Effect of Differential Expression of Genes Induced by Radiation Therapy In Cancer-associated fibroblasts Cell on Patients' Prognosis

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Research

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Abstract

To investigate the effect of radiation therapy on differential expression of genes in tumor-associated fibroblasts and prognosis of patients.

Methods: The tumor-associated fibroblast gene expression profile data chip GSE37318 after radiotherapy treatment was retrieved from the GEO database, and the differentially expressed genes were screened using the limma R software package; GO and KEGG pathway enrichment analysis was performed using the DAVID tool; Protein interaction networks was built by String and Cytoscape software and core genes were obtained; GEPIA was used for prognostic value analysis; Immunohistochemistry was used to detect the expression of the top 5 hub genes in tumor tissues of patients in the radiotherapy and non-radiotherapy groups.

Results: 144 genes were up-regulated and 54 genes were down-regulated, which were mainly enriched in functional pathways such as cell stress, DNA damage, cell cycle, aging, apoptosis, oxidative stress, and p53 signaling pathway. The protein interaction network was constructed and the top 20 hub genes were obtained. Prognostic analysis showed that: Expression of up-regulated PCNA and hub genes that were down-regulated after irradiation, such as MCM10, DLGAP5, FANCI, CENPA, CDC6, FBXO5, NCAPG, and DTL, has a negative correlation with the overall survival time of lung cancer patients ($p < 0.05$). Immunohistochemical results showed that PCNA gene expression was up-regulated in patients with radiotherapy compared with patients without radiotherapy. The test results are consistent with the results of the biochemical analysis.

Conclusion: Radiotherapy can induce differential expression of genes in tumor-associated fibroblasts, and these differentially expressed genes can be used as potential molecular markers for tumor radiotherapy effect and patient prognosis.

Background:

Lung cancer is currently the most malignant tumor with the highest morbidity and mortality in the world, and its morbidity is still increasing year by year, which seriously harms people's physical and mental health [1]. The early symptoms of lung cancer are not obvious, so most of the clinical patients are often in the middle and advanced stages at the time of diagnosis, and radiotherapy and chemotherapy have become the main method of treatment[2–3].

Radiotherapy is a treatment method that uses various energy rays to irradiate tumors to suppress and kill cancer cells. It is an important component of cancer comprehensive treatment, which can significantly improve the cure rate of cancer [4], but it often produces toxic side effects such as tissue damage and bone marrow suppression, and has a high recurrence rate. Therefore, exploring the toxicological mechanism of radiation therapy has important clinical significance. The growth, metastasis and recurrence of tumors are closely related to their microenvironment, and Cancer-associated fibroblasts

(CAF) are the main members of the tumor microenvironment. It is distributed more in the stromal tissue of most tumor types and is a key factor in tumor progression and metastasis. [5–6].

The toxic and side effects of radiation therapy on tumor microenvironment are currently less studied, and changes in tumor infiltrating fibroblasts in patients after radiotherapy are not clear. In view of the wide application of radiotherapy in tumor treatment and the important role of CAF in the development of cancer, this study explores the effect of radiotherapy on CAF gene expression. Through in-depth analysis of microarray data of tumor infiltrating fibroblasts isolated from patients with lung cancer irradiated by radiation therapy in the Gene Expression Omnibus (GEO) database, differentially expressed genes and key signaling pathways in tumor infiltrating fibroblasts before and after radiotherapy are identified in order to provide a new basis for radiotherapy of lung cancer.

1 Materials And Methods

1.1 chip data download

Radiation-treated tumor infiltrating fibroblast gene expression data set GSE37318 was downloaded from the GEO database for screening differentially expressed genes. GPL10191 platform was used by this set of chips and 8 groups of cells were contained. Tumor infiltrating fibroblasts were isolated from patients with non-small cell lung cancer. The cells were subjected to a single dose of 18 Gy ionization irradiation, and the total RNA of the cells was extracted for whole-gene transcriptome analysis after 24 h.

1.2 Identification of differentially expressed genes

The R software limma was used to process the chip data GSE37318, and the default Benjamini-Hochberg method was used for statistical analysis. $\log_2 FC > 1$ and $\text{adj. } p < 0.05$ were used as the screening criteria (FC is the multiple of gene expression difference, $\text{adj. } p$ is the corrected p-value); the volcano map was drawn using the ggplot2 package in the R software, and the heat map was drawn using the heatmap MEV4.9.0 (Multi Experiment Viewer) package in the R software .

1.3 GO analysis and KEGG pathway analysis

The screened differentially expressed genes were introduced into the DAVID [7] online toolkit for re-annotation, and the differentially expressed genes were enriched by GO and KEGG pathway analysis.

1.4 Building protein interaction networks

The STRING database [8] was used to analyze the interaction of differentially expressed genes, the protein interaction network was constructed using Cytoscape 3.5.0 software, and the core submodule network was screened and obtained through the plug-in MCODE, and the core genes were screened using the cytoHubba plug-in, and the difference is considered statistically significant by $p < 0.05$.

1.5 Verification of core differential genes

Patients with clinical stage II and III of non-small cell lung cancer were screened out, and 20 patients each with pre-operative radiotherapy and non-radiotherapy were screened out. See Table 1 for basic information of all patients. They were divided into radiotherapy group and non-radiotherapy group. The resected tumor tissue was taken, paraffin specimens were prepared and sectioned. Immunohistochemical techniques were used to detect and compare the expression of the top 5 hub genes in the two groups of patients. Using the lung cancer-related data in the TCGA database, the GEPIA online tool was used to analyze the expression of the top 10 hub genes in patients with lung adenocarcinoma, lung squamous cell carcinoma, and normal people, and to compare and analyze whether there is differential expression of the hub genes in patients with lung cancer.

Table 1
Basic information of patients in the radiotherapy group and the non-radiotherapy group

group	gender		age	cancer type	stage	TNM	Chemotherapy
	male	female					
Radiotherapy group	11	9	56.1 ± 2.3	10 cases of LUSC and 10 cases of LUAD	IIIA	T1N2M0	none
Non-radiotherapy group	10	10	53.8 ± 3.6	9 cases of LUSC and 11 cases of LUAD	IIIA	T1N2M0	none
<i>P</i> value	> 0.05		> 0.05	> 0.05	/		

1.6 Clinical stage and prognostic value analysis

The online bioinformatics analysis tool GEPIA (Gene Expression Profiling Interactive Analysis) (<http://gepia.cancer-pku.cn/>) [9] was used to analyze the expression of the top 10 core genes in each clinical stage of lung cancer. A violin diagram was drawn for the corresponding gene expression levels in clinical stages. The prognostic value of the selected core genes in lung cancer was analyzed by LogRank, and the Hazard ratio (HR) related to the prognosis was calculated. The difference was statistically significant at $p < 0.05$.

2 Results

2.1 Differentially expressed genes in tumor infiltrating fibroblasts after irradiation

With $|\log_2FC| > 1$ and adj. $P < 0.05$ as the screening criteria, 144 genes with increased expression of tumor infiltrating fibroblasts after irradiation were obtained, and the genes with top 10 expression values were CYFIP2, VWCE, NRG1, CES3, CAF7L, BLNK, KCNN4, FDXR, PIDD1, and CLCA2. 54 genes with reduced expression were obtained, and the genes with top 10 expression values were MKI67, DLGAP5, KIFC1, GTSE1, HJURP, CENPA, KNL1, DTL, FAM111B, and MCM10. The heat map and volcano map of the differential genes are shown in Fig. 1.

2.2 GO and KEGG pathway enrichment analysis

The screened differentially expressed genes were imported into the DAVID database for re-annotation, and the GO (Fig. 2a) and KEGG (Fig. 2b) pathway enrichment analysis were performed. The results show that the down-regulated genes are mainly enriched in biological processes such as DNA replication, strand shift, initiation of DNA replication, and G1 / S transition of the mitotic cell cycle, cell components such as cytoplasm, nucleus, and chromosome centromere, molecular functions such as single-stranded DNA binding, DNA binding, and ATP binding, and Fanconi anemia pathway, homologous recombination and signaling pathways such as p53; up-regulated genes are mainly enriched in biological processes such as DNA damage response, p53-mediated cell cycle arrest, positive regulation of cell proliferation, and positive regulation of GTPase activity, cell components such as nucleosomes, cell junctions, and cytoplasm, molecular functions such as growth factor activity, receptor binding, and protein heterodimerization activity, and functional pathways such as p53 signaling pathways, alcohol abuse, chronic myelogenous leukemia, axon guidance, and ErbB signaling pathways.

2.3 Protein interaction networks

The differentially expressed genes were imported into the STRING database to analyze protein-protein interaction (PPI), and the results were visualized using Cytoscape 3.5.0 software to construct a PPI network, which contains 103 nodes and 376 edges as shown in Fig. 2c.

2.4 Gene enrichment module and Hub gene analysis

MODE plug-in of Cytoscape software was used to perform module analysis on the PPI network and select 2 core modules, and the first 3 core modules were obtained according to the scoring level. Module 1 contains 46 nodes and 286 edges (Fig. 3a). The corresponding proteins of the node genes are HIST1H1C, OGG1, GTSE1, HELLS, MDM2, AEN, KIAA1524, MTFR2, RAD51, HELB, BRCA2, MCM10, CDC6, SESN1, PCNA, LIG1, HJURP, TCF19, CENPA, HSPA4, BLM, ANAPC2, FXXO5, HIST1H2AC, HIST1H2AC, TMPO, HIST1H2BD, FANCI, DTL, PLK3, CCNE2, NCAPG, DLGAP5, KIF15, BCL2L1, BRIP1, KIFC1, CASC5, MKI67, PMCH, DDB2, HIST1H3H, LIN9, CHAF1B, HRAS and KIF21A. Functions are mainly enriched in signal pathways such as DNA replication initiation, cell response to DNA damage stimuli, microtubule-based movement, p53 signaling pathway, homologous recombination, cell cycle, and cancer pathway. Module 2 contains 7 nodes and 11 edges (Fig. 3b), and the corresponding proteins of the node genes are HIST1H1C, GREB1, HIST1H3H, SYNE1, HIST1H2AC, HIST1H2BK and HIST1H2BD. Functions are mainly enriched in biological pathways such as nucleosome assembly, innate immune response and antibacterial humoral response in the mucosa. Module 3 has 11 nodes and 23 edges (Fig. 3c), and the node genes are GAMT, PIDD1, PLK3, CYFIP2, HRAS, SESN1, TP53I3, BBC3, BCL2L1, MDM2, and AEN. Functions are mainly concentrated in mitotic cell cycle checkpoints, cell responses to amino acid stimulation, negative regulation of neuronal apoptosis, p53 signaling pathway, and FoxO signaling pathway .

The plug-in CytoHubba was used to further analyze the constructed PPI network, and the top 10 differentially expressed hub genes were screened. The inter-gene interaction network is shown in Fig. 3d. The top 10 screened genes are NCAPG, MCM10, DTL, DLGAP5, RAD51, RFC4, MKI67, CDC6, PCNA and BLM. Of these genes, only the expression of PCNA was up-regulated after irradiation, and the expression of other genes was down-regulated.

2.5 Experimental verification of differential genes and their correlation with lung cancer

Immunohistochemical results showed that the expression levels of the five hub genes of MCM10, DLGAP5, CDC6, NCAPG and DTL in tumor tissues of the radiotherapy group were significantly higher than those of the non-radiotherapy group (Fig. 4). Among the top 10 hub genes, BLM, RFC4, and PCNA genes were expressed more in lung squamous cell carcinoma than normal people, and the remaining 7 genes were expressed more in lung squamous cell carcinoma and lung adenocarcinoma than normal people (Fig. 5).

2.6 Clinical stage and prognostic value analysis

In order to further explore the clinical staging and prognosis value of the selected core genes for non-small cell lung cancer, we used GEPIA software combined with data from TCGA to analyze the expression of the first 10 core genes in different clinical stages and the prognosis of patients. The results found that the expression levels of all 10 genes in patients with clinical stage II and III were higher than those in clinical stage I (Fig. 6). Among the top 10 core genes, the expression levels of MCM10 (HR = 1.2, $p = 0.13$), DLGAP5 (HR = 1.2, $p = 0.13$), BLM (HR = 1.1, $p = 0.55$), MKI67 (HR = 1.2, $p = 0.031$), CDC6 (HR = 1.1, $p = 0.29$), RAD51 (HR = 1.2, $p = 0.068$), NCAPG (HR = 1.3, $p = 0.018$) and DTL (HR = 1.2, $p = 0.032$) were all negatively correlated with the survival time of lung cancer (Fig. 7). The expression levels of these genes decreased after radiation therapy, suggesting that irradiation can inhibit the expression of genes related to lung cancer, which is beneficial to the survival of patients. In addition, the expression level of the only core gene PCNA that was highly expressed after radiation therapy was negatively correlated with the prognosis of patients with lung cancer (HR = 1.1, $p = 0.41$), suggesting that irradiation can also promote tumor cell proliferation to a certain extent.

3 Discussion

With the continuous development of nuclear technology, medical imaging technology and computer technology, radiotherapy technology has made great progress and has become the main method of comprehensive tumor treatment. 65–75% of cancer patients clinically need radiation therapy during treatment, and about 40% of cancer-cured patients treat radiotherapy as an important part of their disease course management [10]. Radiotherapy works on tumor cells: on the one hand, it can cause damage to biological macromolecules such as proteins, DNA and RNA, and reduce the expression of related genes, promote apoptosis, necrosis, autophagy and aging, and exert the function of tumor killing; on the other hand, tumor cells will start their own repair system after radiation damage. By regulating the expression of certain genes, they respond to radiation damage, mediate tumor radiation resistance, and

promote tumor recurrence and metastasis [11–12]. Analysis of these differentially expressed genes after radiation can not only help us understand the toxic and side effects of tumor radiotherapy, but also provide new biomarkers for tumor radiotherapy effects, patient prognosis, and risk assessment of recurrence and metastasis.

The tumor microenvironment refers to the area between tumor cells and adjacent normal tissues. Its composition mainly includes extracellular matrix, soluble molecules and tumor stromal cells. It plays an important role in tumor cell immune escape, tumor growth, recurrence and metastasis [13]. CAF is a major member of the tumor microenvironment and plays a key role in the progression and metastasis of various tumors such as lung cancer [14].

In view of the current background of radiotherapy, the impact of radiation therapy on tumor-supporting microenvironment is poorly understood. This study used bioinformatics methods to explore the differentially expressed genes in CAF and the key pathways mediated by them after radiotherapy, and obtained 144 up-regulated expression genes and 54 down-regulated expression genes in CAF after irradiation. The analysis results of GO and KEGG showed that the up-regulated expression genes were mainly enriched in functional pathways such as DNA damage response, p53-mediated cell cycle arrest, positive regulation of cell proliferation, p53 signaling pathway, chronic myeloid leukemia, and ErbB signaling. And down-regulated expression genes were mainly enriched in DNA replication, strand shifts, G1 / S transition of mitotic cell cycle, Fanconi anemia pathways, homologous recombination, p53 and other signaling pathways. Radiation therapy acts on the body, causing damage to biological macromolecules such as DNA. The body will initiate damage repair responses by regulating the expression of a series of genes, induce cell cycle arrest, and promote DNA repair and regulate cell proliferation [15–16]. Of course, high-dose radiation therapy will seriously damage the tissue structure of cells, inhibit the expression of many functional genes, cause cell DNA replication and repair defects, cell cycle transition disorders, and cell maturation disorders, etc. [17–18]. However, we also found that the pathways enriched by highly expressed genes are also closely related to the occurrence and development of tumors, such as the activation of the ErbB signaling pathway and the invasion and metastasis of tumors such as lung cancer [19–20]. The p53 signal pathway enriched with low-expressed genes is a classic tumor suppressor pathway [21–22]. Changes in these pathways are likely to be an important cause of tumor recurrence and metastasis after radiotherapy.

We obtained Top 20 genes from the PPI network, which are NCAPG, MCM10, DTL, DLGAP5, RAD51, CENPA, MKI67, CDC6, FANCI, FBXO5, KIF15, HELLS, HJURP, PCNA, GTSE1, CASC5, CCNE2, CHAF1B, BLM and KIFC1. These genes all play an important role in the genesis and development of tumors. NCAPG is highly expressed in patients with prostate cancer and liver cancer, and it is involved in promoting the proliferation and migration of tumor cells. Down-regulating the expression of NCAPG can inhibit the growth of tumor cells [23–24]. MCM10 is an important protein that mediates DNA replication. It is highly expressed in a variety of tumor cells and tissues and is involved in promoting tumor cell proliferation [25–26]. DTL is a homologue of E3 ubiquitin protein ligase. It is highly expressed in liver cancer tumor tissues. Down-regulating the expression of DTL can induce cell cycle arrest and senescence, and inhibit

the growth and colony formation of liver cancer cells [27]. PCNA is the only gene in this Top 20 gene that has an increased expression after radiation and is a cofactor of DNA polymerase δ . The expression of PCNA in lung cancer and other tumors is significantly increased, promoting tumor cell proliferation, migration and invasion [28–29]. In addition, these pivot genes are related to the poor prognosis of tumor, which is consistent with our prognostic analysis results, such as the expression of NCAPG is significantly negatively correlated with the survival of prostate and liver cancer [23–24]; the expression of MCM10 is significantly correlated with the poor prognosis of patients with breast and prostate cancer [25–26];

the expression of DTL is negatively correlated with the prognosis of breast and lung cancers [30]. These findings suggest that down-regulated expression of tumor-related genes after irradiation treatment determines the effect of tumor radiotherapy, while up-regulated genes are likely to play an important role in subsequent tumor recurrence.

Conclusion

The results of the study indicate that the gene expression and biological pathways in CAF have changed after radiotherapy treatment, mainly involving cell stress, DNA damage, cell cycle, aging, apoptosis, oxidative stress, and matrix remodeling. Radiation therapy has both anti-tumor effects and induction of tumor-promoting effects on CAF. The specific mechanism is worthy of further exploration through in vivo experiments.

List Of Abbreviations

CAF: fibroblasts

GEO: Gene Expression Omnibus

GEPIA : Gene Expression Profiling Interactive Analysis

HR: Hazard ratio

PPI: protein-protein interaction

Declarations

Ethics approval and consent to participate :

This article does not contain any studies with animals performed by any of the authors. All methods are carried out in accordance with relevant guidelines and regulations.

Informed consent was obtained from all subjects.

All experimental protocols were approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University

Consent for publication: Not applicable.

Availability of data and material:

All data are available. Please contact us to access if it is needed.

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Authors' contributions:

JX: research design and drafting the manuscript

KL: literature search and helping to draft the manuscript

HN: help modify articles and collate references

XW: review and revision of the manuscript and writing guidance

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References

- 1 Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA: A Cancer Journal for Clinicians, 2018, 68(6): 394- 424. DOI: 10.3322/caac.21492.
- 2 Li M Q, Gan L, Song A, et al. Rethinking pulmonary toxicity in advanced non-small cell lung cancer in the era of combining anti-PD-1/PD-L1 therapy with thoracic radiotherapy[J]. Biochimica et Biophysica Acta-Reviews on Cancer, 2019, 1871(2): 323-330. DOI: 10.1016/j. bbcan.2019.02.004.
- 3 Yamaguchi O, Kaira K, Hashimoto K, et al. Radiotherapy is an independent prognostic marker of favorable prognosis in non-small cell lung cancer patients after treatment with the immune checkpoint inhibitor, nivolumab[J]. Thoracic Cancer, 2019, 10(4): 992-1000. DOI: 10.1111/1759-7714.13044.
- 4 Jadon R, Higgins E, Hanna L, et al. A systematic review of dose-volume predictors and constraints for late bowel toxicity following pelvic radiotherapy[J]. Radiation Oncology, 2019, 14(1): 57. DOI: 10.1186/s13014-019- 1262-8.

- 5 LYU L, Zhang S Z, Hu Y, et al. Invasive pituitary adenoma-derived tumor-associated fibroblasts promote tumor progression both in vitro and in vivo[J]. *Experimental and Clinical Endocrinology & Diabetes*, 2018, 126(4): 213-221. DOI: 10.1055/s-0043-119636.
- 6 Yamao T, Yamashita Y I, Yamamura K, et al. Cellular senescence, represented by expression of caveolin-1, in cancer-associated fibroblasts promotes tumor invasion in pancreatic cancer[J]. *Annals of Surgical Oncology*, 2019, 26(5): 1552-1559. DOI: 10.1245/s10434-019-07266-2.
- 7 Huang D W, Sherman B T, Lempicki R A. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources[J]. *Nature Protocols*, 2009, 4 (1): 44-57. DOI: 10.1038/nprot.2008.211.
- 8 Szklarczyk D, Gable A L, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets[J]. *Nucleic Acids Research*, 2019, 47(D1): D607-D613. DOI: 10.1093/nar/gky1131.
- 9 Tang Z F, Li C W, Kang B X, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses[J]. *Nucleic Acids Research*, 2017, 45 (W1): W98-W102. DOI: 10.1093/nar/gkx247.
- 10 Thompson M K, Poortmans P, Chalmers A J, et al. Practice-changing radiation therapy trials for the treatment of cancer: where are we 150 years after the birth of Marie Curie?[J]. *British Journal of Cancer*, 2018, 119(4): 389-407. DOI: 10.1038/s41416-018-0201-z.
- 11 Park S Y, Kim J Y, Jun Y, et al. Strategies to tackle radiation resistance by penetrating cancer stem cell line of scrimmage[J]. *Recent Patents on Anti-Cancer Drug Discovery*, 2018, 13(1): 18-39. DOI: 10.2174/1574892812666171003150410.
- 12 Kim B M, Hong Y, Lee S, et al. Therapeutic implications for overcoming radiation resistance in cancer therapy[J]. *International Journal of Molecular Sciences*, 2015, 16 (11): 26880-26913. DOI: 10.3390/ijms161125991.
- 13 Shojaei S, Hashemi S M, Ghanbarian H, et al. Effect of mesenchymal stem cells-derived exosomes on tumor microenvironment: tumor progression versus tumor suppression[J]. *Journal of Cellular Physiology*, 2019, 234 (4): 3394-3409. DOI: 10.1002/jcp.27326.
- 14 Hao J, Zeltz C, Pintilie M, et al. Characterization of distinct populations of carcinoma-associated fibroblasts from non-small cell lung carcinoma reveals a role for ST8SIA2 in cancer cell invasion[J]. *Neoplasia*, 2019, 21 (5): 482-493. DOI: 10.1016/j.neo.2019.03.009.
- 15 Minten E V, Yu D S. DNA repair: translation to the clinic [J]. *Clinical Oncology*, 2019, 31(5): 303-310. DOI: 10.1016/j.clon.2019.02.007.
- 16 Xu F, Li X, Yan L L, et al. Autophagy promotes the repair of radiation-induced DNA damage in bone marrow hematopoietic cells via enhanced STAT3 signaling[J]. *Radiation Research*, 2017, 187(3): 382-396.

DOI: 10.1667/RR14640.1.

17 Cornforth M N, Loucas B D. A cytogenetic profile of radiation damage[J]. Radiation Research, 2019, 191(1): 1- 19. DOI: 10.1667/RR15205.1.

18 Mishyna M, Volokh O, Danilova Y, et al. Effects of radiation damage in studies of protein-DNA complexes by cryo-EM[J]. Micron, 2017, 96: 57-64. DOI: 10.1016/j.micron.2017.02.004.

19 Yu T, Li J, Yan M, et al. MicroRNA-193a-3p and -5p suppress the metastasis of human non-small-cell lung cancer by downregulating the ERBB4/PIK3R3/mTOR/ S6K2 signaling pathway[J]. Oncogene, 2015, 34(4): 413- 423. DOI: 10.1038/onc.2013.574.

20 Elizalde P V, Russo R I, Chervo M F, et al. ErbB-2 nuclear function in breast cancer growth, metastasis and resistance to therapy[J]. Endocrine Related Cancer, 2016,23(12): T243-T257. DOI: 10.1530/ERC-16-0360.

21 Chen Y N, Ren C C, Yang L, et al. MicroRNA let7d5p rescues ovarian cancer cell apoptosis and restores chemosensitivity by regulating the p53 signaling pathway via HMGA1[J]. International Journal of Oncology, 2019, 54(5): 1771-1784. DOI: 10.3892/ijo.2019.4731.

22 Qin Q P, Wang S L, Tan M X, et al. Novel tacrine platinum(II) complexes display high anticancer activity via inhibition of telomerase activity, dysfunction of mitochondria, and activation of the p53 signaling pathway [J]. European Journal of Medicinal Chemistry, 2018, 158: 106-122. DOI: 10.1016/j.ejmech.2018.09.008.

23 Arai T, Okato A, Yamada Y, et al. Regulation of NCAPG by miR-99a-3p (passenger strand) inhibits cancer cell aggressiveness and is involved in CRPC[J]. Cancer Medicine, 2018, 7(5): 1988-2002. DOI: 10.1002/cam4.1455.

24 Zhang Q, Su R X, Shan C, et al. Non-SMC condensin I complex, subunit G (NCAPG) is a novel mitotic gene required for hepatocellular cancer cell proliferation and migration[J]. Oncology Research, 2018, 26(2): 269-276. DOI: 10.3727/096504017X15075967560980.

25 Cui F L, Hu J P, Ning S Y, et al. Overexpression of MCM10 promotes cell proliferation and predicts poor prognosis in prostate cancer[J]. The Prostate, 2018, 78 (16): 1299-1310. DOI: 10.1002/pros.23703.

26 Mahadevappa R, Neves H, Yuen S M, et al. DNA replication licensing protein MCM10 promotes tumor progression and is a novel prognostic biomarker and potential therapeutic target in breast cancer[J]. Cancers (Basel), 2018, 10(9): 282. DOI: 10.3390/cancers10090282.

27 Chen Y C, Chen I S, Huang G J, et al. Targeting DTL induces cell cycle arrest and senescence and suppresses cell growth and colony formation through TPX2 inhibition in human hepatocellular carcinoma cells[J]. OncoTargets and Therapy, 2018, 11: 1601-1616. DOI: 10.2147/OTT.S147453.

28 Li P, Wang Q A, Wang H N. MicroRNA-204 inhibits the proliferation, migration and invasion of human lung cancer cells by targeting PCNA-1 and inhibits tumor growth in vivo[J]. International Journal of Molecular Medicine, 2019, 43(3): 1149-1156. DOI: 10.3892/ijmm.2018.4044.

29 Hou X F, Xu L P, Song H Y, et al. ECRG2 enhances the anti-cancer effects of cisplatin in cisplatin-resistant esophageal cancer cells via upregulation of p53 and downregulation of PCNA[J]. World Journal of Gastroenterology, 2017, 23(10): 1796-1803. DOI: 10.3748/wjg.v23.i10.1796.

30 Perez-Peña J, Corrales-Sánchez V, Amir E, et al. Ubiquitin-conjugating enzyme E2T (UBE2T) and denticleless protein homolog (DTL) are linked to poor outcome in breast and lung cancers[J]. Scientific Reports, 2017, 7(1): 17530. DOI: 10.1038/s41598-017-17836-7.

Figures

Figure 1

Heatmap (left) and volcano map (right) of differential genes; red represents gene up-regulation and blue represents gene down-regulation.

Figure 2

a: GO analysis result of differentially expressed genes; b: KEGG analysis result of differential genes; c: protein-protein interaction network of differential genes.

Figure 3

Module analysis and hub gene of PPI network (a) module 1 (b) module 2 (c) module 3 (d) Interaction networks of the top 10 differentially expressed hub genes. Red circle (up-regulated proteins) green circle (down-regulated proteins) and the color depth of the line represents the strength of the interaction among genes

Figure 4

Comparison of the expression levels of the top five hub genes MCM10, DLGAP5, CDC6, NCAPG and DTL in tumor tissues between the radiotherapy group and the non-radiotherapy group.

Figure 5

Comparison of the expression levels of the first 10 hub genes in patients with lung adenocarcinoma–lung squamous cell carcinoma, and normal people

Figure 6

The expression levels of the first 10 hub genes in patients with lung cancer at various clinical stages

Figure 7

Prognostic values of the expression of 9 related genes in lung cancer patients