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Bioinformatics screening of therapeutic targets in chemoresistance human colon cancer HCT8 cell line

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ABSTRACT

Background: Chemoresistance is still one of the main challenges in treatment of cancers, including colon adenocarcinoma (COAD). COAD is a common cancer with a high mortality. The goal of this study was to identify and evaluate the differentially expressed mRNAs (DEmRNAs) associated with both 5-fluorouracil (5-Fu) and cisplatin (DDP) resistance in human COAD cell line.

Methods: Common DEmRNAs, DEmiRNAs, and DElncRNAs were obtained from the gene expression profile GSE173606 between acquired two chemoresistance (5-Fu and DDP) and sensitive HCT8 cells. PPI network of overlapped DEmRNAs was obtained based on STRING database and Cytoscape and Gephi software. Degree algorithm in CytoHubba was used to determine hub genes. The hub genes were evaluated in TIMER2.0 and GEPIA databases. miRTarBase database was used to find DEmiRNAs which target the common DEmRNAs. Then DElncRNAs which have interaction with the selected DEmiRNAs were obtained from RNAInter database. LncRNA-miRNA-mRNA competing endogenous RNA (ceRNA) network visualized using Cytoscape software.

Results: A high number of common DEmRNAs (about 1780) in chemoresistance HCT8 cells were identified. TIMER2.0 database showed that the expression of high-expressed hub genes, including EGFR, TGFB1, ESR1, ICAM1, PECAM1, CAV1, and CCL5 has significant positive correlations with tumor infiltration of cancer-associated fibroblasts in COAD. CeRNA networks included the low-expressed mRNAs (as targets of upregulated miR-675-3p, miR-6084, and miR-1182) whose expression is also downregulated in COAD tissues based on GEPIA database. MDM2 (as a target of downregulated miR-4635 and miR-4306 in the ceRNA network) was an upregulated gene in both chemoresistance cells and COAD tumor tissues. RNAactDrug database confirmed the association of the high expression of four mRNAs of the ceRNA network (i.e., EFNB2, F2RL2, FLT1, and ADGRF1) with decreased drug sensitivity of DDP.

Conclusion: The results of this study can offer therapeutic targets. For example, inhibition of CCL5, oncogenic miR-675-3p, and MDM2 might be a good choice for gene targeted therapy to overcome the multi-drug resistance in COAD. Moreover, it can provide multiple mRNAs and miRNAs for predicting chemoresistance COAD.

Keywords: 5-Fluorouracil, Cisplatin, Colon adenocarcinoma, Drug resistance, HCT8 cell line

INTRODUCTION:

Colon adenocarcinoma (COAD) is one of the leading cancer-dependent causes of death in both men and women worldwide. Surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy are the common types of treatment for people with COAD [1]. 5-fluorouracil (5-Fu) and cisplatin (DDP) are two examples of chemotherapy drugs. 5-Fu by damaging the RNA and DNA leads to cell death [2]. DDP by modulation of DNA synthesis disturbs the S phase of the cell cycle and repair mechanisms leading to cell death [3]. However, a heterogenous population of cancer cells and complex interconnection between cancer cells and noncancer cells inside the tumor microenvironment (TME) usually trigger drug resistance. Drug resistance decreases the therapeutic efficacies of chemotherapy drugs as well as increases metastasis and recurrence [4]. According to studies, resistance mechanisms can be different among cancer patients; therefore, finding common approaches for reversing drug resistance is challenging. On the other hand, cancer cells usually develop different resistance mechanisms against different chemotherapy drugs. Various drug resistance cellular mechanisms have been reported, including increased drug efflux, increased drug metabolism, and apoptosis escape [4, 5]. The mentioned modified mechanisms are because of genetic factors either mutations or transcriptome changes or both of them [6]. Moreover, multi-drug resistance happens frequently in cancers [7]; therefore, determining common mutations and differentially expressed genes (DEGs) associated with chemoresistance can be useful in overcoming multi-drug resistance. This study using gene expression profile GSE173606 data looked for genes with differential expression between 5-Fu resistance and sensitive human COAD HCT8 cell line as well as between DDP resistance and sensitive cells. This research aimed to explore the potential common hub genes related to chemoresistance because targeting the key upregulated genes could be effective in reversing the resistance to chemotherapy drugs. In addition, the correlation of hub genes with cancer-associated fibroblasts (CAFs) infiltration to TME was investigated. Overlapped mRNA-miRNA-lncRNA ceRNA networks associated with chemoresistance were constructed to find key miRNAs. The identified common genes can be used in diagnostic and therapeutic approaches.

2. Methods:

2-1 Data processing and determining DEGs

The GSE173606 data package containing the microarray data of acquired 5-Fu and DPP-resistant and sensitive HCT8 cell line from the GEO database were obtained. DEGs with $|\log2FC| > 0$ and adjusted P value ≤ 0.05 between drug resistance and sensitive groups were identified via GEO2R online tools and R program. DEGs with $|\log2FC| \geq 1$ were selected for further analysis.

2-2 Determining hub genes

We used STRING database [8] for construction of a protein-protein interaction (PPI) network for DEmRNAs associated with both 5-Fu and DDP resistance in the HCT8 cell line. The results were visualized with CytoScape software. To select the hub genes, CytoHubba, as a plug-in of CytoScape, was employed to identify the hub genes. Degree method as a network centrality measure was used to identify hub genes participating in PPI network. Genes with the higher interactions have the higher values (Score > 100) in Degree method and regarded as hub genes.

2-3 Evaluations of hub genes

We evaluated the correlation between upregulated hub genes and CAF infiltration. According to TIMER2.0 database [9], different methods (EPIC, XCELL, TIDE, and MCP-COUNTER) were performed for the association analysis. The Gene Expression Profiling Interactive Analysis (GEPIA) database was used for the evolution of the expression of the identified hub genes.

2-4 Construction of common ceRNA networks

Targets of common DEmiRNAs (mRNAs and lncRNAs) were determined from the miRTarBase [10] and RNAInter [11]. Overlapping of the targets with common DEmRNAs or DElncRNAs was found by Venn diagram. Finally, the mRNA-miRNA-lncRNA ceRNA network was visualized using the Cytoscape software platform. The drug sensitivity analysis of DEmRNAs was analyzed using the RNAactDrug database [12].

3. Results:

3-1 Different gene expression between resistant and sensitive cells

About 1544 genes, including 1203 mRNAs and 341 ncRNAs were high-expressed and 1900 genes, including 1272 mRNAs and 628 ncRNAs were low-expressed in 5-Fu resistance cells. In DDP resistance cells, 1328 high-

expressed and 1029 low-expressed mRNAs and 434 high-expressed and 608 low-expressed ncRNAs were detected (Fig 1). Venn diagram showed the 1780 overlapped DEmRNAs in the 5-Fu and DPP resistance cells. These common DEmRNAs and their related DEmiRNAs and DElncRNAs (with |log2FC| ≥ 1 and adjusted P value ≤ 0.05) were selected for future analyses.

3-2 PPI network construction of common 5-Fu and DDP related genes and selection of hub genes

Degree algorithm in CytoHubba was used to determine the interactions. The PPI network for the top 100 genes was reconstructed by Gephi software and reanalyzed to identify the hub genes (Fig. 2). Seven overexpressed common hub genes, including EGFR, TGFB1, ESR1, ICAM1, PECAM1, CAV1, and CCL5 and five downregulated, including CD44, CXCL8, CCL2, HIF1A, and PTGS2 were determined (Table 1). CCL5 and ICAM1 were also high-expressed in COAD compared to normal tissues and CCL2 and PTGS2 were also low-expressed in COAD compared to normal tissues based on GEPIA database. However, EGFR, TGFB1, ESR1, PECAM1, and CAV1 were downregulated and CD44, CXCL8, and HIF1A were upregulated in COAD compared to normal tissues based on GEPIA database (Supplementary Figure 1).

TIMER2.0 database showed positive correlations (0.75 ≥ methods) for all the seven high-expressed hub genes with CAFs infiltration (Fig. 3).

3-3 mRNA-miRNA-lncRNA ceRNA network associated with chemoresistance in human colon cancer HCT8 cell line

As depicted in Fig. 4, there are 8 downregulated and 9 upregulated miRNAs in common ceRNA networks of chemoresistance cells. Based on miRTarBase database, 50 high-expressed mRNAs have interaction with the 8 miRNAs and 18 low-expressed mRNAs have interaction with the 9 miRNAs. The high-expressed lncRNAs namely HOXA-AS3 can act as a competing endogenous RNA for miR-4306 and cause the upregulation of its targets.

According to RNAactDrug database, the expression of EFNB2, F2RL2, FLT1, ADGRF1, TCF4, and CCDC80 are negatively related to the drug sensitivity of DDP ($P < 0.01$). mRNAs of EFNB2, F2RL2, and ADGRF1 genes were increased in both chemoresistance cells and in COAD tissues. However, mRNAs of TCF4 and CCDC80 genes were increased in chemoresistance cells and decreased in COAD tissues based on GEPIA database (Fig.5).

4. Discussion

Although there have been reported several routes (such as small molecules or natural anticancer agents) for reversing drug resistance in cancer, chemoresistance is still an unsolved problem in cancer treatment [13, 14]. Therefore, finding the key genes and molecular mechanisms involved in drug resistance seems to be necessary. This study aimed to identify the key genes

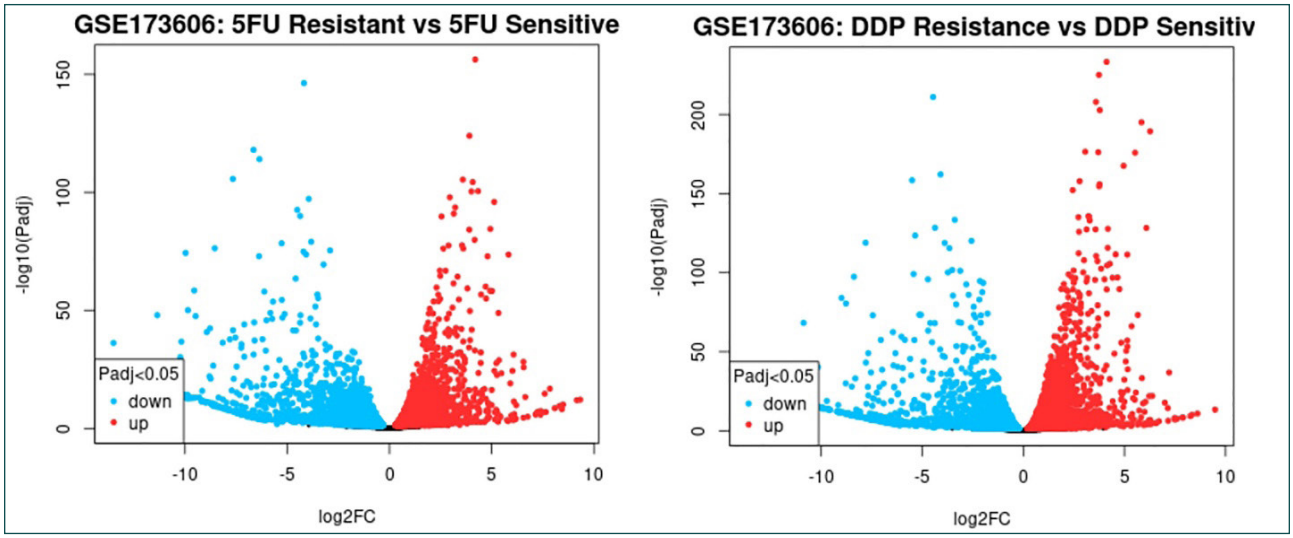

Figure 1. Tolcano plots of GSE173606 for acquired 5-Fu and DDP resistance COAD cells obtained from GEO2R analysis. X axis indicates the log2FC (or log2 fold change) of genes and Y axis is related to the adjusted P value which is the negative log10 of the adjusted P value. Red dots represent upregulated genes (log2FC > 0) and blue dots represent downregulated genes (log2FC < 0). In this study, genes with log2FC ≥ 1 were considered high-expressed and genes with log2FC ≤ -1 were considered low-expressed genes in chemoresistance cells.

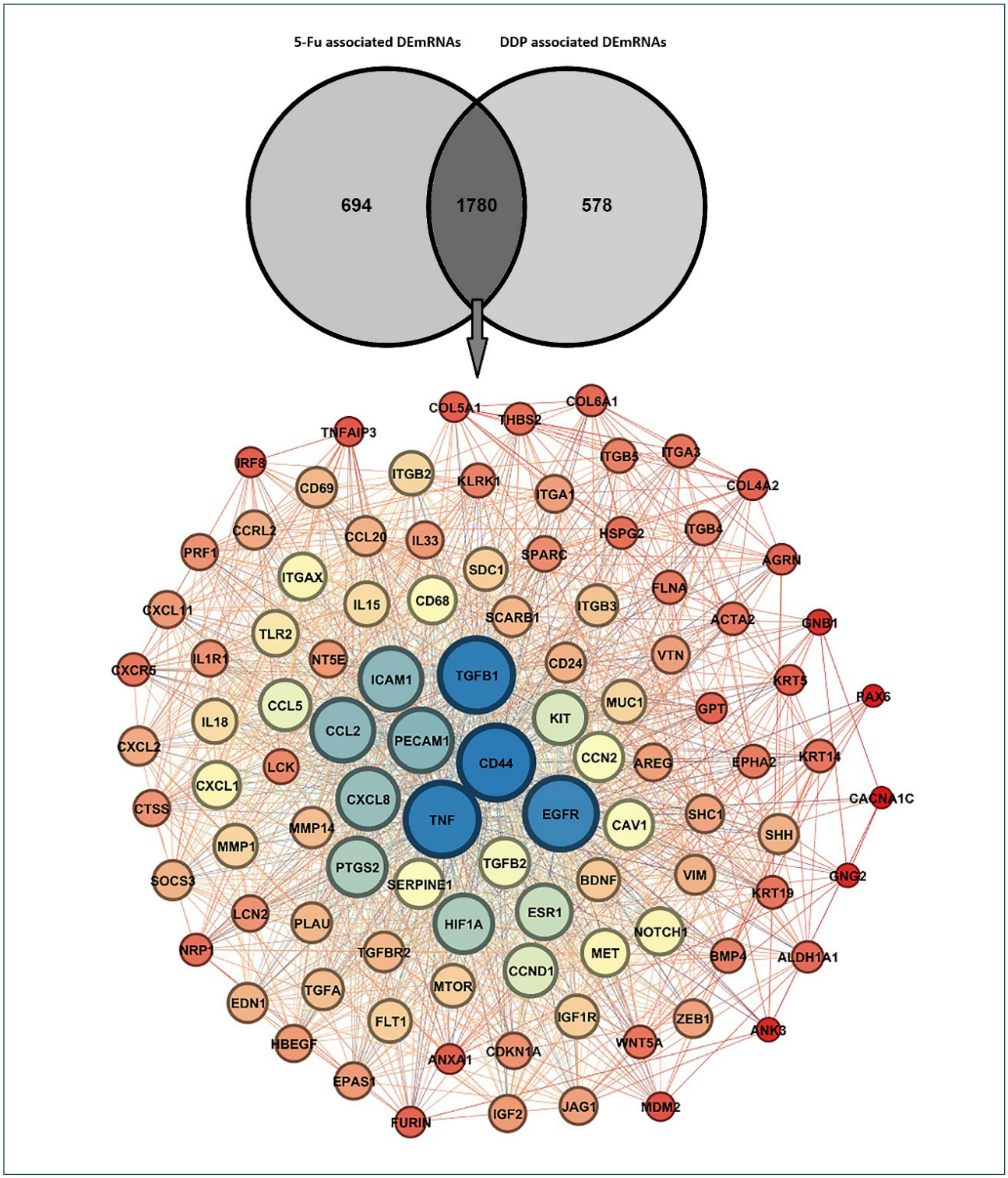

Figure 2. PPI network construction. Venn diagram showed the 1780 overlapped DEmRNAs in the 5-Fu and DPP resistance cells. PPI network of top 100 common chemoresistance associated DEmRNAs in Gephi (version 0.1) based on Degree algorithm, genes with more interactions are depicted with the high intensity of blue color and larger circles.

Table 1. Common identified hub genes in acquired 5-Fu and DDP- resistance COAD cells based on Degree algorithm in CytoHubba.

Rank	Name	Score	Log2FC	
			5-Fu-R compared to 5-Fu-S	DDP-R compared to DDP-S
1	EGFR (epidermal growth factor receptor)	243	1.29	1.72
2	CD44 (CD44 molecule)	170	-2.69	-2.84
3	TGFB1 (transforming growth factor beta 1)	163	1.50	1.78
4	ESR1 (estrogen receptor 1)	141	3.08	2.33
5	CXCL8 (C-X-C motif chemokine ligand 8)	140	-2.45	-4.00
6	CCL2 (C-C motif chemokine ligand 2)	134	-4.16	-4.06
7	ICAM1 (intercellular adhesion molecule 1)	124	1.38	1.60
7	HIF1A (hypoxia inducible factor 1 subunit alpha)	124	-1.10	-1.07
9	PECAM1 (platelet and endothelial cell adhesion molecule 1)	119	1.10	2.04
10	PTGS2 (prostaglandin-endoperoxide synthase 2)	115	-3.5	-6.5
11	CAV1 (caveolin 1)	112	2.81	2.53
12	CCL5 (C-C motif chemokine ligand 5)	101	1.71	1.90

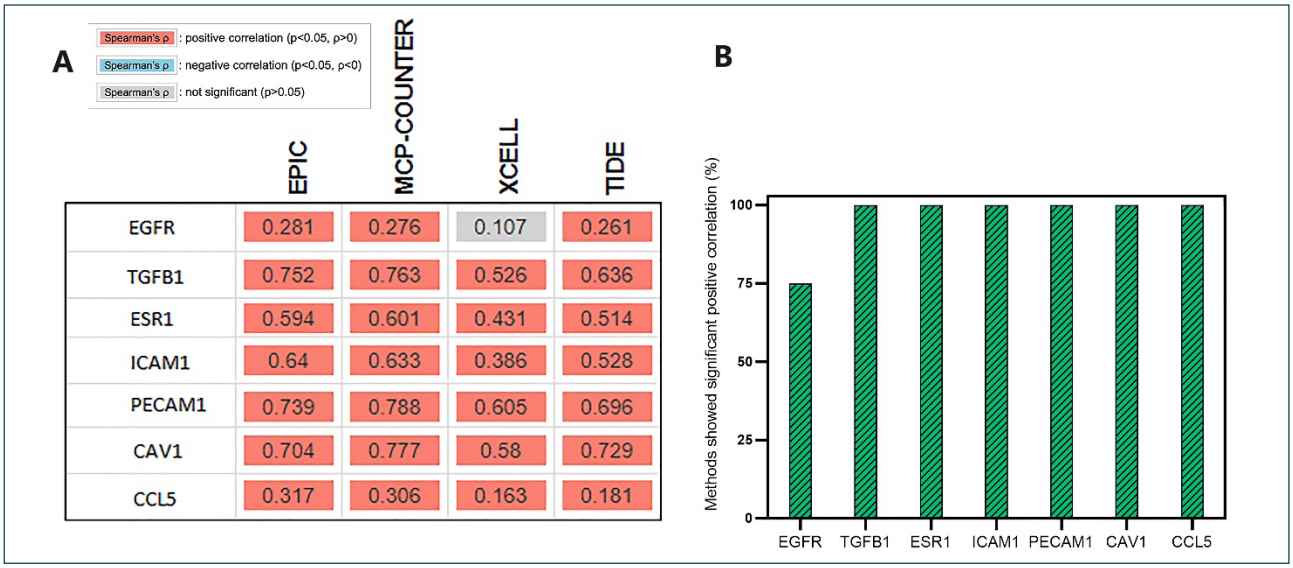

Figure 3. Correlation of high-expressed hub genes (log2FC > 1, P value < 0.05) with CAFs infiltration in COAD. A) According to TIMER2.0 database, different methods (EPIC, XCELL, TIDE, and MCP-COUNTER) were performed for the association analysis. B) Bar chart represents the percentages of methods showed positive correlation between upregulated hub genes and CAFs tumor infiltration for each gene.

involved in the multi-drug resistance COAD HCT8 cell line. A high number of common DEmRNAs (about 1780) in 5-Fu and DDP resistance HCT8 cells was identified. Seven overexpressed common hub genes, including EGFR, TGFB1, ESR1, ICAM1, PECAM1, CAV1, and CCL5 and five downregulated, including CD44, CXCL8, CCL2, HIF1A, and PTGS2 were determined.

Ye et al. [15] showed that overexpression of ESR1 or ESR-α was related to decreased chemosensitivity to 5-Fu and the present study reinforced this. The blockade of the three upregulated hub genes, namely EGFR, TGFB1, and CCL5 has been suggested as some treatment routes in COAD in previous studies [16-18]. CCL5 is a significantly high-expressed gene in COAD chemoresistance cells and COAD tissues. Overexpression of CCL5 has been associated with immune cell infiltration, drug resistance, and metastasis in several studies [19, 20]. According to the TIMER2.0 database, the upregulated hub genes (EGFR, TGFB1, ESR1, ICAM1, PECAM1, CAV1, and CCL5) can recruit CAFs to the TME milieu. The TME can have an essential role in drug resistance properties [21]. CAFs are the main cellular components of TME. Many studies showed that CAFs can have a role in chemoresistance, radiotherapy, and immunotherapy resistance. Paracrine (such as secreting exosomes and cytokines) and contact-dependents signaling between CAFs and cancer cells have been suggested as an underlying mechanism in producing therapy resistance TME [22]. Additionally, CAFs by secreting cytokines and ECM proteins can produce immunosuppressive and remodeled ECM [23]. In this study, the relationship between the hub genes and CAFs recruitment emphasizes the effect of TME in chemoresistance.

In ceRNA networks, three upregulated miR-675-3p (log2FC > 2), miR-6084 (log2FC > 1.6), and miR-1182 (log2FC > 1.2) target mRNAs which their expression is downregulated both in chemoresistance cells and in COAD compared to normal tissues. These mRNAs include IGF1R and EHD3 (targets of miR-675-3p and miR-6084), ZNF704 (target of miR-675-3p), TNFAIP2 (a target of miR-6084), and CD1D and KLHL3 (targets of miR-1182). The oncogenic role of miR-675-3p in COAD

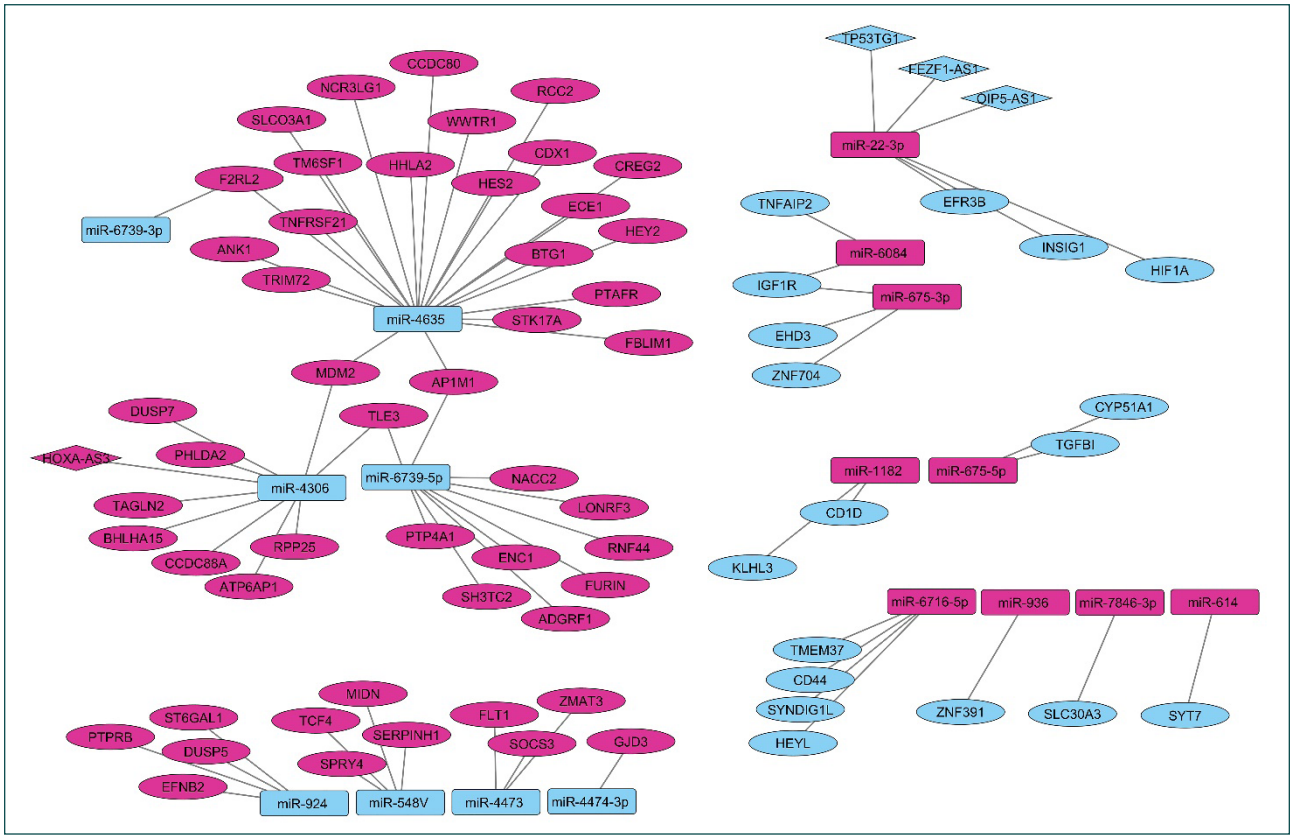

Figure 4. PCommon mRNA-miRNA–lncRNA network in chemoresistance cells visualized using the Cytoscape software. Purple represents upregulated and blue represents downregulated genes. LncRNAs are depicted in diamond, miRNAs in rectangle, and mRNAs in circle.

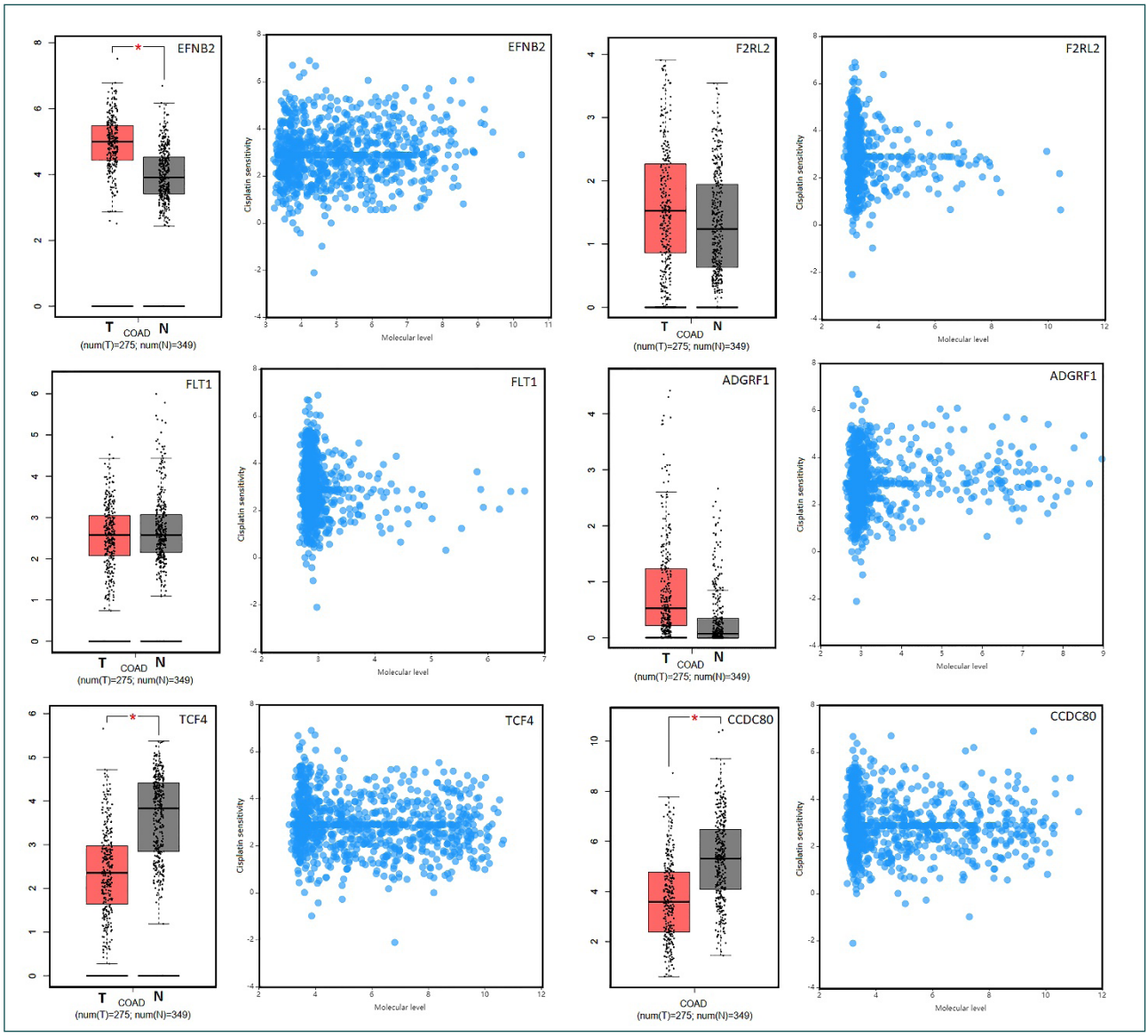

Figure 5. The molecular level of EFNB2, F2RL2, FLT1, ADGRF1, TCF4, and CCDC80 genes (which are high-expressed in chemoresistance cells) are negatively related to drug sensitivity of DDP based on RNAactDrug database ($P < 0.01$). mRNAs of EFNB2, F2RL2, and ADGRF1 genes are upregulated in COAD tissues. However, mRNAs of TCF4 and CCDC80 genes are decreased in COAD tissues based on GEPIA database.

has been confirmed previously [24]. Therefore, the knockdown of miR-675-3p might be a good choice for gene targeted therapy in chemoresistance COAD. EHD3, as a target of miR-675-3p, is frequently downregulated by several tumor types and confers lower chemotherapy response rates in COAD [25].

In the ceRNA network, MDM2 is under the control of two downregulated microRNAs (miR-4635 and miR-4306). It was reported negative association between MDM2 overexpression and chemosensitivity [26]. MDM2 is a crucial inhibitor of the TP53 tumor suppressor [27] which is an upregulated gene in both COAD tumor tissues (according to GEPIA) and chemoresistance cells in this study. Normally, TP53 causes cell cycle arrest or apoptosis in damaged cells but most cancer cells lose this crucial brake because of many reasons such as mutations or MDM2 upregulation [28]. Blocking of TP53 through MDM2 prevents cell cycle arrest or apoptosis induction in chemotherapy induced DNA damages. MDM2 inhibitors led to TP53 reactivation and increased

chemosensitivity [26]. TLE3 as another upregulated mRNA in the ceRNA network is under the control of two downregulated microRNAs (miR-6739 and miR-4306); however, high TLE3 expression is associated with increased chemosensitivity in taxane-treated breast and ovarian cancers [29].

The expression of EFNB2, FLT1, F2RL2, and ADGRF1 was negatively correlated with the drug sensitivity of DDP according to the RNAactDrug database. Considering that the mentioned four genes are upregulated in COAD based on GEPIA, they can be also suggested as the genes in targeted therapy to overcome the chemoresistance in COAD. EFNB2 and FLT1 are overexpressed genes in several cancers and correlated with the malignant progression of tumors, including COAD. So, blocking EFNB2 and FLT1 has been suggested as alternative therapies for COAD [30,31]. Silencing of EFNB2 inhibited cell growth and migration of COAD cells [31].

5. Conclusion

In general, the common DEGs in chemoresistance cells were screened out. It was found some mRNAs and miRNAs that their manipulation could increase the drug sensitivity of COAD cells; for example, the knockdown of EGFR, TGFB1, CCL5, and miR-675-3p. EGFR, TGFB1, and CCL5, as upregulated hub genes, by recruiting the CAFs can induce immune abnormalities in TME, which is important in chemoresistance. In addition to therapeutic potential, some hub genes (e.g., EGFR, TGFB1, ESR1, PECAM1, CAV1, CD44, CXCL8, and HIF1A) can be used as chemoresistance diagnostic markers because their changes in chemoresistance cells are in the contrary of the COAD tissues (based on GEPIA). Further research is needed to confirm these results obtained from bioinformatic methods.

Conflict of Interests: The authors report there are no competing interests to declare.

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