ZINC SUPPLEMENTATION MAY REDUCE THE RISK OF HEPATOCELLULAR CARCINOMA USING BIG DATA NETWORK ANALYSIS

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ABSTRACT

Hepatocellular carcinoma (HCC) is a primary liver cancer with poor survival rates. Gene expression data of HCC are investigated to screen target genes and core genes, which are employed to propose a new strategy for the treatment of HCC. New concepts such as gene data streams, gene characteristic strength (CS), gene impact factor (GIF) and gene force (GF) are proposed. Together with gene community network (GCN), a novel algorithm, that is, called gene force algorithm (GFA), is presented to screen feature genes, target genes and core genes. The fifteen target genes are obtained, which can be divided into three clustering sets including HAMP Cluster = {HAMP, Trans, AQP4, VIPR1}, MT Cluster ={MT1H, MT1B, MT1G, MT1E, MTIL, RNAHP, DNASE1L3} and GPC3 Cluster ={GPC3}. The core genes of each clusters are HAMP, Metallothionein genes (MTs) and GPC3 respectively, where MTs is a general name for a group of metallothionein genes. According to the relationship between the three core genes and the metals including copper, iron and zinc, a treatment strategy for HCC is proposed, namely, "Supplement Zinc after surgery" for HCC patients. The proposed treatment method can be used to regulate the expression levels of HCC core genes.

KEYWORDS

Core gene, gene community network, gene regulation, HCC, zinc supplementation

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of the world-cancer-related death. [1]. It is vital to develop efficient and safe treatments of HCC for clinical services in order to save or prolong patients' lives. It has been recognized that gene therapy is one of the most promising solutions to the treatments of HCC.

The traditional feature genes approaches only consider the differences of the gene expressions between tumour and normal tissues [2]; however, the degrees of the differences are not taken into

account. The intensity of the correlations between genes has not been clearly defined and interpreted. The absolute values of Pearson's correlation coefficients are usually used to assess the correlation of genes [3], which fail to distinguish the positive correlation from the negative correlation. Target genes are usually obtained by using biological experiments [4], but it is noted that the experiments are difficult to implement and the coverage is often small. There seems not to be an agreed opinion on the reason caused tumour. One opinion is that oncogenes being active and anti-oncogenes being inactive will cause tumour [5]. Another one emphasizes that the mutation of the oncogenes and anti-oncogenes will lead to cancer [6]. Recently, gene therapy has been applied in gene replacement, gene correction, gene augmentation, and gene inactivation [7]. Unfortunately, the conventional gene therapy methods [8] seem to be difficult to implement and their effectiveness has yet proved significantly.

Currently, the big data [9-16] study is overwhelming, we can consider using the network method to solve biological data. As a result, there is a strong motivation to investigate gene therapy from the viewpoint of big data, providing a new idea for the treatment of HCC. In this study, the gene community network (GCN) proposed in [17-21] can be divided into gene positive network (GPN or POS network) and gene negative network (GNN or NEG network), which are employed here to describe the facilitating impact between the same type genes and the restraining impact between the opposite type genes. The gene characteristic strength (CS) and the CS threshold (CST) are then defined, from which the genes with CS values being greater than the CST are described as feature genes. The genes impact factor (GIF) is next defined as the sum of all the Pearson correlation strength between this gene and all other genes. The gene force (GF) is defined as the multiplication of CS and GIF. Together with the GCN, the concepts of the gene data stream, CS, GIF and GF pave the way for developing a novel algorithm, called GF algorithm (GFA), in order to screen the target genes and core genes. Finally, a simple and safe method is presented to regulate gene expression levels, providing a new insight into the treatment of HCC.

2 MATERIAL AND METHODS

2.1 Data

The HCC data are available from the NCBI database GSE3500[22]. There are 3964 genes with different expression in 156 liver tissues (74 non-tumour liver tissues and 82 HCC tissues).

2.2 Basic concept

In order to develop an algorithm to screen target genes, a number of important concepts such as gene community network, gene characteristic strength, gene impact factor, and gene forces are proposed and discussed.

A Gene Force(GF) is determined by both the total impacts between the characteristics strength (CS) and the gene impact factor (GIF). The greater the gene force, it is more likely for this gene to become a target gene. The GF is defined as the multiplication of the CS and GIF, that is,

$$GF = CS * GIF$$

Where $GIF = \sum |p_{ij}|$, and the absolute value of p_{ij}, which is the Pearson correlation between gene i and gene j, is greater 0.2, and $CS = |\text{mean(Tumor)} - \text{mean(Normal)}|$.

2.3 Core gene algorithm (CGA)

Many different HCC target genes can be obtained from different angles or different method. But it is difficult to adopt measures for each target gene. They follow the leader, we mainly directed against the most influential of these target genes, which called core genes. And the algorithm steps (see Figure 2) are as follows:

(a) The feature genes are selected from the original gene.
After Replacing missing values and calculating the CS value of all original genes, the K genes of the largest CS values as feature genes. In this paper, K = 100.

(b) The candidate target genes are choosing from feature genes.
From the feature genes, select the genes which rather than the CS mean value as a candidate target gene.

(c) The target genes are screened from the candidate target genes.
The target genes, which CS value are greater than the GF mean value, are screened in the features genes.

(d) Finally, the core genes are picked from the target genes.
The GCN network is built based on the target genes, and the core genes can be gotten after analysis of the network.

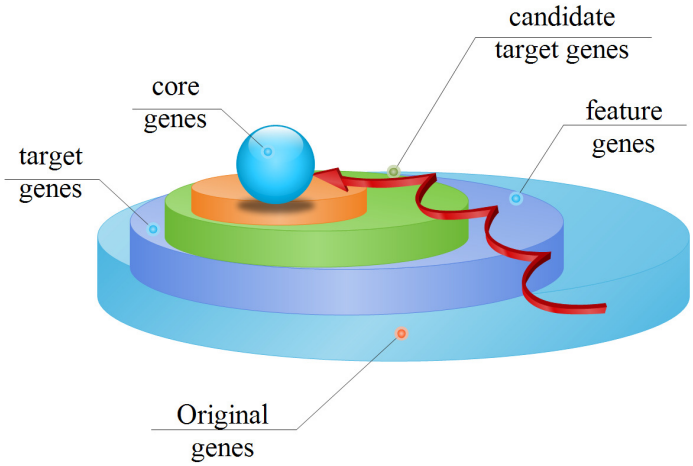


Figure 1. The relationship of five categories of genes.

3 RESULTS

3.1 HCC target genes

Based on the CGS algorithm proposed, we can screen HCC target genes following the steps:

(a) Calculate the CS values of the original genes, and sort the genes in descending order according to the CS values. One can obtain the top 100 (K=100) genes with larger CS values.

(b) The CS average value of the top 100 genes is 2.37, which is set as the threshold CST=2.37, leading to 30 (R=30) candidate target genes with CS values larger than CST.

(c)The average GF value of the 30 candidate target genes obtained is calculated as 129.68, which is set as the threshold GFT=129.68. We thus can obtain the 13 (Q=13) target genes, described by table 1 whose GF values are larger than GFT.

Table 1. The target genes

GID	GName	CS	GIF	GF
GENE7X	HAMP	38.75	12.91	500.1
GENE86X	MT1H	13.24	17.58	232.71
GENE531X	RNAHP	11.15	17.7	197.3
GENE3131X	MT1G	12.7	15.54	197.3
GENE1288X	RNAHP	13.71	13.91	190.66
GENE3579X	GPC3	18.3	10.19	186.46
GENE2088X	MT1L	10.64	16.1	171.31
GENE414X	Trans	10.1	15.64	157.98
GENE3558X	AQP4	10.81	14.08	152.2
GENE1911X	Trans	9.46	16.05	151.85
GENE676X	ADH4	9.53	14.51	138.24
GENE244X	MT1E	8.95	15.42	138.03
GENE976X	VIPR1	7.79	17.19	133.91

Trans: Transcribed locus; the duplicate genes are the same ones.

In Table 1, the 13 target genes are shown with names, and the values of CS, GIF and GF. If the Transcribedlocus and the duplication of genes are extracted, the set of the target genes can be given by TGS= {HAMP, MT1H, RNAHP, MT1G, RNAHP, GPC3, MT1L, AQP4, ADH4, MT1E, VIPR1}, where the genes are listed in descending order in terms of GF values.

3.2 HCC core genes

On the basis of the 13 target genes, we can construct the GCN networks for further analysis in order to screen the most important HCC target genes, called HCC core genes. Firstly, we calculate the Pearson correlation of target genes (see table 2).

Table 2. Pearson correlation of the target genes

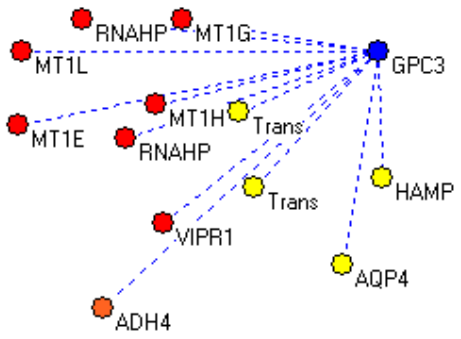
	GPC3	Trans	Trans	HAMP	AQP4	MT1L	MT1H	RNAHP	RNAHP	MT1G	MT1E	VIPR1	ADH4
GPC3	1.00	-0.35	-0.33	-0.29	-0.31	-0.40	-0.38	-0.37	-0.26	-0.34	-0.35	-0.42	-0.37
Trans	-0.35	1.00	0.84	0.76	0.81	0.63	0.76	0.75	0.59	0.61	0.66	0.73	0.49
Transc	-0.33	0.84	1.00	0.63	0.66	0.59	0.71	0.75	0.55	0.71	0.61	0.70	0.51
HAMP	-0.29	0.76	0.63	1.00	0.89	0.47	0.54	0.51	0.42	0.49	0.44	0.53	0.37
AQP4	-0.31	0.81	0.66	0.89	1.00	0.54	0.62	0.58	0.52	0.51	0.56	0.59	0.38
MT1L	-0.40	0.63	0.59	0.47	0.54	1.00	0.84	0.85	0.79	0.69	0.83	0.67	0.50
MT1H	-0.38	0.76	0.71	0.54	0.62	0.84	1.00	0.95	0.77	0.77	0.83	0.76	0.61
RNAHP	-0.37	0.75	0.75	0.51	0.58	0.85	0.95	1.00	0.80	0.75	0.83	0.77	0.60
RNAHP	-0.26	0.59	0.55	0.42	0.52	0.79	0.77	0.80	1.00	0.55	0.74	0.51	0.35
MT1G	-0.34	0.61	0.71	0.49	0.51	0.69	0.77	0.75	0.55	1.00	0.66	0.62	0.49
MT1E	-0.35	0.66	0.61	0.44	0.56	0.83	0.83	0.83	0.74	0.66	1.00	0.63	0.45
VIPR1	-0.42	0.73	0.70	0.53	0.59	0.67	0.76	0.77	0.51	0.62	0.63	1.00	0.68
ADH4	-0.37	0.49	0.51	0.37	0.38	0.50	0.61	0.60	0.35	0.49	0.45	0.68	1.00

The correlation matrix is denoted by P. To cluster the target genes, we set different threshold values. The thresholds were obtained by the methods as follows:

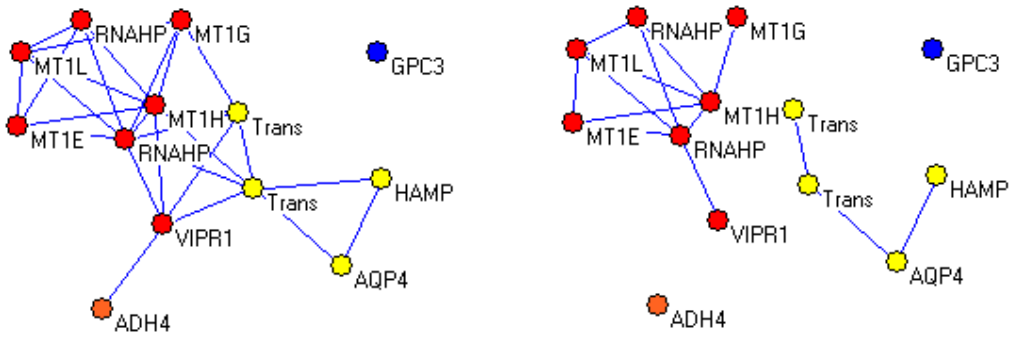
(1) the positive threshold "T1" <=the positive minimum of maximum values in each column "mm1". In this paper, the T1= [0.6787 0.7688 0.8047 0.8339 0.8434 0.8923 0.9998 0.9998 0.9998 0.9998 0.9998 0.9998 0.9998 0.9998]

(2) the negative threshold "T2" <= the negative maximum of maximum values in each column "mm2", and for this data analysis, T2= [-0.2641 -0.2884 -0.3088 -0.3337 -0.3432 -0.3484 -0.3534 -0.3693 -0.3723 -0.3781 -0.403 -0.4177 -0.9999]

Three threshold values were chosen for clustering, that is : -0.2641, 0.6787, 0. 7688.Based on different threshold, we get different GCN Networks (see Figure 2).



(a)GNN network (T= -0.2641)



(b)GPN network (T=0.6787)

(c) GPN network (T=0.7688)

Figure 2.GCN networks of target genes.

In the figure 2, (a) when T=-0.2642, the GPC3 is negative related to other genes;(b) when T=0.6787, we get a big cluster {HAMP, Trans, Trans, AQP4, MT1H, MT1B, MT1G, MT1E, MTIL, RNAHP, VIPR1, ADH4}. (c) when T=0. 7688, the big cluster is divided to two clusters {HAMP, Trans, Trans, AQP4}and {MT1H, MT1B, MT1G, MT1E, MTIL, RNAHP, VIPR1, ADH4}.

According the analysis above, the target genes are obtained, which can be divided into three clustering sets (see Figure 3) including HAMP Cluster = {HAMP, Trans, AQP4, VIPR1}, MT Cluster = {MT1H, MT1B, MT1G, MT1E, MTIL, RNAHP, DNASE1L3} and GPC3 Cluster ={GPC3}. The core genes of each clusters are then derived, that is, HAMP, MTs and GPC3 respectively, where MTs is a general name for a group of metallothionein genes including MT1H, MT1B, MT1G, MT1E, and MTIL.

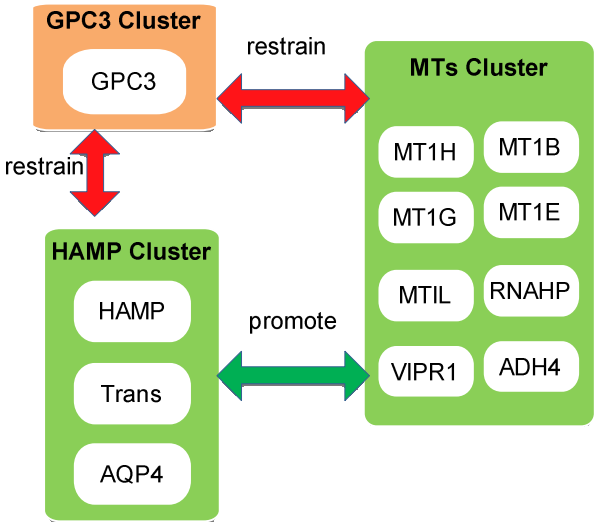


Figure 3 Three clusters and core genes

At last, we get three core genes HAMP, MTs and GPC3. The HAMP genes is to regulate the iron homeostasis in the human body [23], and is the Iron overload brings negative impact for the HCC [24]. And the aquaporin plays a role in the hepatocellular [25].

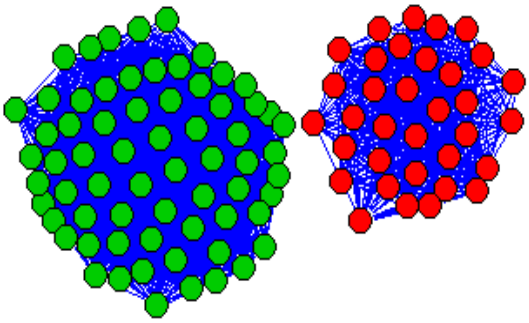
The Metallothionein genes (MTs) is very important role to adjust the heavy metal in the body [26], and the HCC patients with high zinc- Color rate [27]. In addition, the RNAPH [28], which has another name DDX42, is apoptosis in the HCC patients.

And the GPC3 gene is a very significant oncogene，which is over- expressed in the fetal liver [29] and is normal in healthy adults, and it's a new biomarker for live cancer [30].

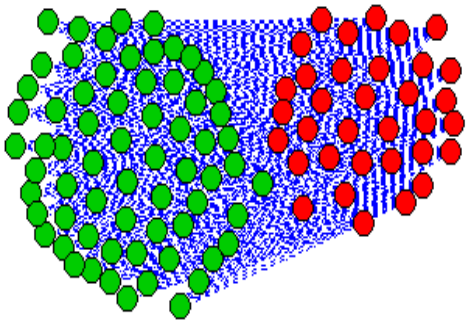
4 DISCUSSION

4.1 Correlations between oncogenes and anti-oncogenes

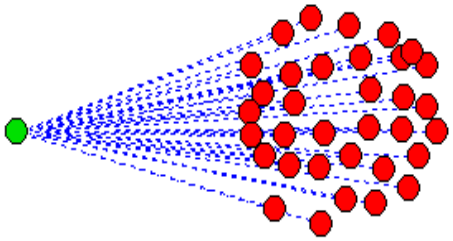
A single gene generally cannot perform a function independently. There is mutual promotion or mutual inhibition relationship between genes. Oncogenes and tumour anti-oncogenes are obviously in opposite types. In order to illustrate the correlations between oncogenes and anti-oncogenes, we use the top 100 feature genes, including 34 oncogenes and 66 anti-oncogenes (see Figure 3)



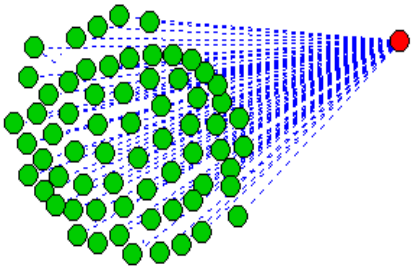
(a) The positive relationship between oncogenes and anti-oncogenes.



(b) The negative relationship between oncogenes and anti-oncogenes (macro diagram).



(c). An oncogene and anti-oncogenes have negative relationship



(d). An anti-oncogene and proto-oncogenes have negative relationship

Figure 4. The relationship between oncogenes and anti-oncogenes

In the figure 4, the green nodes represent anti-oncogenes, the yellow nodes represent oncogenes, the solid lines represent positive correlation, and dotted lines represent negative correlation, respectively. The lines with Pearson correlation strength defined by equation (2) being smaller than 0.2 are regarded as noises, which are removed in Figures 3.

To summarize, one can conclude that: a) Genes in the same type are positively related, indicating a mutual promotion relationship. b) Genes with opposite types are negatively related, which inhibit each other.

4.2 The importance of zinc supplementation

The aim of the treatment is to make the core genes GPC3, HAMP and MTs to come back to the normal expression level, that is, we have to do something such that $GPC3\downarrow\ HAMP\uparrow MTs\uparrow$. In the same time, we also hope the contents of trace elements such as irons, coopers and zincs to recover to normal content levels. As a result, we have to make $Fe^{2+}\downarrow Cu^{2+}\downarrow Zn^{2+}\uparrow$.

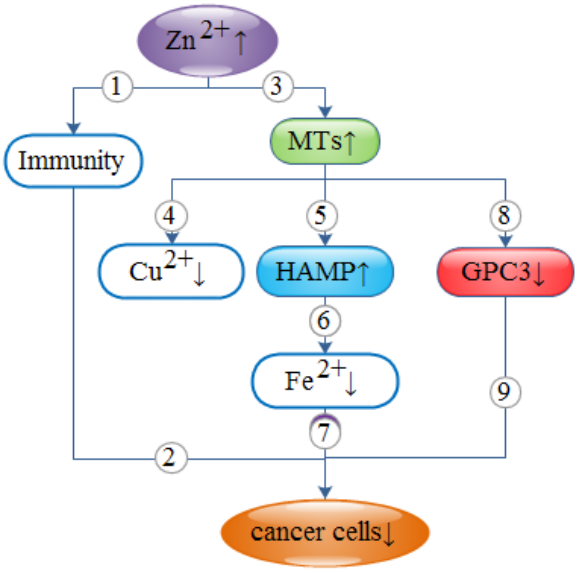


Figure 5. Supplement Zn for HCC patients.

The principle of the zinc supplementation is illustrated as follows (see figure 5) ：

i) Zinc deficiency associates with many symptoms of liver cancer patients [31] and Zn supplementation can improve immunity of patients.

ii) To some degree, the immune system can inhibit cancer cell proliferation.

iii) Adding Zn can make the expression level of MTs up-regulate [32].

iv) The high-level expression of MTs can make metallothionein proteins bond free copper ions, leading to the reduction of the content of free copper ions in serum.

v) MTs and HAMP mutually promote so that the up-regulation of the expression level of MTs will stimulate the up-regulation of the expression level of HAMP.

vi) The up-regulation of the expression level of HAMP will lower the content of irons in the serum.

vii) As the reduction of irons in the serum, the transported oxygen is decreased so that the proliferation speed of the cancel cells slow down, and even some cancer cells die due to insufficient supply of oxygen.

viii) MTs and GPC3 inhibit mutually so that the up-regulation of the expression level of the MTs will lead to the down-regulation of the expression level of the GPC3.

ix) The low-level expression of the GPC3 may slow down the proliferation of cancer cells. Papers in this format must not exceed twenty (20) pages in length. Papers should be submitted to the secretary AIRCC. Papers for initial consideration may be submitted in either .doc or .pdf format. Final, camera-ready versions should take into account referees' suggested amendments.

5 CONCLUSION

Big data is a new and main trend, and this paper proposes a gene networks to analysis the biology big data. And the article presents result of Hepatocellular carcinoma (HCC), which is a primary malignancy of the liver cancer, as follows:

(1) To bring the liver cancer under control, we should rely mainly on the suppressor genes (e.g. HAMP) supplemented by oncogenes (e.g. GPC3).

(2) Generally, two genes in the proto-oncogenes group or suppressor genes group are positively related. But if one is proto-oncogene and another is suppressor one, they are negatively correlated.

(3) According the relationship between the HCC core genes (HAMP, MTs, GPC3) and the trace metals (Cu, Fe, Zn) in human body , suitable zinc supplementation may reduce the risk of liver cancer.

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