Mitochondria-Related Core Genes and TF-miRNA-hub mrDEGs Network in Breast Cancer

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Research

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Abstract

Background

Mitochondria-nuclear cross talk and mitochondrial retrograde regulation are involved in the genesis and development of breast cancer (BC). Therefore, mitochondria can be regarded as a promising target for BC therapeutic strategies. In the present study, we aimed to construct regulating network and seek the potential biomarkers of BC diagnosis, prognosis and also the molecular therapeutic targets from the perspective of mitochondrial dysfunction.

Methods

The microarray data of mitochondria-related encoding genes of BC were downloaded from GEO including GSE128610 and GSE72319. GSE128610 was treated as test set and validation sets consisted of GSE72319 and TCGA, which were used for identifying mitochondria-related differential expressed genes (mrDEGs). We performed enrichment analysis, PPI network, hub mrDEGs, and overall survival analysis and constructed transcription factor (TF)-miRNA-hub mrDEGs network.

Results

A total of 23 up-regulated and 71 down-regulated mrDEGs were identified and validated. Enrichment analyses indicated that mrDEGs were associated with several cancer-related biological processes, Moreover, 9 hub mrDEGs were identified and validated in tissues. Finally, 5 hub coregulated mrDEGs, 21 miRNA and 117 TF were used to construct TF-miRNA-hub mrDEGs network. MAZ, HDGF and SP2 could regulate 3 hub mrDEGs. hsa-mir-21-5p, hsa-mir-1-3p, hsa-mir-218-5p, hsa-mir-26a-5p, and hsa-mir-335-5p regulated 2 hub mrDEGs. Overall survival analysis suggested that the up-regulated FN1 and down-regulated DDR2 conferred to poor BC prognosis.

Conclusion

TF-miRNA-hub mrDEGs has instruction significance for the etiology exploration of BC. The identified hub mrDEGs, such as FN1 and DDR2, were likely to regulate mitochondrial function and might be novel biomarkers of BC diagnosis and prognosis as well as the therapeutic targets.

1. Introduction

Mitochondria, the only extranuclear organelle carried with genetic material, plays an important role in carcinogenesis through its communication and retrograde regulation of nucleus (1). The Reactive Oxygen Species in mitochondria were suggested to promote proliferation, migration and apoptosis of tumor cells (2). The mitochondria in BC cells could exert retrograde regulation of nucleus by transmitting signal to them, facilitating the bidirectional communication between each other (3), and making mitochondria an anticancer drug target for tumor. In addition, mitochondria from non-cancer cell lines has been shown to suppress multiple carcinogenic pathways and reverse the carcinogenic properties of tumor cells under the

same nuclear background, including cell proliferation, viability in hypoxia, anti-apoptosis property, resistance to anticancer drugs, invasion, colony formation, and enhancing the response of tumor cells to treatment (4). These findings emphasize that mitochondria have critical regulatory roles for cancer cell property. The correction of mitochondrial function is a promising target of anticancer therapy (4).

miRNA could participate in the whole signal pathway of tumorigenesis and tumor progression, including the regulation of mitochondrial function in tumor progression(5, 6). miRNAs can also be regarded as one of the driving factors to trigger Otto Warburg effect, thus affecting tumor progression(6). Transcription factors (TF) are regulatory factors at the transcription level, which can regulate the progression of breast cancer(7, 8), and also modulate mitochondria biogenesis and mitochondria-to-nuclear communication(9, 10). Since the transcription of mRNA and miRNAs are regulated by transcription factors (TFs) and the expression of TFs is modulated by miRNAs(11), and they were closely related to mitochondria function, it is of great importance to construct regulating network for “TF-miRNA-hub mrDEGs network” to explore mitochondria dysfunction of BC.

Breast cancer (BC) is the most common cancer and the leading cause of cancer-related death in female (12, 13). The exploration of potential biomarkers and regulation mechanism for early diagnosis and therapeutic targets of BC has important scientific significance and application value. In recent years, it remains rare about the differential analysis and network regulation mechanism of mitochondria-related encoding genes in BC. Hence, the model and network construction for predicting early BC diagnosis and prognosis via bioinformatics would greatly benefit the identification of potential mitochondrial diagnostic biomarkers, therapeutic targets, and pathogenic mechanism for BC. In the present study, two microarray datasets of mitochondria-related genes in BC were collected from Gene Expression Omnibus (GEO), of which one served as test set and the other served as validation set. Then the mitochondria-related differential expressed genes (mrDEGs) were screened out and validated by TCGA database. Our study aims to focus on mrDEGs, construct potential TF-miRNA-mrDEGs network and seek potential diagnostic and prognostic biomarkers as well as the molecular therapeutic targets of BC from the perspective of mitochondrial dysfunction.

2. Materials And Methods

2.1 Data collection

To identify mrDEGs involved in BC genesis and development, two datasets (GSE128610 and GSE72319) were collected from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/gds/). GSE128610 contained three BC cell lines (MDA-MB-468) and three BC-free epithelial cell lines (MCF10A). GSE72319 was composed of three triple-negative BC cell lines (SUM159) and three benign BC cell lines (A1N4). Both of them adopted transmitochondrial cybrid system (Cybrid), which is well acknowledged in mitochondrial function research currently. For the Cybrid model, the nucleus in experimental and control groups were both replaced by other cells' to eliminate the interference of nuclear encoding genes in mitochondrial function research (14, 15). BC data was downloaded from The Cancer Genome Atlas (TCGA,

https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), including 1112 BC tissues and 113 normal breast tissues.

2.2 Data processing

In this research, GSE128610 was treated as test set, while GSE72319 and TCGA data respectively served as the first and second validation set. To identify mrDEGs, the original microarray datasets of GSE128610 and GSE72319 were analyzed using GEO2R, and TCGA data was processed by edgeR and SangerBox. The screening criteria were set at |logFC|>=1, P<0.05 and P. adjust<0.05. GSE72319 and TCGA were successively used to verify mrDEGs in GSE128610 through the “MATCH function”. FunRich 3.1.3 was used to paint the heatmap to visualize these validated mrDEGs.

2.3 GO enrichment analysis and KEGG mapping

Gene ontology (GO) analysis was performed for validated mrDEGs by Search Tool for the Retrieval of Interacting Genes (STRING), including cellular component, molecular function, and biological process. P<0.05 was considered as statistical significance. KEGG mapper (https://www.genome.jp/kegg/mapper.html) was applied to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway map of mrDEGs.

2.4 Protein–protein interaction (PPI) network construction and modeling analysis

PPI network of validated mrDEGs was constructed by STRING. The cut-off value of Interaction score was set at 0.4, and PPI network was visualized. Subsequently, the classical models were screened out by Molecular Complex Detection (MCODE) plug-in of Cytoscape_3.7.2 based on the criteria of score > = 3 and nodes > = 3. The function enrichment analysis of single model was performed by STRING, and P < 0.05 was considered as cut-off criteria.

2.5 Screening and tissues identification of hub mrDEGs

The hub mrDEGs were selected by cytohubba plug-in of Cytoscape_3.7.2 according to MCC>=6. Next, Ualcan (http://ualcan.path.uab.edu/) was employed to validate the expression levels of the mrDEGs of BC tissue samples.

2.6 Construction of TF-hub mrDEGs network and miRNA-hub mrDEGs network

NetworkAnalyst (https://www.networkanalyst.ca/), a website for comprehensive gene expression analysis, meta-analysis & network biology, was applied to find TF and miRNA targeting hub mrDEGs (16). Hub mrDEGs were uploaded to NetworkAnalyst to acquire the TF targeting hub mrDEGs from ENCODE database and miRNA-hub mrDEGs pairs from TarBase and miRTarBase. Two interaction lists were downloaded and Cytoscape_3.7.2. was used to visualize TF-hub mrDEGs network and miRNA-hub mrDEGs network respectively.

2.8 TF-miRNA- hub mrDEGs network construction

In order to construction a TF-miRNA-hub mrDEGs network, TF-hub mrDEGs network and miRNA-hub mrDEGs network were overlapped. Coregulated hub mrDEGs of TF and miRNA were selected to build TF-miRNA-hub mrDEGs network and visualize by Cytoscape_3.7.2.

2.8 Survival analysis based on coregulated hubmrDEGs by TF and miRNA

The correlation between coregulated hub mrDEGs and the overall survival of 1402 BC cases was analyzed applying Kaplan Meier plotter (http://kmplot.com/analysis/). The hazard ratios with 95% confidence intervals and P values of log rank test were calculated and displayed in the figure. P<0.05 was regarded to be statistically significant.

3. Results

3.1 The identification and validation of mrDEGs in BC

The flow diagram was shown in Figure 1. GSE128610 and GSE72319 datasets were analyzed online by GEO2R. The mrDEGs in BC were identified based on the cut-off criteria of |logFC|>=1, P<0.05 and P. adjust<0.05. We found out 1756 up-regulated and 3225 down-regulated mrDEGs in GSE128610. Subsequently, 251 up-regulated and 1162 down-regulated mrDEGs in GSE128610 were validated by GSE72319. After initial validation, they were further verified by TCGA data, and then 23 up-regulated and 71 down-regulated mrDEGs were finally identified (Table 1, Figure 2).

3.2 GO function enrichment and KEGG pathway map for mrDEGs

GO enrichment analysis of 94 validated mrDEGs was performed by STRING. The results were presented in Table 2 and Table S1. The mrDEGs in BC were shown to play roles in cancer-related biological processes, such as neural crest cell migration involved in autonomic nervous system development, regulation of cell migration, cell surface receptor signaling pathway and cell differentiation. However, no significant molecular function or cellular component was observed.

The mrDEGs were mapped in KEGG pathway. They were suggested to participate in the following cancer-related pathways: PI3K-ALT pathway, TGF-beta pathway, evading apoptosis, and resistance to chemotherapy (Figure 3).

3.3 mrDEGs-related PPI network construction and Modeling analysis

PPI network of 94 mrDEGs in BC was constructed by STRING database, with a total of 94 nodes and 60 edges (Figure 4a). Three models were found to meet the criteria of score>=3 and nodes>=3 by MOCODE plug-in of Cytoscape software (Figure 4b). GO and KEGG enrichment analyses were carried out for the three models respectively (Table 3 and Table S2). The results showed that model 1 was mainly associated with the structure and function of nerve cells, model 2 was involved in extracellular matrix, and model 3 took parts in tissue development and gene expression.

3.4 Selection and tissues validation of hub mrDEGs

We screened out 9 hub mrDEGs according to MCC>=6 by cytoHubba plug-in of Cytoscape. Among them, up-regulated hub mrDEGs contained FN1, BGN, EFNA3, COL5A2 and SEMA3F, and down-regulated hub mrDEGs comprised RHOQ, SEMA3A, NRP1 and DDR2 (Table 4). Consistent results were obtained after we validated and visualized the expression of 9 hub mrDEGs by Ualcan with tissue samples (Figure 5).

3.5 Analysis of TF-hub mrDEGs network

In order to explore the potential regulatory relationships of hub mrDEGs, we predicted the TF targeting hub mrDEGs with ENCODE database. The result demonstrated that 8 hub mrDEGs were matched excepting for COL5A2. The Cytoscape software was applied to visualize the TF-hub mrDEGs network, and 167 associations between 8 hub mrDEGs and 121 TF were predicted (Figure 6). As is shown, MAZ could regulate 5 hub mrDEGs (eg. EFNA3, SEMA3A and SEMA3F), and SP2, MXD4, KLF9, KLF16, HDGF and ARID4B regulated 3 hub mrDEGs.

3.6 Analysis of miRNA-hub mrDEGs network

Subsequently, miRNA-hub mrDEGs pairs of 9 hub mrDEGs were performed with TarBase and miRTarBase. Finally, only 6 hub mrDEGs were mapped excepting for BGN, SEMA3F and SEMA3A, next 31 associations between 25 miRNA and 6 hub mrDEGs were obtained by Cytoscape software (Figure 7). We found that hsa-mir-21-5p could interact with 3 hub mrDEGs (eg. COL5A2 and DDR2).

3.7 Construction of TF-miRNA-hub mrDEGs network analysis

The hub mrDEGs (FN1, EFNA3, NRP1, RHOQ and DDR2) which coregulated by TF and miRNA were selected and their interactive regulators were extracted, and then building a TF-miRNA-hub mrDEGs network by Cytoscape (Figure 8). A total of 5 hub mrDEGs, 21 miRNA and 117 TF were included in the TF-miRNA-hub mrDEGs network. Next, we analyzed the interactive results of TF-mrDEGs and miRNA-hub network respectively (Table 5). We found that MAZ, HDGF and SP2 could regulate 3 hub mrDEGs. Simultaneously, hsa-mir-21-5p, hsa-mir-1-3p, hsa-mir-218-5p, hsa-mir-26a-5p, and hsa-mir-335-5p regulated 2 hub mrDEGs. In addition, FN1, EFNA3, and NRP1 were the highest degree score in interaction network.

3.8 Survival analysis of coregulated mrDEGs by TF and miRNA

Kaplan Meier plotter was adopted to analyze overall survival curve of with the expression of 5 coregulated hub mrDEGs by TF and miRNA in BC. Two hub mrDEGs with statistical significance were identified in the survival analysis ($P<0.05$, n=1402), including FN1 (HR = 1.28 (1.03 − 1.59), P = 0.023) and DDR2 (HR = 0.77 (0.62 − 0.96), P = 0.017). Therefore, the up-regulated FN1 and down-regulated DDR2 might confer to poor BC prognosis (Figure 9).

4. Discussion

In this study, the microarray data of mitochondria-related genes in BC collected from GEO were used to identify the mrDEGs further validated in TCGA. Moreover, GO enrichment analysis and KEGG pathway mapping for validated mrDEGs were performed to explore the potential function of mrDEGs in breast carcinogenesis. Based on that, we constructed the PPI network, discovered, and validated the hub mrDEGs. Furthermore, we constructed the TF-miRNA-hub mrDEGs network and analyzed the correlation between coregulated hub mrDEGs and the overall survival of BC patients to investigate the influence of coregulated hub mrDEGs on BC prognosis.

Mitochondria play a critical role in multiple cell processes, and mitochondrial dysfunction may affect the occurrence and development of BC. The initiation and metastasis of BC could be altered by regulating the genetic background of mitochondria, making mrDEGs potential therapeutic targets (17). Here, we utilized multiple bioinformatics tools to analyze the microarray data of mitochondria-related genes, and found out 23 up-regulated and 71 down-regulated mrDEGs in BC. They were closely associated with mitochondrial dysfunction in breast carcinogenesis. GO enrichment analysis demonstrated that 94 mrDEGs were enriched in cancer-related biological processes, such as neural crest cell migration involved in autonomic nervous system development, regulation of cell migration, cell surface receptor signaling pathway, cell differentiation and regulation of cell communication. These biological processes conformed to tumor cell properties, including unlimited cell proliferation, cell invasion and migration, and reduced intercellular adhesion (18), suggesting that mrDEGs were tightly linked to breast carcinogenesis. KEGG pathway mapping showed that mrDEGs might participate in cancer-related regulation pathways, including PI3K-ALT pathway, TGF-beta pathway, evading apoptosis, and resistance to chemotherapy. The relationship of them with BC could be listed as follows: (1) Inhibiting PI3K-ALT pathway may induce mitochondria-mediated cell apoptosis of BC (19); (2) Ligand-dependent or cell-autonomous activation of the TGF-β pathway in stromal cells could induce metabolic reprogramming, enhance oxidative stress, mitochondrial autophagy and aerobic glycolysis, and decrease Cav-1, which can spread to adjacent fibroblasts and maintain BC cell growth (20); (3). Regarding the well-known property of unlimited proliferation in BC cells, the GSTs gene mapped in evading apoptosis pathway could regulate cell apoptosis by its interaction with various protein partners (21); (4). MITF, a differential gene identified in our research, is able to enhance mitochondrial oxidative phosphorylation (22). It has been reported that enhanced mitochondrial oxidative phosphorylation may induce the resistance to chemotherapy of BC cells (23), thus these genes could be related to drug resistance of BC cells. Overall, the validated mrDEGs mentioned above might be enriched in the pathways for BC progression through regulating mitochondrial function.

PPI network analysis indicated that three interaction networks could be classical models to predict BC occurrence. Model 1 consisted of SEMA3F, EFNA3, SEMA3A and NRP1, which were mainly associated with the structure and function of nerve cells. EFNA3 was induced by HIF under anoxic conditions, and then Ephrin-A3 protein encoded by EFNA3 was aberrantly accumulated to promote the metastasis of BC cells (24). Model 2 was composed of BGN, DDR2 and COL5A2, which were mainly involved in extracellular matrix of cells. COL5A2 related to extracellular matrix remodeling was up-regulated during ductal carcinoma in situ developed to invasive ductal carcinoma, leading to BC progression (25). Model 3

included ZEB1, VIM and FN1, which participated in tissue development and gene expression. ZEB1 increased the expression of vascular endothelial growth factor (VEGF) via paracrine to stimulate angiogenesis in BC (26). ZEB1 also promoted epithelial mesenchymal transformation (EMT), proliferation and migration of BC (27). All the three models with different function took their parts in BC progression.

In our study, 9 hub mrDEGs were screened out based on MCC method, including up-regulated FN1, BGN, EFNA3, COL5A2 and SEMA3F as well as down-regulated RHOQ, SEMA3A, NRP1 and DDR2. Ualcan was used to validated and visualized the expression of 9 hub mrDEGs. TF-miRNA-hub mrDEGs network was constructed, which has important instruction significance to explore the potential regulating mechanism of hub mrDEGs of BC. The results of TF-miRNA-hub mrDEGs network showed that MAZ of TF nodes can interact with 3 hub mrDEGs including EFNA3, NRP1, RHOQ, which implied its significance in BC. Myc-associated zinc finger protein (MAZ) has been considered as a transcription factor with C2H2 zinc finger motif that can be bind with a GA box(28, 29), and plays an important role in the progression of BC(29, 30).The study suggested that the transactivation and transcriptional alteration of MAZ could modulated the process of aerobic glycolysis in tumor(31). NRP1 targeting hub mrDEGs of MAZ could be located in mitochondria and regulate mitochondrial function and iron-dependent oxidative stress(32). A GEO dataset (GSE115118) about miRNA mitochondria sublocalization indicated that hsa-mir-218-5p, hsa-mir-26a-5p, hsa-mir-335-5p regulated by NRP1 could be located in mitochondria. Thus, we predicted that MAZ has potential to impact on mitochondria function by interacting with NRP1 and these miRNAs to regulate the progression of BC. Further study needs to be done to explore these hub mrDEGs function in BC.

The results of survival analysis showed that up-regulated FN1 and down-regulated DDR2 suggested poor BC prognosis ($P<0.05$) with the potential to be a significant biomarker. FN1 has been demonstrated to be up-regulated in BC epithelial cells without mitochondria DNA (33). FN1 was also a core gene of mrDEGs network and its encoded fibronection distributed in BC cell matrix affecting tumor progression (33). Meanwhile, FN1 could regulate EMT of BC cells (34) and might be one of the key genes in BC invasion and migration (35). In addition, TF-miRNA-hub mrDEGs network showed that FN1 has the highest degree score and also can interact with hsa-mir-218-5p and hsa-mir-26a-5p which have been sublocated in mitochondria (GSE115118). DDR2 was activated by fibrillar collagen to regulate the synthesis of extracellular matrix and wound healing (36), exerting important roles in microenvironment. DDR2 was involved in hypoxia-induced cancer metastasis by accelerating migration, invasion and EMT of BC cells (37). TF-miRNA-hub mrDEGs network showed that DDR2 could interact with hsa-mir-21-5p, hsa-mir-548a-3p and hsa-mir-129-2-3p which also have been sublocated in mitochondria (GSE115118). Further investigations on these genes would help to elucidate BC etiology from the perspective of mitochondrial dysfunction, and thus to identify diagnostic and prognostic biomarkers and also molecular targets for BC targeted therapy.

In summary, we employed bioinformatics analyses to discover mrDEGs in BC. Then enrichment analyses for these genes were carried out and three interaction networks were constructed to serve as classical models for predicting breast carcinogenesis. We also selected 5 coregulated hub mrDEGs by TF and

miRNA, including FN1, DDR2, NRP1, EFNA3 and RHOQ, to construct TF-miRNA-hub mrDEGs network to explore the potential pathogenic mechanism of hub mrDEGs of BC.

Abbreviations

mrDEGs: mitochondria-related differential expressed genes; BC: breast cancer; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein network. TF, transcription factor.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the results of this manuscript are available from the corresponding author upon reasonable request.

Competing Interests

All authors disclose no conflicts of interest that might bias their work.

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Authors' contributions

Yuan Yuan and Qian Xu conceived and designed this study. Li-rong Yan and Ang Wang were responsible for the data analysis and performed data interpretation. Li-rong Yan wrote the paper. Qian Xu, Zhi Lv and Yuan Yuan revised the manuscript.

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Tables

Table 1 Validation of mrDEGs via GSE72319 and TCGA

mrDEGs	Total	Genes name
up-regulated	23	BGN LSR SEMA3F RCN3 PADI3 DEPDC7 OASL ISG15 H2AFJ EFNA3 FN1 COL5A2 CHTF18 COL9A3 FAM132A SERINC2 KRT81 ISYNA1 NXPH4 ASPM CELSR1 GPC2 HES4
down-regulated	71	NAP1L2 EPB41L4B ZEB1 MSRB3 QKI DOCK11 JADE1 SNCA PROS1 TACC1 CXCL2 CCDC136 RHOQ MECOM PIK3R1 PYGO1 SYNM EHHADH MEF2C MDFIC MBNL2 ZDHHC2 SLC16A7 FGD4 TMEM47 MAP3K8 ANK2 VIM EHBP1 ARHGEF40 PTPN21 DSEL DDR2 KCTD12 MITF CACNA2D1 MGST1 FIGN SAMD5 NR3C2 FAM126A VKORC1L1 MYLK ARHGEF28 AKAP12 CLIP4 ZNF106 CPS1 DLC1 PIP5K1B LPIN1 BHLHE41 SATB1 MCTP1 NRP1 CCDC50 DPP4 MARK1 SEMA3A NR3C1 EMP1 SACS NFIB LRCH2 LIMCH1 RECK BICC1 PDLIM3 MAP7D3 PARD3B GPAM

mrDEGs, mitochondria-related differential expressed genes

Table 2 GO enrichment analysis of mitochondria-related differential expressed genes

Term ID	Term description	FDR	Genes
GO:1901166	neural crest cell migration involved in autonomic nervous system development	0.00015	FN1, NRP1, SEMA3A, SEMA3F
GO:0030334	regulation of cell migration	0.0054	CXCL2, DDR2, DLC1, EPB41L4B, FN1, LIMCH1, MCTP1, MEF2C, MYLK, NRP1, PIK3R1, RECK, SEMA3A
GO:0007166	cell surface receptor signaling pathway	0.006	ARHGEF28, BGN, CELSR1, CXCL2, DDR2, EFNA3, FN1, GPC2, ISG15, MAP3K8, MARK1, MEF2C, MITF, NRP1, OASL, PIK3R1, PYGO1, RHOQ, SEMA3A, SEMA3F, SNCA, VIM, ZEB1, ZNF106
GO:0030154	cell differentiation	0.0172	ANK2, ARHGEF28, ASPM, BHLHE41, CCDC136, CELSR1, CPS1, DOCK11, EFNA3, FN1, GPC2, HES4, KRT81, LSR, MARK1, MECOM, MEF2C, MGST1, MITF, NAP1L2, NFIB, NRP1, PIK3R1, PYGO1, QKI, SATB1, SEMA3A, SEMA3F, VIM, ZEB1
GO:0010646	regulation of cell communication	0.0114	AKAP12, ANK2, ARHGEF28, ARHGEF40, ASPM, BGN, BICC1, CXCL2, DEPDC7, DLC1, DPP4, FAM132A, FGD4, FN1, GPC2, JADE1, MAP3K8, MCTP1, MDFIC, MECOM, MEF2C, NRP1, PIK3R1, PIP5K1B, RHOQ, SEMA3A, SEMA3F, SNCA, ZEB1

Table 3 Enrichment analysis of models

Category	Term ID	Term description	FDR	Genes
Module 1	GO:0021612	facial nerve structural organization	3.66E-08	NRP1, SEMA3A, SEMA3F
	GO:0021637	trigeminal nerve structural organization	3.66E-08	NRP1, SEMA3A, SEMA3F
	GO:0021785	branchiomotor neuron axon guidance	3.66E-08	NRP1, SEMA3A, SEMA3F
	GO:0038191	neuropilin binding	9.71E-05	SEMA3A, SEMA3F
	hsa04360	Axon guidance	4.53E-08	EFNA3, NRP1, SEMA3A, SEMA3F
Module 2	GO:0030198	extracellular matrix organization	0.00093	BGN, COL5A2, DDR2
	GO:0030199	collagen fibril organization	0.0011	COL5A2, DDR2
	GO:0001503	ossification	0.0241	COL5A2, DDR2
Module 3	GO:0019221	cytokine-mediated signaling pathway	0.0121	FN1, VIM, ZEB1
	GO:0045664	regulation of neuron differentiation	0.0121	FN1, VIM, ZEB1
	GO:0009888	tissue development	0.0246	FN1, VIM, ZEB1
	GO:0010628	positive regulation of gene expression	0.0268	FN1, VIM, ZEB1
	GO:0045666	positive regulation of neuron differentiation	0.027	FN1, ZEB1

Table 4 Hub mrDEGs of MCC>=6

Genes name	MCC	Differentially expressed
FN1	25	up-regulated
EFNA3	12	up-regulated
NRP1	12	down-regulated
BGN	10	up-regulated
RHOQ	9	down-regulated
COL5A2	8	up-regulated
SEMA3A	7	down-regulated
DDR2	6	down-regulated
SEMA3F	6	up-regulated

mrDEGs, mitochondria-related differential expressed genes

Table 5 Hub mrDEGs of coregulated by miRNA and TF

TF	Hub mrDEGs	Gene counts	miRNA	Hub mrDEGs	Gene counts
MAZ	EFNA3, NRP1, RHOQ	3	hsa-mir-218-5p	NRP1, FN1	2
HDGF	DDR2, EFNA3, RHOQ	3	hsa-mir-26a-5p	NRP1, FN1	2
SP2	EFNA3, NRP1, RHOQ	3	hsa-mir-335-5p	NRP1, RHOQ	2
ARID4B	FN1, NRP1	2	hsa-mir-1-3p	NRP1, FN1	2
BCL11A	EFNA3, NRP1	2	hsa-mir-21-5p	DDR2, RHOQ	2
BCL11B	EFNA3, NRP1	2	hsa-mir-338-3p	NRP1	1
CREB3L1	EFNA3, RHOQ	2	hsa-mir-16-5p	NRP1	1
CTBP2	EFNA3, NRP1	2	hsa-mir-148a-3p	NRP1	1
DRAP1	EFNA3, FN1	2	hsa-mir-181a-5p	NRP1	1
GATAD2A	EFNA3, FN1	2	hsa-mir-124-3p	NRP1	1
KLF8	EFNA3, NRP1	2	hsa-mir-152-3p	NRP1	1
MBD1	FN1, NRP1	2	hsa-mir-26b-5p	FN1	1
MLLT1	DDR2, FN1	2	hsa-mir-200b-3p	FN1	1
MXD4	NRP1, RHOQ	2	hsa-let-7g-5p	FN1	1
TGIF2	FN1, RHOQ	2	hsa-mir-200c-3p	FN1	1
TSHZ1	EFNA3, NRP1	2	hsa-mir-548a-3p	DDR2	1
WT1	EFNA3, NRP1	2	hsa-mir-129-2-3p	DDR2	1
ZBTB7A	EFNA3, RHOQ	2	hsa-mir-375	RHOQ	1
ZFP2	EFNA3, FN1	2	hsa-mir-210-3p	EFNA3	1
ZNF2	EFNA3, NRP1	2	hsa-mir-224-5p	EFNA3	1
ZNF324	EFNA3, FN1	2	hsa-mir-330-3p	EFNA3	1
ZNF423	EFNA3, NRP1	2			
ZNF580	FN1, NRP1	2			
ZNF589	DDR2, RHOQ	2			
ZNF610	EFNA3, NRP1	2			

mrDEGs, mitochondria-related differential expressed genes; TF, transcription factor.

Figures

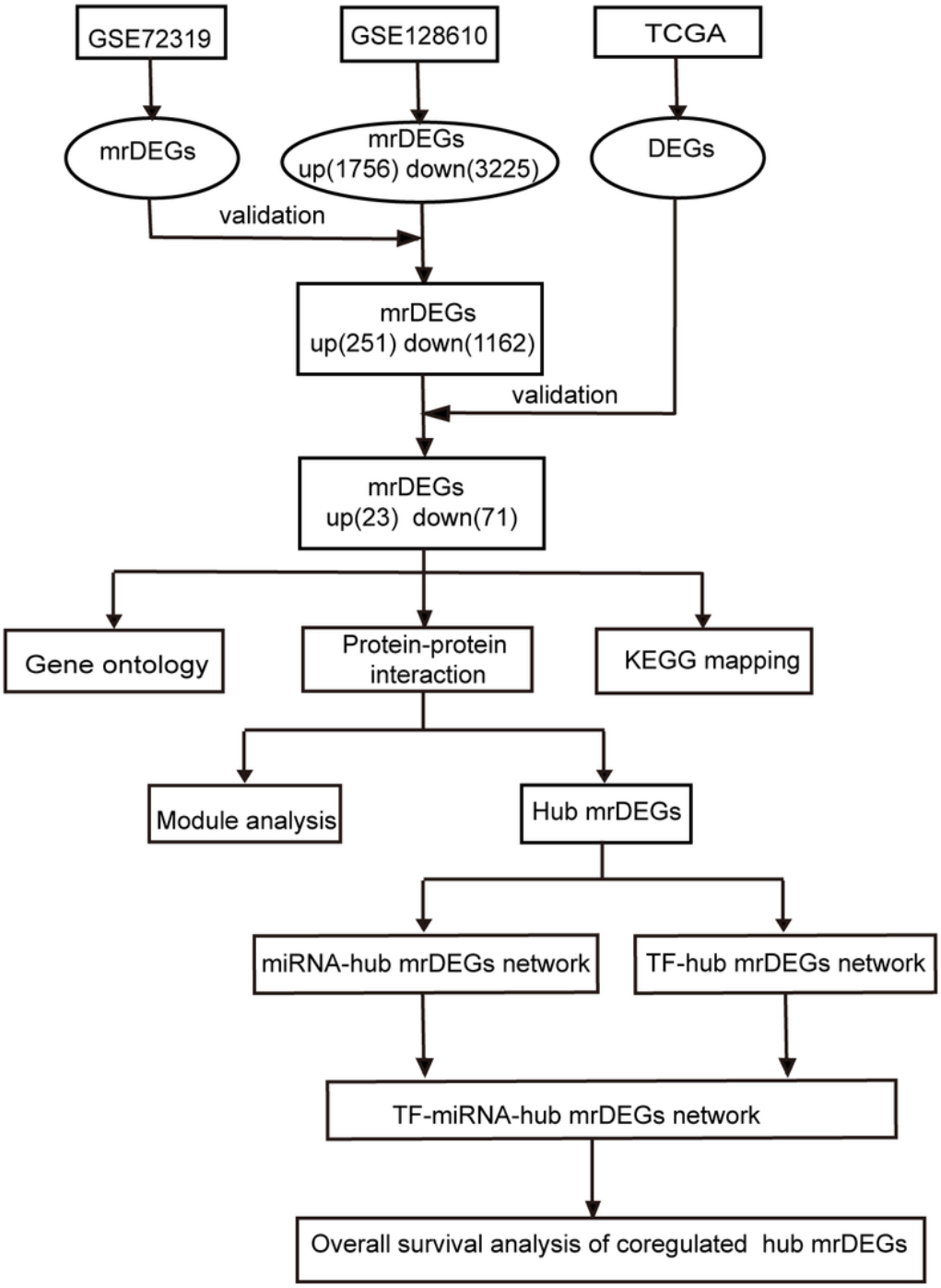

Figure 1

Flow diagram of bioinformatics analysis. mrDEGs, mitochondria-related differential expressed genes. DEGs, differential expressed genes.

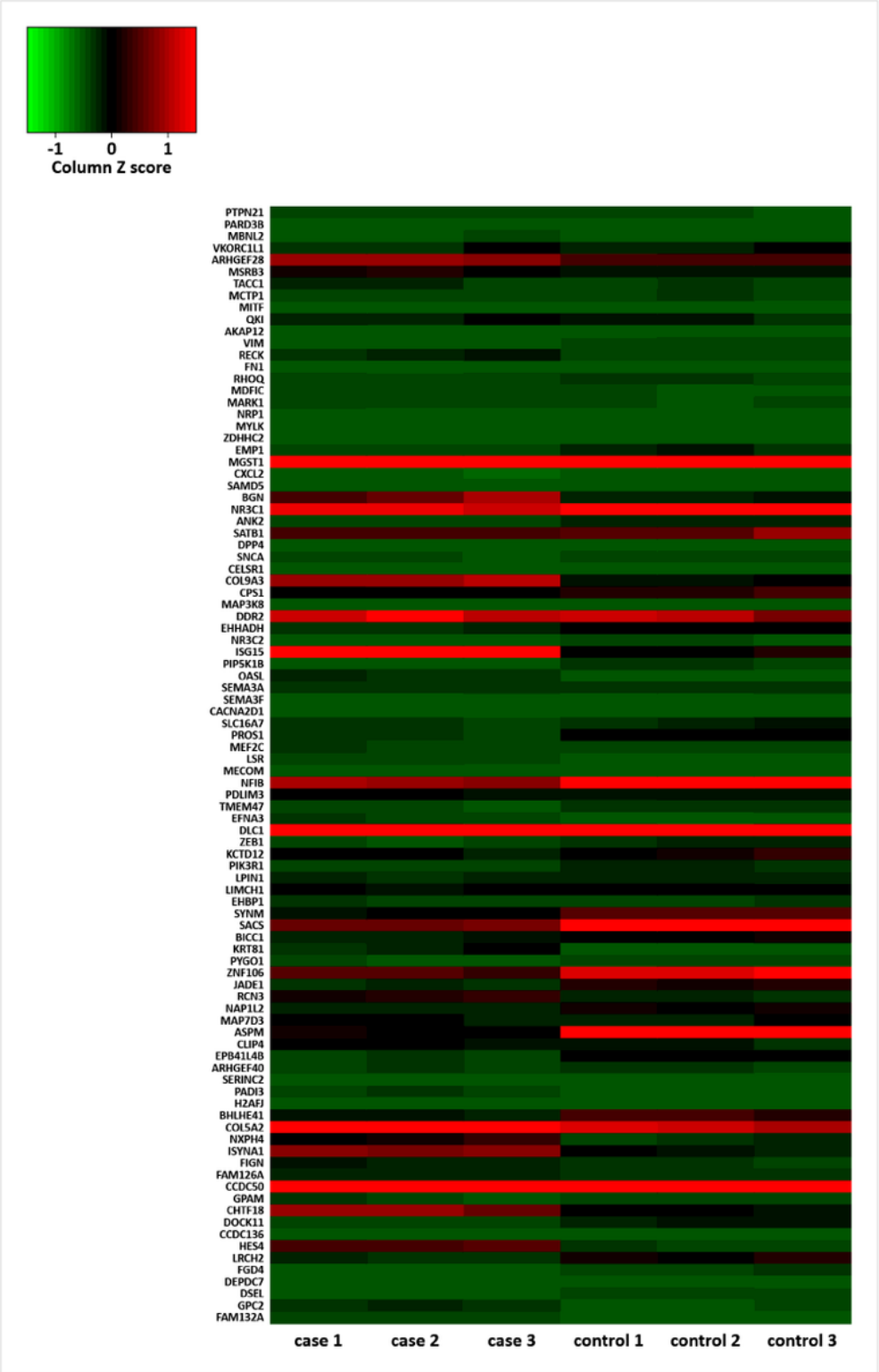

Figure 2

Heatmap of 94 mitochondria-related differential expressed genes.

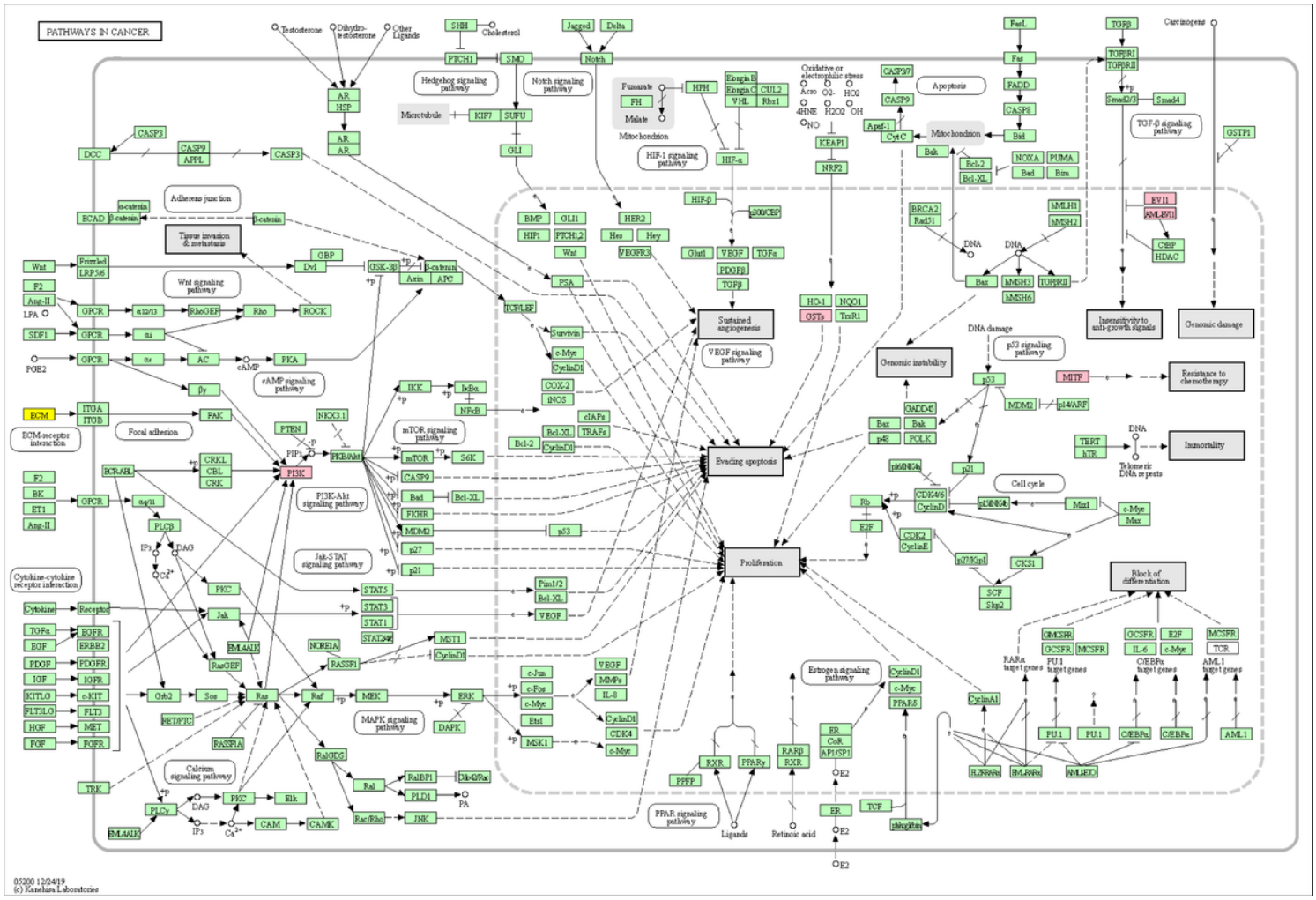

Figure 3

mrDEGs were annotated in KEGG pathway. Pink represented down-regulated mrDEGs and yellow represented up-regulated mrDEGs. mrDEGs, mitochondria-related differential expressed genes.

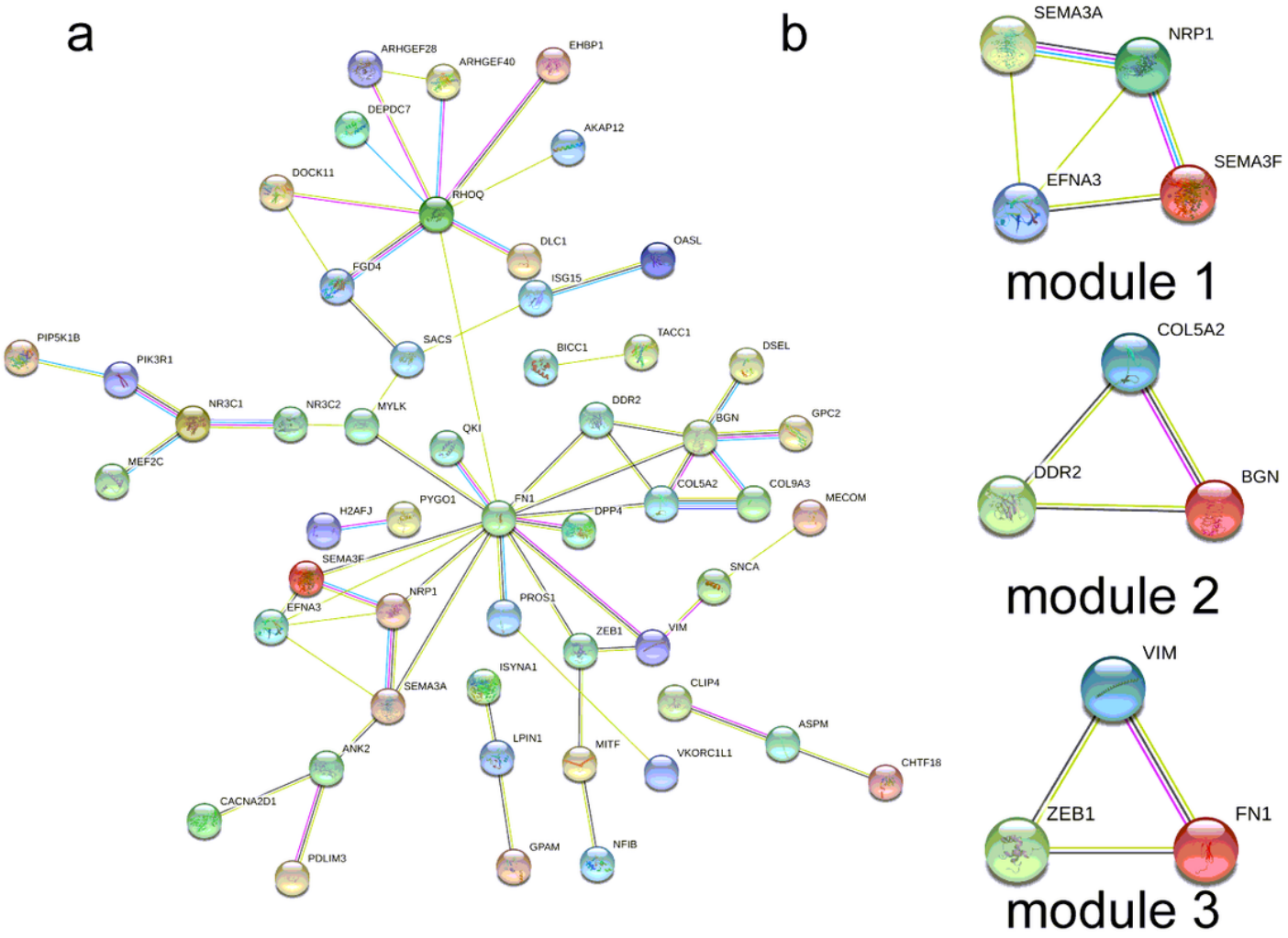

Figure 4

protein-protein interaction network and top 3 modules of 94 mrDEGs in breast cancer. a. PPI network; b. Top modules 1-3. mrDEGs, mitochondria-related differential expressed genes.

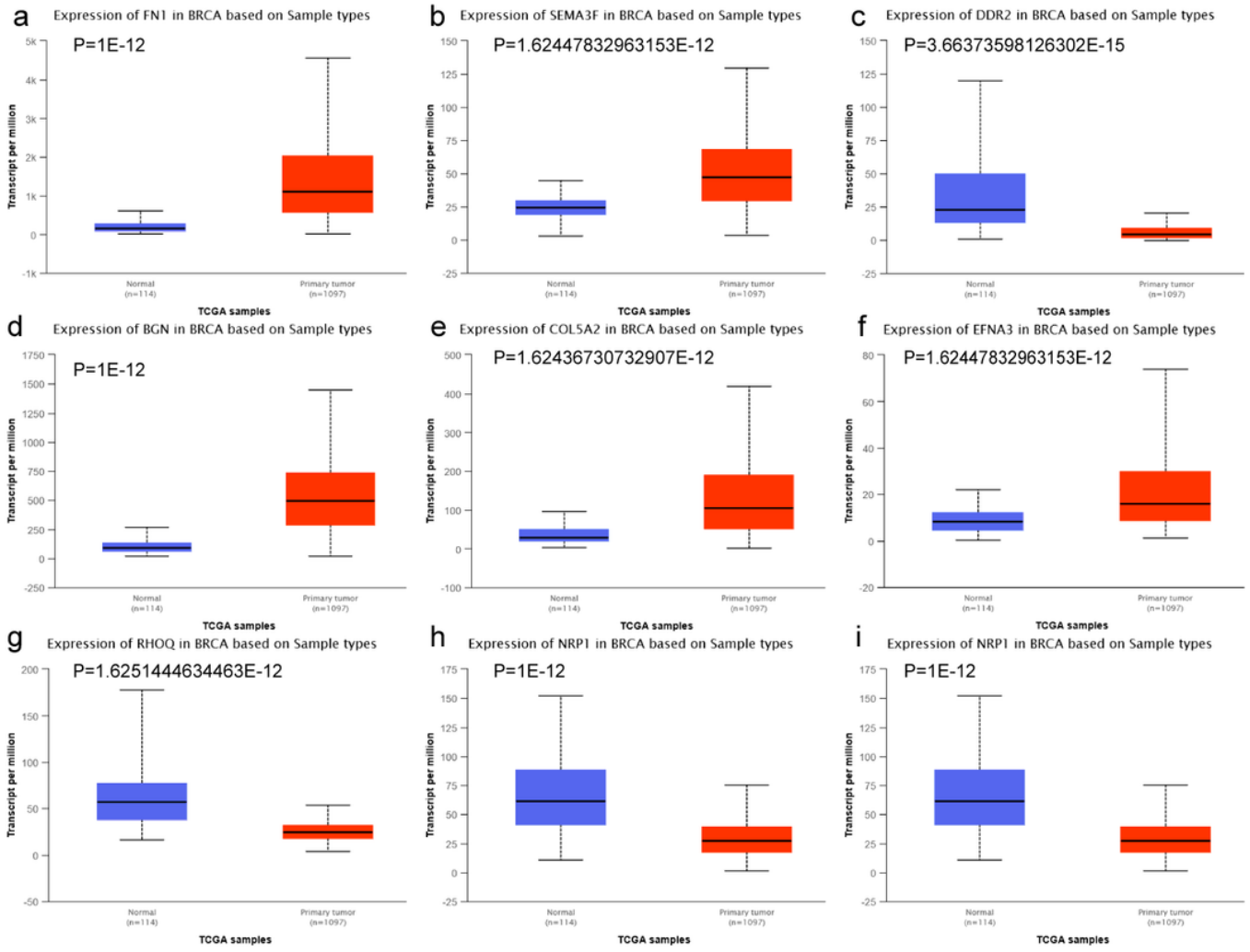

Figure 5

The expression levels of hub mrDEGs in tissue samples. FN1(a), SEMA3F(b), DDR2(c), BGN(d), COL5A2(e), EFNA3(f), RHOQ(g), NRP1(h), SEMA3A(i). mrDEGs, mitochondria-related differential expressed genes.

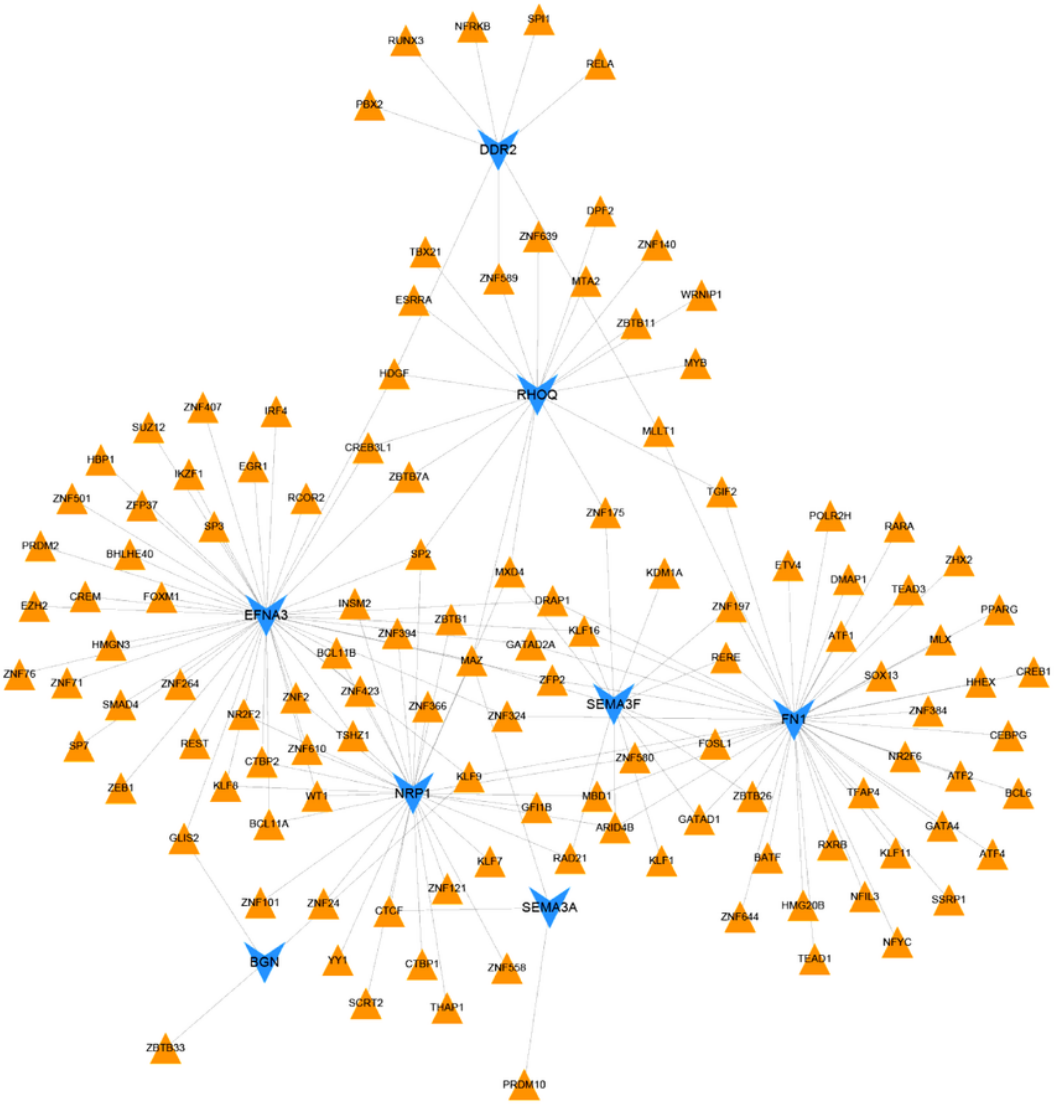

Figure 6

TF-hub mrDEGs interaction network. Blue color represented mrDEGs and orange color represented TF. mrDEGs, mitochondria-related differential expressed genes; TF, transcription factor.

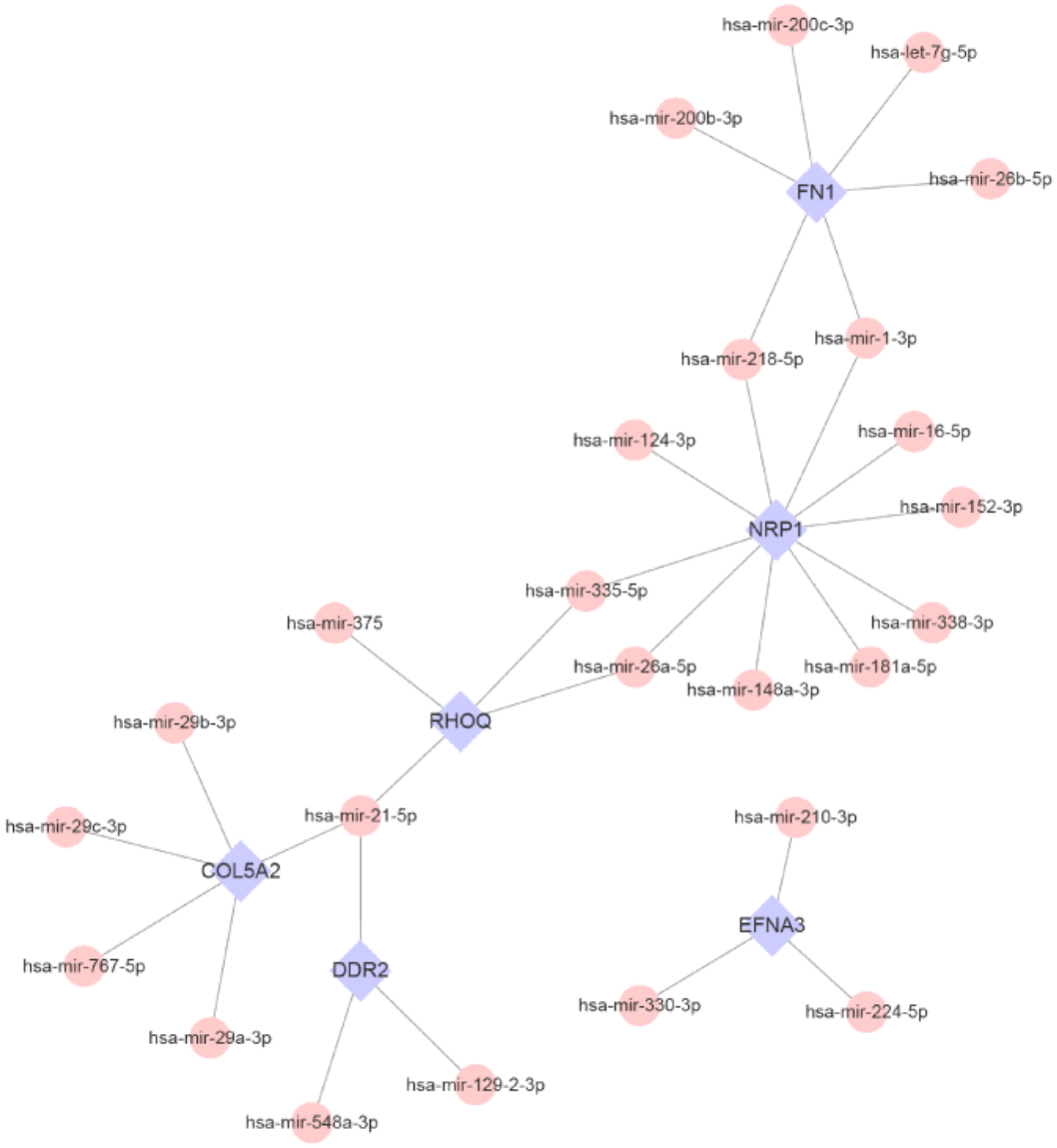

Figure 7

miRNA-hub mrDEGs interaction network. Blue color represented mrDEGs and pink color represented miRNA. mrDEGs, mitochondria-related differential expressed genes.

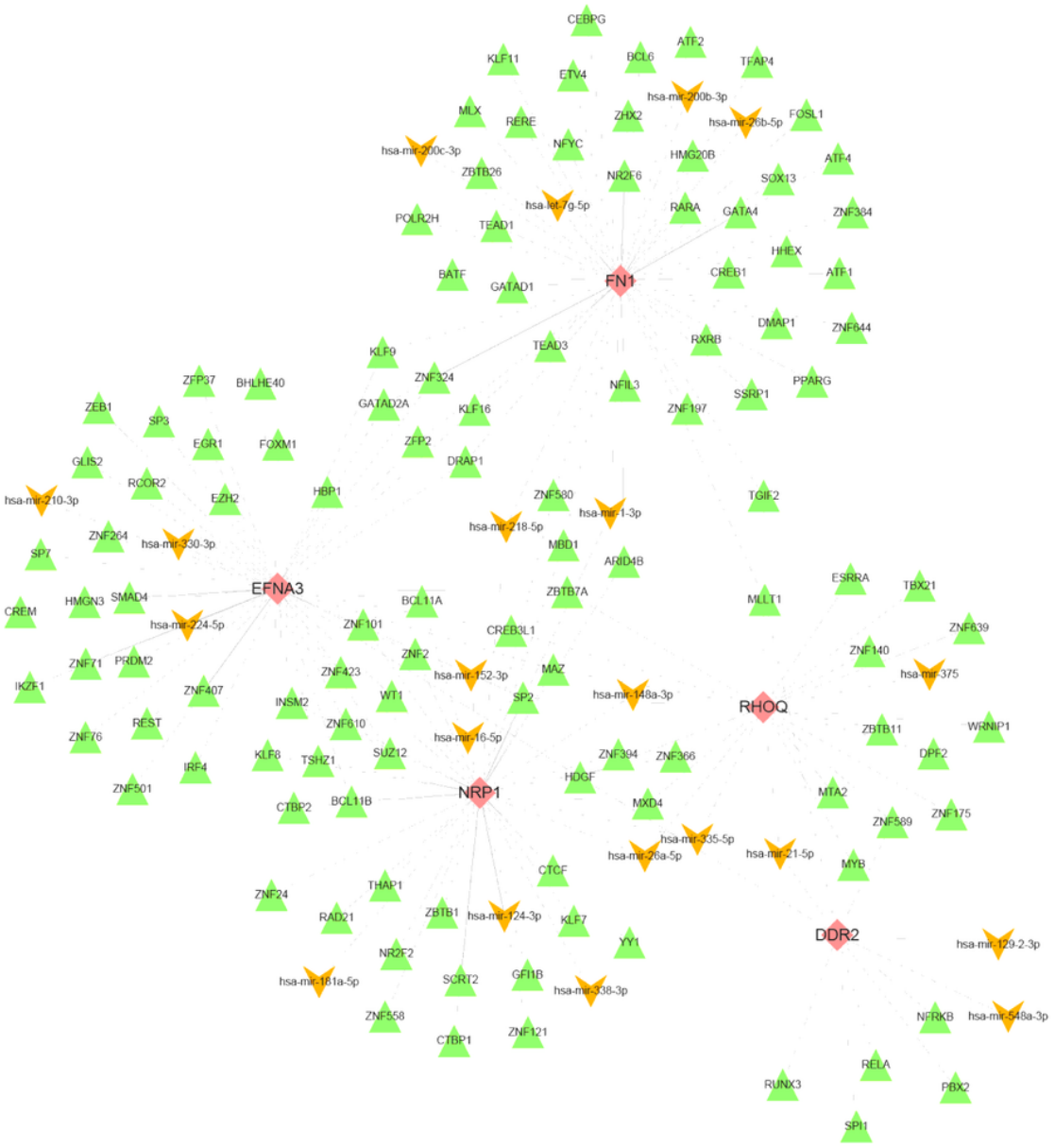

Figure 8

TF-miRNA-hub mrDEGs interaction network. Pink color represented mrDEGs, orange color represented miRNA and green color represented TF. mrDEGs, mitochondria-related differential expressed genes; TF, transcription factor.

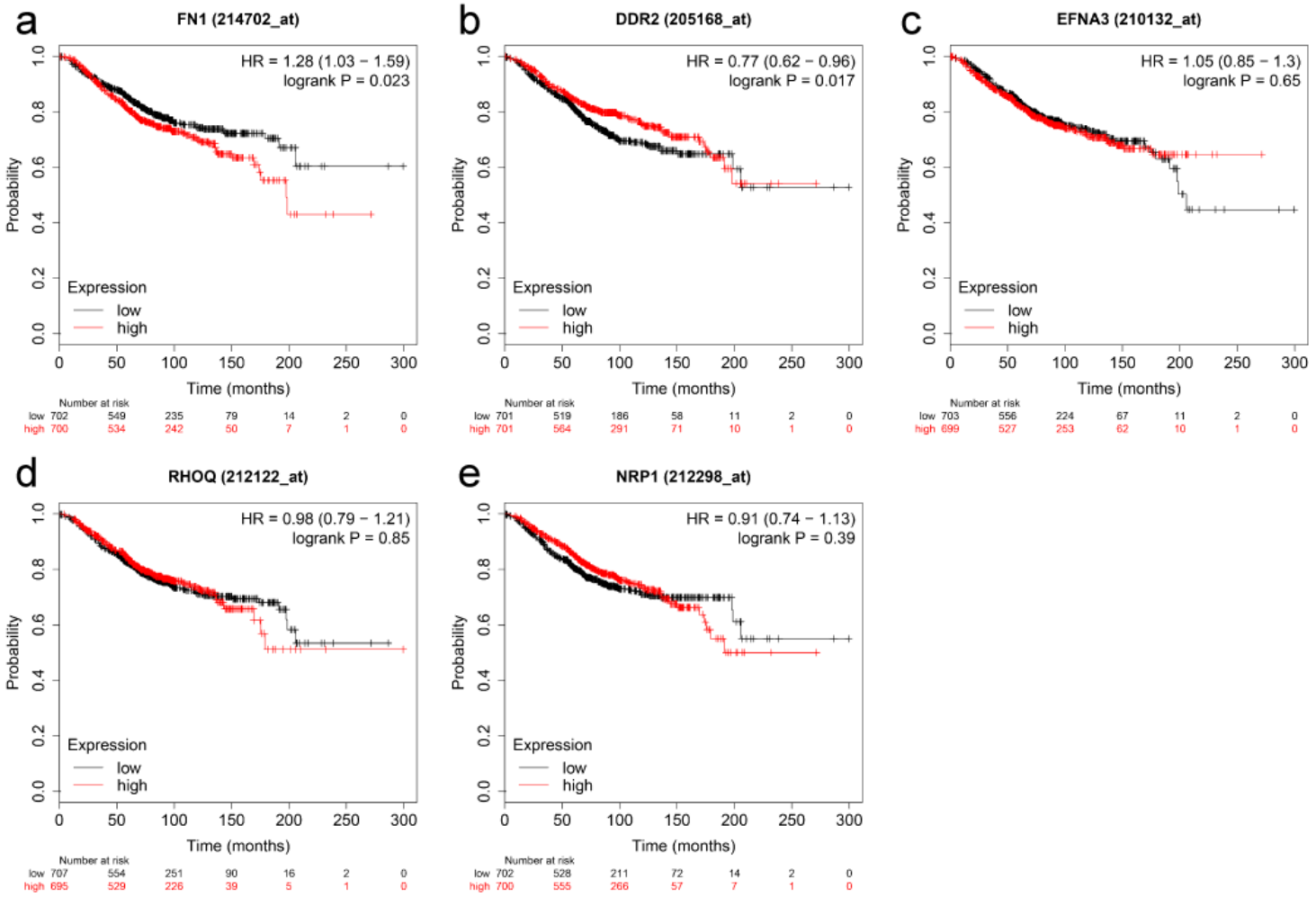

Figure 9

Overall survival analysis of 5 hub mrDEGs coregulated by TF and miRNA were performed by Kaplan-meier plotter. Red color represented higher expression and black color represented lower expression. The up-regulated FN1 have significant worse overall survival rate (P<0.05). FN1(a), DDR2(b), EFNA3(c), RHOQ(d), NRP1(g). HR, hazard ration; CI, confidence interval; mrDEGs: mitochondria-related differential expressed genes.

Supplementary Files

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- Supplementarymaterials.docx