in HCC by GRNInfer. Arrowhead represents activation relationship, empty circle represents inhibition relationship.

cycle progression, regulation of mitosis, meiosis, protein amino acid autophosphorylation, centrosome separation and cell division. Inhibited *CCNA2* in the HCC biological process is involved in cell cycle progression, mitosis, mitotic G2 checkpoint, positive regulation of transcription, cell division. Compared to the same and different activated & inhibited *CDKN3* networks with GO analysis, our results demonstrated different molecular networks of *CDKN3* cell cycle modules, stronger cell division and the weaker mitotic G2 checkpoint as the only characteristics in non-malignancy associated hepatitis/cirrhosis.

Thirdly, we identified the different novel molecules of *CDKN3* cell cycle modules in HCC compared to non-malignancy associated hepatitis/cirrhosis. The different upstream *NEK2, CCNE2, ZWINT, CCNB2, CHAF1A, CCNB1* activated *CDKN3*, and *MCM2, CKS1B, KATNB1, CDKN2C* inhibited *CDKN3*; different downstream *CDKN3* activated *NUSAP1, CCNA2, CDC2, CKS1B* and inhibited *NEK2, BIRC5, CCNE2, CDC6, KATNB1, CDKN2C* in HCC. To interpret further molecular mechanisms of the *CDKN3* cell cycle module in HCC, we analysed GO of the different activated and inhibited molecules from HCC. For example, activated *NUSAP1* in the HCC biological process is relevant to cytokinesis after mitosis, cell cycle progression, mitotic chromosome condensation, establishment of mitotic spindle localization, positive regulation of mitosis and cell division progression. Inhibited *BIRC5* in the HCC biological process is involved in G2/M transition of during the cell cycle, cytokinesis, apoptosis, anti-apoptosis, cell cycle progression, mitosis, protein complex localization, positive regulation of exit from mitosis, spindle checkpoint, negative regulation of caspase activity, positive regulation of progression through mitosis of the cell cycle and establishment of chromosome localization. Compared to the same and different activated and inhibited *CDKN3* networks with GO analysis, our results indicate different molecular network of *CDKN3* cell cycle modules, stronger ell cycle progression and weaker cytokinesis, and as a result formation of multinucleate cells in HCC.

In conclusion, our high expression molecules of HCC did not contain *CDKN3* interaction proteins, whereas they completely covered *CDKN3* pathway proteins by comparison of similarities. We identified same and different novel activated and inhibited upstream and downstream genes related to *CDKN3* in the cell cycle module between

non-malignancy associated hepatitis/cirrhosis and HCC, on condition that our *CDKN3* network covered *CDKN3* pathways and matched cell cycle enrichment analysis by the CapitalBio MAS 3.0 software from published data. Comparing same and different activated & inhibited *CDKN3* networks with GO analysis between non-malignancy associated hepatitis/cirrhosis and HCC, our results suggested *CDKN3* cell cycle networks (1) with stronger DNA replication and weaker ubiquitin-dependent protein catabolism as common characteristics in both non-malignancy associated hepatitis/cirrhosis and HCC; (2) with stronger cell division and the weaker mitotic G2 checkpoint in non-malignancy associated hepatitis/cirrhosis and (3) with stronger cell cycle and weaker cytokinesis - and as a result formation of multinucleate cells in HCC. Thus, it is most useful to identify *CDKN3* cell cycle networks for understanding of molecular mechanisms between non-malignancy associated hepatitis/cirrhosis and HCC transformation.

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