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Targeting the NCAPD3 gene activates EGFR and ASNS as two pivotal contributors to gastric cancer progression

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ABSTRACT

Aim: This study was conducted to discover the effect of NCAPD3 knockdown on the gene expression profile of gastric cancer.
Background: Gastric cancer, a potentially fatal disease, requires thorough evaluation for targeted interventions. Through the post-analysis of microarray data, it is crucial to further examine the impact of NCAPD3 (Non-SMC condensin II complex subunit D3) inhibition in gastric cancer, emphasizing the need for a more comprehensive analysis of this knockdown.
Methods: The use of Cytoscape and its plug-ins for protein-protein interaction network analysis enables the identification of genes that significantly affect network stability. These hub-bottlenecks are regulated due to the NCAPD3 inhibition and some of them act as compensators in this condition. The hub-bottlenecks pathways identified by ClueGO indicate their relationships in underlying mechanisms of knockdown. These identified central differentially expressed genes could be considered eligible targets for therapeutic interventions. Some of them play compensative roles while others are regulated in NCAPD3 knockdown.
Results: It can be concluded that some of the hub-bottlenecks contribute to compensation mechanisms including NPM1, PTEN, EGFR, HSPA5, and ASNS, while the other ones including HSPA4, DHX9, CAV1, MAP1LC3B, and SRSF1 are among the regulated genes.
Conclusion: In particular, the up-regulation of EGFR and ASNS genes in the knockdown scenario could significantly impact and deteriorate cancer treatment outcomes after comprehensive validation studies.

Keywords: Gastric cancer, Network analysis, Gene expression, NCAPD3 knockdown.

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Introduction

Digestive system malignancies are growing in the general population (1). One of them is gastric cancer which despite the presence of many treatment options available, still accounts for the second leading cause of death worldwide (2). The high mortality rate and the lack of diagnostic approaches for this cancer with its complex nature have spurred significant research activity in recent years (1). One of the challenges with this malignancy is its asymptomatic feature until it reaches advanced levels. In addition, prognosis failure is due to this characteristic of this type of cancer and a more accurate approach is required for early diagnosis (3).

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Molecular diagnosis is one of the routes to establishing whole through analysis of cancer triggers and developments if studied in more depth and via complementary assessments. These studies could be based on protein-protein interaction network analysis and gene ontology evaluations. In this way, it is possible to identify and validate the potential biomarkers suggested by studies such as gene expression profiling. In a protein-protein interaction network analysis, key genes are detected based on two important parameters including degree and betweenness centrality. These two features are provided by applied software in Cytoscape which explores them through topological analysis. The genes with high values of degree and betweenness centrality are called hub-bottlenecks. These fundamentals are essential for the integrity and strength of a scale-free network. In a PPI network, which is a scale-free network, these nodes could be deadly for organisms if removed and in other words, dysregulated in the system. In humans, cancer trigger and development could be related to the dysfunctional hub-bottleneck and therefore this requires further studies (4-6).

Gene knockdown is another way to identify the contribution of that specific gene in cancer pathology and its effects on other cancer genes. For instance, the knockdown of gene, EGFR, revealed inhibition in tumorigenesis of gastric cancer and its invasive behavior. The role of this gene is oncogenic and it relates to many types of cancers. The up-regulation of this gene has been reported for many cancers so far but still, there are some remaining facts about this gene to be studied (7). On the other hand, NCAPD3 is a major player in many kinds of diseases including gastric cancer. The characteristics of its contribution remain to be thoroughly explored. It has been found that the inhibition of this gene could result in the activation of certain genes regulating apoptosis in AGS (8). This study explores the impact of NCAPD3 knockdown on the gene expression profile of gastric cancer, aiming to identify the biological processes and expression profiles affected by the inhibition of this gene. This alteration is further studied in the protein-protein interaction network scale to better understand the underlying mechanism of this gene knockdown.

Methods

Data collection

The NCAPD3 knockdown in gastric cancer gene expression study sourced from the Gene Expression Omnibus database (GEO) (https://www.ncbi.nlm.nih.gov/geo/) was conducted as part of the primary investigation. AGS cells were utilized, with a total of six samples including three controls and three samples with NCAPD3 knockdown being compared. The data were published in 2024 under the series identifier GSE261264 and utilized the platform GPL15207 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE261264). More details of the experiment are presented in the report of Zhang SY et al (8).

Pre-evaluation analysis

The microarray data was analyzed by GEO2R and R programming software as R Studio in our study. This statistical software uses the Limma R package for the array analysis. Before this, it is essential to download some related packages for gene expression profiles. T-test, Log (fold change), Fold Change, and FDR are the statistical criteria used by these applications. Data visualization was carried out by the statistical methods of box plot, Uniform Manifold Approximation and Projection (UMAP), Mean-diff, and moderated t-statistical. These plots were depicted by RPubs.

PPI network analysis

The significant differentially expressed genes (DEGs) were identified based on adjusted p-value <0.05 and fold change > 2. The significant DEGs were divided into two down-regulated and up-regulated groups. Two networks for topological analysis were designated in Cytoscape and its plug-ins (9). Both networks are scale-free and with specific parameters that indicate the centrality of the DEGs in that network. These parameters, degree and betweenness centrality, are crucial for identifying hub and bottleneck nodes within the network. Degree centrality quantifies how many genes are directly connected to the gene of interest, while betweenness centrality measures the gene's position within the network, identifying whether it serves as a critical bridge facilitating connections. Essentially, a gene's betweenness centrality reflects its importance as a connector between pairs of nodes along the shortest paths in the network. The networks were constructed using a cutoff of 0.4 based on data from the STRING database. The STRING database (https://string-db.org/) provides information from different sources

including compound query, disease query, and protein query (10). The top 10% of the nodes of the main connected components of the PPI networks based on degree value were identified as the hubs. The bottlenecks were determined as the top 5% of the nodes of the main connected components of the PPI networks. The common hubs and bottlenecks were introduced as hub-bottlenecks.

Gene ontology enrichment

Topological analysis indicated that in the PPI network, essential nodes are related to biological processes that could be important in the underlying mechanisms of the knockdown. These terms are investigated by ClueGO+CluePedia, the enrichment analyzers in Cytoscape (11). These two plug-ins apply specific statistical methods to derive the related data. Statistical analysis: The gene ontology results were obtained based on the Kappa score and corrected p-values using the Bonferroni step-down (12). The PPI networks were constructed based on a confidence score of 0.4. The significant DEGs are selected considering adjusted p-value < 0.05 and fold change > 2.

Results

Pre-evaluation analysis

Box plotting is helpful for analyzing the data for cross-comparison between two groups of samples. In this way, it is possible to identify whether it is possible to go further in data analysis (Figure 1). The grouped samples are colored in green and purple indicating two conditions of knockdown and control. The groups are comparable as they are median-centered.

Using dimensionality reduction approaches such as UMAP, it is possible to predict the position of the studied samples and their relationships (Figure 2). UMAP plotting of this study revealed that the two conditions are grouped well as two groups with acceptable distances.

The mean-diff plot is another way of data visualization; in this method, it is possible to gain information regarding the nature of regulation and how significant the genes are differentially expressed (see Figure 3). In Figure 3, the mean difference plotting indicates that points that are away from the mean are significantly different in the expression pattern.

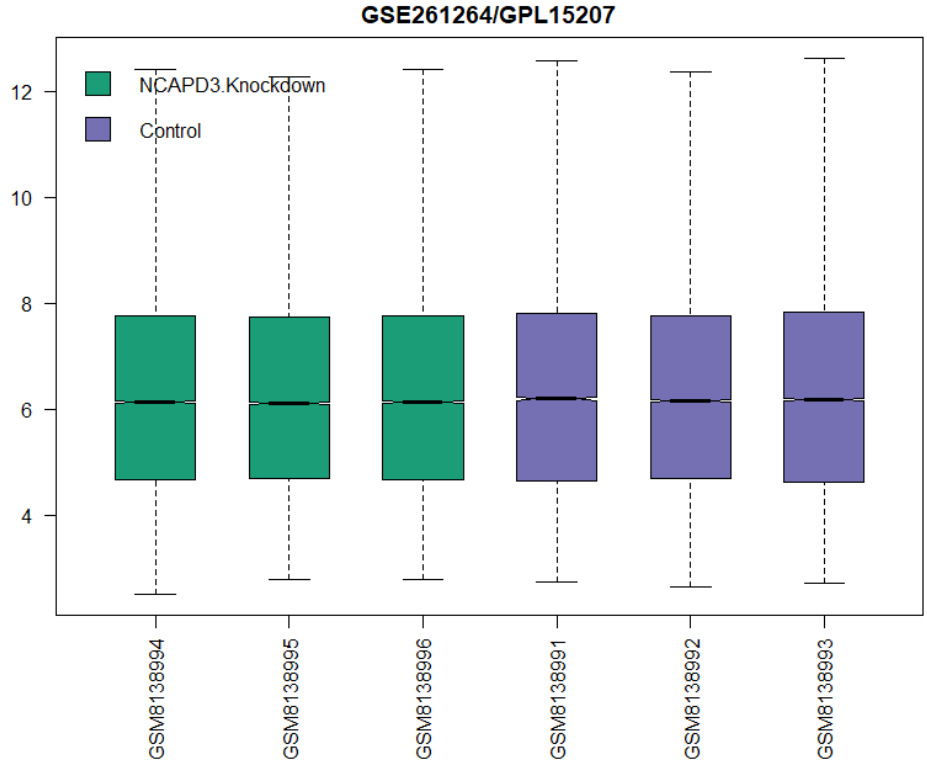

Figure 1. Boxplot of comparison between gene expression profiles of AGS cells with NCAPD3 knockdown versus control samples.

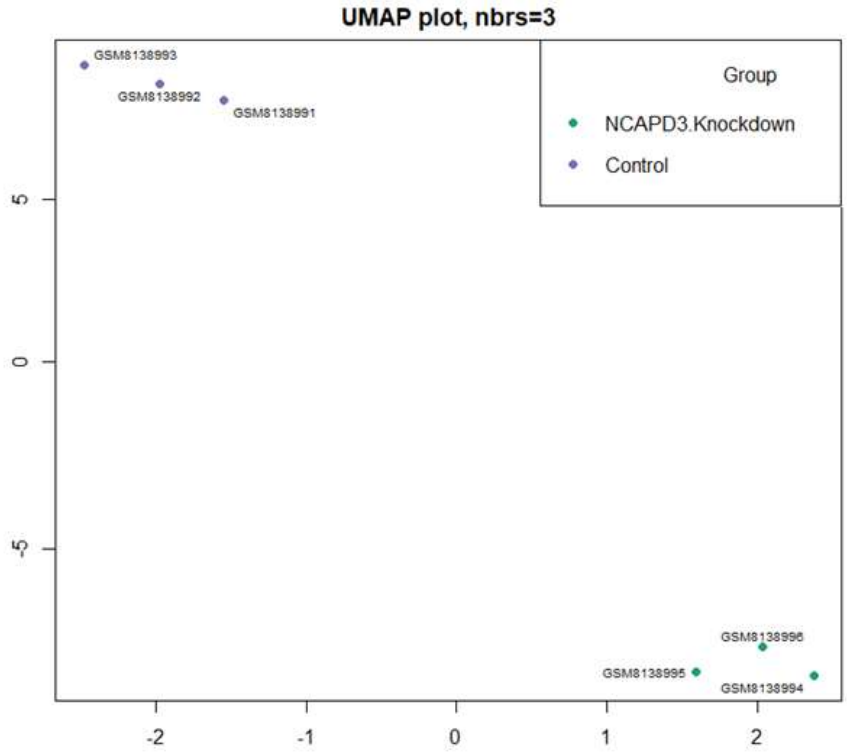

Figure 2. UMAP presentation of AGS cells with NCAPD3 knockdown versus control samples

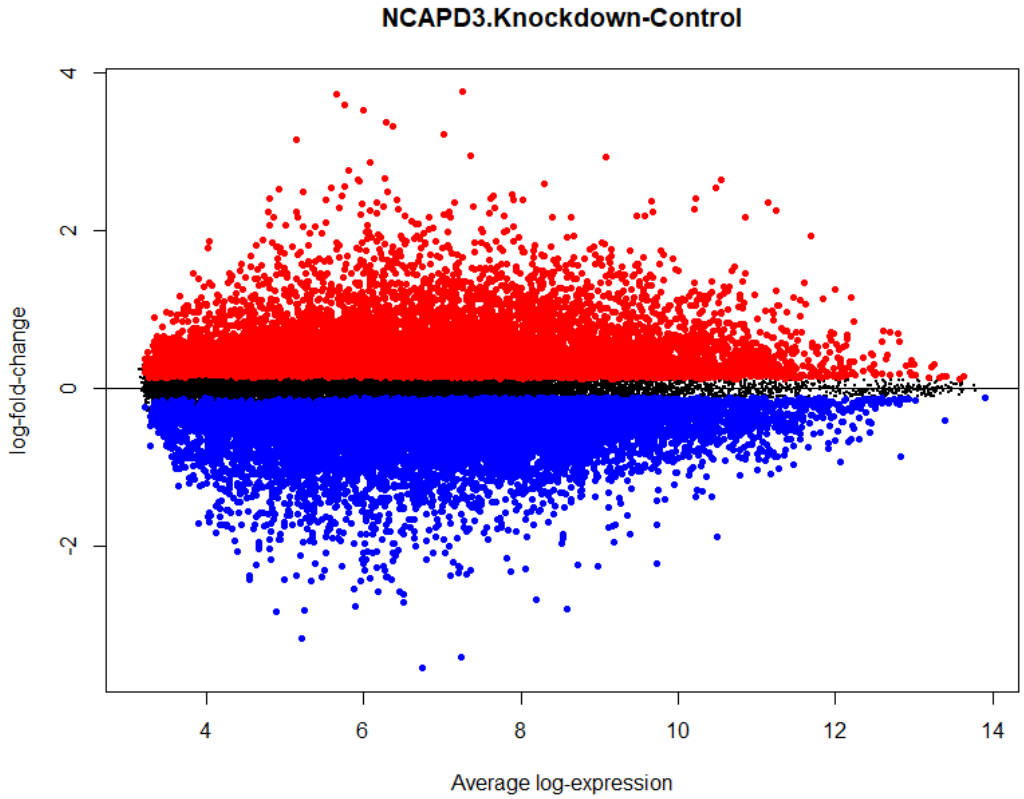

Figure 3. Mean difference plot of significant up-regulated in red and down-regulated genes in blue related to comparison between gene expression profiles of AGS cells with NCAPD3 knockdown versus control samples. Points with black color have no significant difference.

The farther they are, the more significant features they have in the expression pattern.

The next visualization method is through the application of Moderated t statistic. This plotting shows the quality of the limma test (Figure 4). In this analysis, the points aligned on the straight line indicate that the predicted distribution corresponds closely to the experimental outcome.

PPI network analysis

Two networks of up-regulated and down-regulated genes were constructed. The first one is 317 nodes with 595 connections while the second one is 394 nodes with 920 connections. Centrality analysis of both networks was conducted by "Network Analyzer". The detections concluded in two tables that are ranked based on degree value and nodes are either hubs-bottlenecks or hub-none-bottlenecks. Regardless of whichever they are, these nodes are valuable for maintaining network integrity and

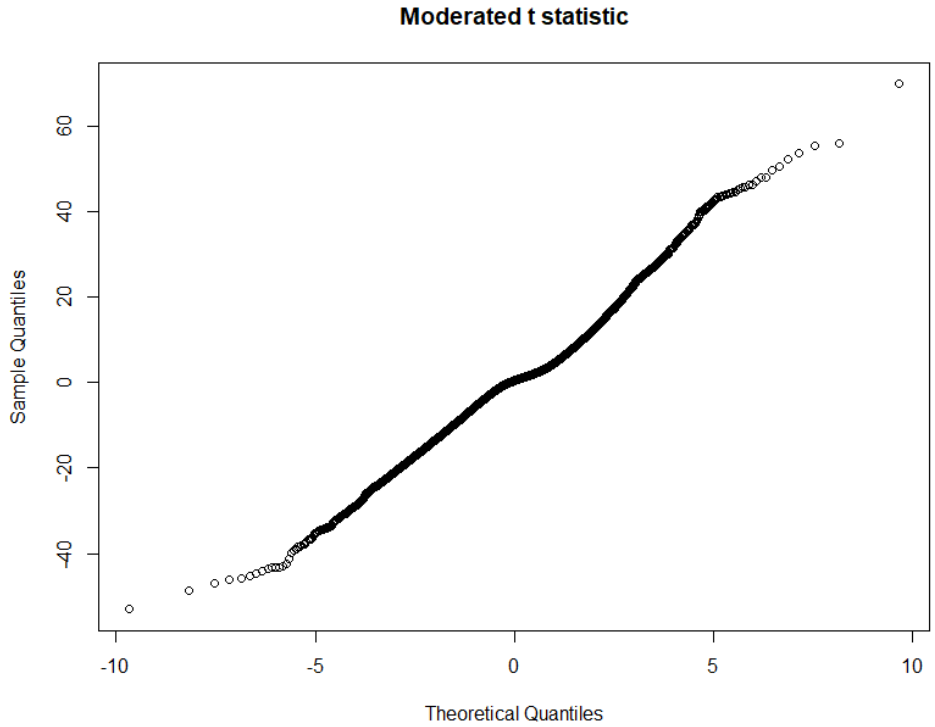

Figure 4. Moderated t statistic for comparison between gene expression profiles of AGS cells with NCAPD3 knockdown versus control samples; the points lie along a straight line.

Table 1. The top 10% of the down-regulated genes relative to the main connected component of the down-regulated PPI network. The hub-bottlenecks are assigned with an asterisk.

Row	Gene	Degree	Betweenness centrality
1	NPM1*	41	0.1
2	PTEN*	33	0.1
3	HSPA4*	33	0.1
4	DHX9*	29	0.09
5	SRSF1*	28	0.09
6	PA2G4	28	0.06
7	CREBBP	27	0.08
8	RRP1B	25	0.03
9	MDM2	24	0.06
10	DDX28	23	0.02
11	CHD4	23	0.05
12	RUVBL1	22	0.06
13	RRS1	22	0.02
14	PIK3R1	21	0.05

ensuring its uninterrupted function.

In the first examination, a network of downregulated nodes was assessed and a table of highly ranked genes was obtained (Table 1). A number of 14 hub nodes and 5 hub-bottleneck nodes are listed in Table 1. The next step was identifying the important DEGS of up-regulated genes in the second PPI network of the study. The hubs and hub-bottleneck nods are reported in Table 2.

Gene ontology enrichment: Pathway analysis of the hub-bottlenecks was examined by ClueGO+CluePedia in Figure 5. Five groups of pathways including "negative regulation of potassium ion transmembrane transporter activity", "cellular response to dsRNA", "ER-nucleus signaling pathway", "positive regulation of post-transcriptional gene silencing", and "peptidyl-tyrosine autophosphorylation" have been identified as the clusters of pathways.

Discussion

The benefit of expression data analysis is to better understand potential biomarkers in terms of physical interactions on a network scale. In this sense, the gene expression data from gastric cancer profile with a gene knockdown is evaluated for bioinformatic exploration.

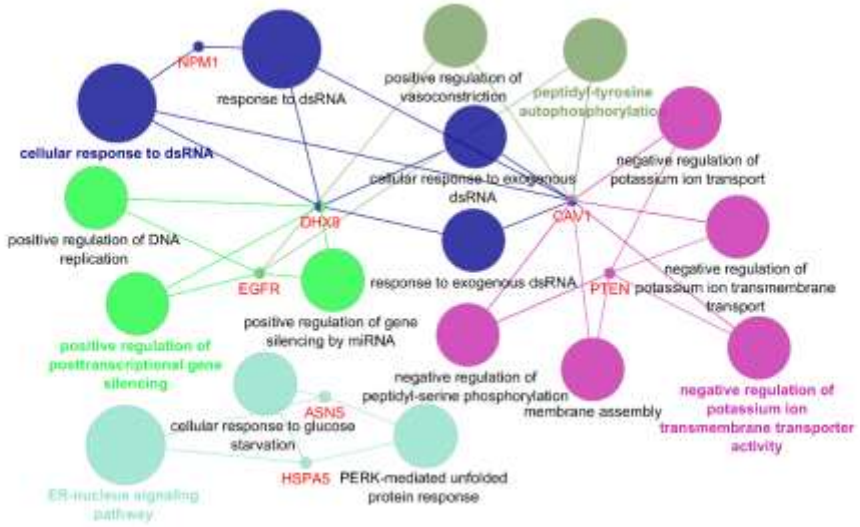

Figure 5. Pathway analysis of hub-bottleneck DEGs, different groups are colored differently. The associated genes are presented in red.

Table 2. The top 10% of the up-regulated genes relative to the main connected component of work. The hub-bottlenecks are assigned with an asterisk.

Row	Gene	Degree	Betweenness centrality
1	EGFR*	55	0.4
2	HSPA5*	24	0.09
3	ASNS*	21	0.06
4	THBS1	21	0.02
5	CAV1*	21	0.09
6	PLAUR	18	0.04
7	MAP1LC3B*	18	0.08
8	SLC3A2	17	0.03
9	TRIB3	17	0.05
10	ATM	16	0.1
11	SLC7A11	16	0.02
12	HSPA8	16	0.08
13	SLC7A5	15	0.01
14	PSAT1	15	0.01
15	MET	15	0.02
16	VCL	14	0.07

The gene NCAPD3 was knocked down and the evaluation of its effect on the gene expression profile of gastric cancer was explored in terms of PPI network analysis. This gene has been reportedly introduced as a great contributor to many types of diseases and is also highly expressed in gastric cancer (8). As depicted in Figures 1-4, the result from comparison of gene expression profiles indicated that two conditions are appropriate for further analysis and PPI examination. This evaluation was handled by GEO2R and R Programming and the significant DEGs were introduced to the Cytoscape software.

Two networks of differentially expressed genes have been derived from statistical analysis of two conditions of normal gastric cancer and gastric cancer with a knockdown gene of NCAPD3. Since critical data of the networks are extracted and listed in Tables 1 and 2, the networks have not been shown. The PPI network reveals that there are potential biomarkers in these networks where any dysregulation in these genes and their related pathways could have an important impact on cancer regulation following NCAPD3 knockdown. Moreover, many other genes have received plenty of attention as targets for analyzing the possible therapeutic outcome of their inhibition (13-15).

In this context, in our study, genes that are down-regulated and up-regulated are identified as separate networks to enhance understanding of their roles in network changes after knockdown. The introduced hub-bottlenecks are integral to understanding the effects of knockdown on cancer-related changes. To better grasp their roles and expression alterations post-knockdown, we conducted a literature survey to analyze previous findings and ascertain the consequences of NCAPD3 inhibition.

The first down-regulated gene to start with is Nucleophosmin-1 (NPM1), which is the highest-ranked down-regulated gene with a degree of 41 and a betweenness of 0.1. NPM1 has so many valuable roles with some of them including the biogenesis of ribosomes and genomic stability maintenance. The progression of gastric cancer is highly related to the expression of this gene. In previous studies, the expression of this gene both at gene and protein level revealed heterogeneity. It has been reported mostly down-regulated in most of the samples rather than up-regulation (16, 17). This gene could play a substantial role in diagnosis and prognosis prediction contribution to gastric cancer. It has also been mentioned that patients with high expression of this gene have a better chance of cure than those with low expression levels of this gene in gastric cancer (16, 18).

The next gene is phosphatase and tensin homologue (PTEN) which is the second-ranked down-regulated gene in this condition. PTEN acts as a tumor suppressor gene (19). Low expression levels of this gene are reported in gastric cancer (20). The molecular role of this gene is to block the pathway of PI3K/Akt in the development of gastric cancer (21). Heat shock protein A4 (HSPA4) as the other downregulated gene plays a role in gastric cancer cell development control. This gene is from the family of HSP110 and its role is to modulate the immune response in the gastrointestinal tract (22). There is an indispensable link between the gastric cancer ulcer healing system and this gene participation (23). In addition, the up-regulation of this gene in gastric cancer has been reported (24). Overall, this gene known as the stress gene has a great contribution to different kinds of cancer advancement (25). DExH-box helicase-9 (DHX9) is the next critical gene that is known as an oncoprotein with a close relationship with tumorigenesis. DHX9 is significantly increased in most reported cancers (26). Not much information is available on the relationship between this gene and gastric cancer. Serine and arginine-rich splicing factor-1 (SRSF1) is the last hub-bottleneck down-regulated gene. The contribution of this gene to the proliferation of cancer cells has been highlighted with high expression level in different cancers but not yet gastric cancer (27-29).

The next category is the up-regulated hub-bottlenecks assigned for the literature review perspective. The first high-ranked hub-bottleneck is epidermal growth factor receptor (EGFR), which interestingly has been reported before as one of the candidate genes for targeting in gastric cancer (7). In the primary study, the expression levels of this gene have been very high which could pinpoint the effect of NCAPD3 on other tumor-promoting genes. This could be due to mechanisms in which the tumor tries to compensate the absence of NCAPD3 by up-regulating another malignant gene at great expression level. Heat shock protein family A member-5 (HSPA5) is another up-regulated gene in the knockdown condition. This gene has also remained understudied for gastric cancer

though its role and up-regulation in other cancers are established and ultimately with poor prognosis outcomes (30).

Asparagine synthetase (ASNS) is highly expressed under knockdown conditions. In addition, the knockdown of this gene has also been in focus for some cancer studies including gastric cancer and AGS cells (31). Further, this gene has been reported as resistant in various cancers as chemotherapeutic approaches. The role of this gene is the proliferation of tumor cells and promoting metastatic behavior (31). The increment in expression levels of this gene after the knockdown of NCAPD3 could suggest compensation mechanism activation. The next up-regulated hub-bottleneck is caveolin-1 (CAV1). Low levels of this gene could promote gastric cancer development (32). MAP1LC3B due to its higher value of betweenness centrality was considered as an up-regulated hub-bottleneck. Lymph node metastasis in gastric cancer is related to the low expression of this gene (33). The knockdown of NCAPD3 has a contradictory effect on the gene expression profile of gastric cancer; AGS cells. It both activates the compensation mechanism and regulation process by regulating some hub-bottlenecks and their related pathways. In the context of compensation mechanisms, dysregulation is observed in hub-bottlenecks such as NPM1, PTEN, EGFR, HSPA5, and ASNS, whereas regulation is noted in HSPA4, DHX9, CAV1, MAP1LC3B, and SRSF1.

Conclusion

In summary, investigations into protein-protein interaction networks demonstrate that targeting NCAPD3 could lead to mixed outcomes in gastric cancer treatment. However, the knockdown of NCAPD3 is accompanied by the regulation of several oncogenes. It elevates the expression levels of genes such as EGFR and ASNS, which have pivotal roles in cancer development. It seems examination of new effective target genes to control gastric cancer is a subject for future research.

Conflict of interests

The authors declare that they have no competing interests.

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