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ORIGINAL ARTICLE

Study of lung cancer regulatory network that involves erbB4 and tumor marker gene

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KEYWORDS

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Abstract Our purpose is to screen out serum tumor markers closely correlated to the nature of solitary pulmonary nodule (SPN) and to draw a regulatory network containing genes correlated to lung cancer. Two hundred and sixty cases of SPN patients confirmed through pathological diagnosis were collected as subjects, factors closely correlated to the nature of SPN were screened out from eight tumor markers through Fisher discriminant method, and functional annotation and pathway analysis were conducted on erbB4 as well as its tumor marker genes by GO and KEGG databases. Four key tumor markers: CYFRA21-1, CA125, SCC-Ag and CA153 were successfully screened out and the first three proteins' corresponding gene were KRT19, MUC16 and SERPINB3 while that of CA153 was not found. GO analysis on erbB4, KRT19, MUC16 and SERPINB3 showed that they covered three domains, cell components, molecular function and biological process; meanwhile, combined with KEGG database and based on signal pathway of erbB4, a regulatory network of lung cancer cells escaping from apoptosis was successfully made. This study indicates that serum tumor marker genes play an important role in the occurrence and development of lung cancer, besides, this study primarily discussed the molecular mechanism of these tumor markers in predicting tumor, which provides a basis for in-depth information about lung cancer.

1. Introduction

Lung cancer, a malignant tumor with highest morbidity and mortality currently, tops the five most common tumors' list among males and ranks the second place among females, and it is still the top of the four cancers threatening people's life according to 2015 cancer statistics report in China (Chen et al., 2016). And it is mainly because of the high misdiagnosis rate and missed diagnosis rate for early lung cancer. Expression of the early lung cancer usually is solitary pulmonary nodule (SPN) (Siegel et al., 2013), therefore, accurate discrimination of benign and malignant SPN is an effective method to reduce the mortality of lung cancer.

SPN is a roundlike solid lesion with a diameter no more than 3 cm. Data from American College of Chest Physicians (ACCP) show that among malignant SPN primary lung cancer accounts for 75%, and adenocarcinoma is the most common tumor followed by squamous cell carcinoma (Wahidi et al.,

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2007). For patients with early primary lung cancer, their five-year survival rate will be up to 80% if they can be diagnosed early (Vazquez et al., 2009; Varoli et al., 2008); due to difficult qualification of SPN, about 50% lung cancer patients, however, miss optimal therapeutic time leaving them a relatively low five-year survival rate. Therefore, it is the key to distinguishing benign SPN from malignant SPN for secondary prevention of lung cancer, which still is a diagnostic difficulty.

CT imaging examination is the preferred approach for SPN identification, usually from nodule's size, location, internal feature and surrounding environment. However, it's not reliable to identify SPN only through imaging approach and laboratory diagnostic methods like tumor marker diagnosis is necessary. Tumor marker is chemical substance reflecting tumor's existence and commonly used markers are carcino-embryonic antigen (CEA), carbohydrate antigen (CA) (like CA125, CA199 and CA153) and neuron-specific enolase (NSE). Reasonable usage of these markers will be helpful for early SPN diagnosis and treatment. Currently, however, there are few studies on inside molecular mechanism of these markers participating in lung cancer cell regulation. Our preliminary study analyzed the structural and functional changes of erbB4 before and after mutation (Chen and Zhao, 2016), and based on the original signal pathway of erbB4, tumor marker genes were added to the regulatory network in this study to uncover the internal molecular regulatory mechanism for prediction of tumor occurrence and development, which lays foundation for in-depth cancer suppressor research in the future.

2. Material and methods

2.1. Subjects

In this study, continuous data of patients who went to Affiliated Cancer Hospital of Zhengzhou University between 2012 and 2014 were collected. And through analysis of their medical records, 260 cases of SPN patients were selected including 145 cases of malignant SPN (according to the pathological diagnosis there were 88 cases of adenocarcinoma, 32 cases of squamous cell carcinoma, eight cases of adenosquamous carcinoma, two cases of nonsmall-cell lung cancer, four cases of neuroendocrine carcinoma, two cases of mucoepidermoid carcinoma, four cases of mucoepidermoid, one case of carcinoma mucocellulare, one case of metastatic renal cell carcinoma, one case of metastasis breast cancer, one case of carcinoma sareomatodes and one case of unclassified lung cancer) and 115 cases of benign PSN (56 cases of inflammation, 13 cases of tuberculosis, 14 cases of inflammatory pseudotumor, 11 cases of mycotic infection, five cases of hamartoma, four cases of angeioma, three cases of pulmonary abscess, two cases of epithelial tumor, one case of bronchiectasia, one case of glioma peripheral of chest wall, one case of lymphadenoma, one case of secondary osteogenic sarcoma of lung, one case of coccus infection, malignant dyskaryosis and one case of fibrocartilage). All above patients were confirmed by CT imaging diagnosis and pathological diagnosis.

2.2. Content measurement of serum tumor markers

In this study eight seismological indexes, including CEA, NSE, cytokeratin 19 fragment (CYFRA21-1), CA125, CA199, CA724, squamous cell carcinoma antigen (SCC-Ag) and CA153, in patients' blood samples were detected. The detection of every index was in accordance with instruction on kit; CEA and CYFRA21-1 were detected by ELISA method; CA125, CA199, CA724, SCC-Ag and CA153 were detected by Roche E601 automatic immuno-analyzer and NSE was detected by radio immunoassay.

2.3. Methods

2.3.1. Fisher discriminant analysis

Fisher discriminant analysis was put in 1930s by British statistician Fisher who first defined Fisher discriminant analysis and applied it to discriminant analysis of iris, and this method has been improved continuously so till now it is still considered as one of the best feature extraction. Its fundamental idea is to conduct linear project on sample data making their between-class scatter maximum and within-class scatter minimum. Fisher discriminant method in SPSS 23.0 software was used to screen serologic tumor markers, aiming to screen out factors correlated to SPN nature.

2.3.2. GO analysis of genes correlated to lung cancer

Corresponding genes of tumor markers correlated to SPN nature were searched through literature search and AmiGo homepage (http://geneontology.org/) was visited to conduct Go analysis with screening condition being "Homo sapiens". The screened out serum tumor markers and erbB4 were primarily analyzed and every gene was conducted functional annotation from cell components, molecular function and biological process, aiming to identify their functions and provide a basis for further researches.

2.3.3. KEGG analysis of genes correlated to lung cancer

After visiting KEGG database homepage (http://www.kegg.jp/kegg/pathway.html), key words "lung cancer" and "erbB4" were input to search signal pathway; literature search was used to find key factors running by corresponding genes of tumor markers, and starting from these key factors and based on the signal pathway of erbB4, all signal pathways were linked together through using key nodes, and functions of every tumor marker were added as well.

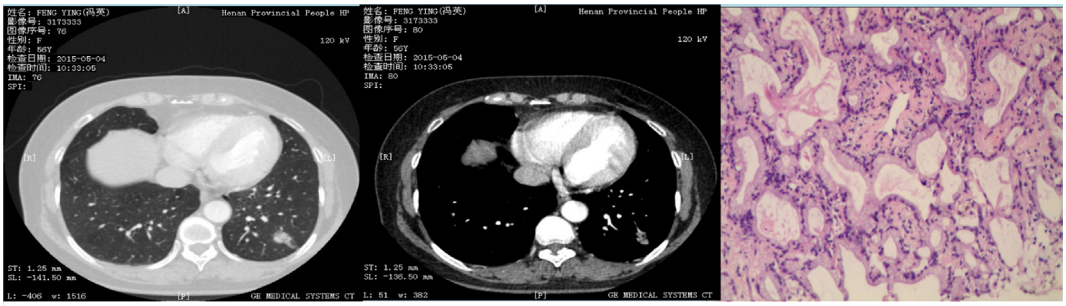
3. Results

3.1. CT imaging maps and histopathological slice of different pathological type SPN patients

Patients highly suspected as malignant SPN not only underwent CT examination but histopathological examination while the benign SPN patients only underwent histopathological examination. Figs. 1–3 respectively are CT imaging map and histopathological slice of lung adenocarcinoma, squamous cell carcinoma and inflammatory pseudotumor.

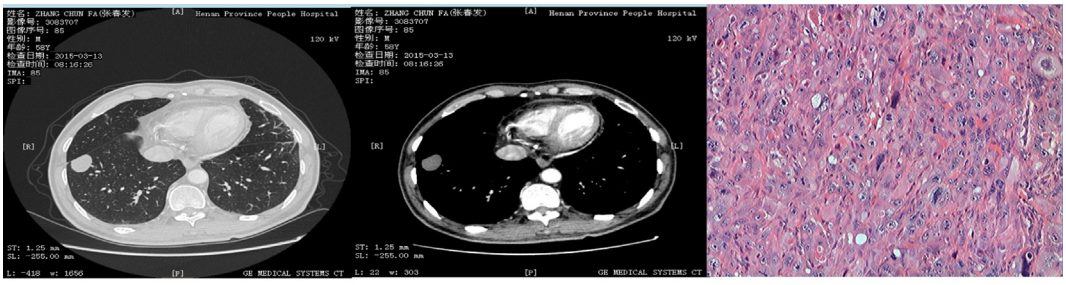
3.2. Content measurement of serum tumor markers

Measurement results of eight tumor markers for totally 260 patients in malignant SPN group and benign SPN group are listed in Table 1 and the eight tumor markers were CEA,



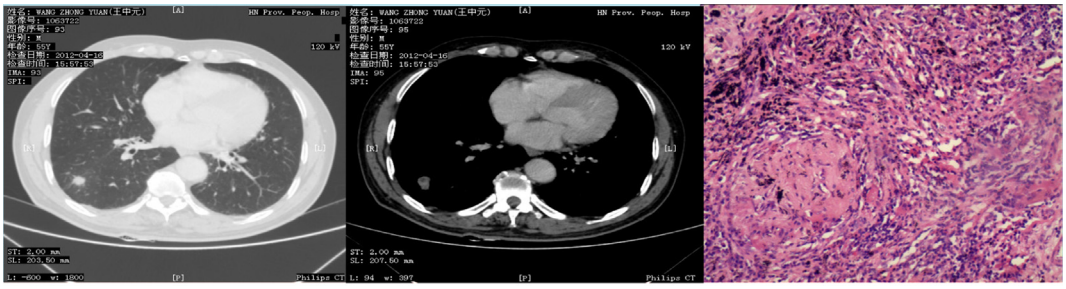
(a) Lung window, (b) Mediastinal window (c) Histopathologyical slice.

Figure 1 CT imaging map and histopathological slice of lung adenocarcinoma.



(a) Lung window (b) Mediastinal window (c) Histopathological slice.

Figure 2 CT imaging map and histopathological slice of lung squamous cell carcinoma.



(a) Lung window (b) Mediastinal window (c) Histopathological slice.

Figure 3 CT imaging map and histopathological slice of inflammatory pseudotumor.

Table 1 Measurement results of eight serum tumor markers of SPN patients.

Serum tumor markers	Malignant SPN group	Benign SPN group	*t*	*P*
CEA (ng/mL)	14.61 ± 2.58*	3.23 ± 0.87	4.18	0.000
NSE (ng/mL)	15.01 ± 0.64*	12.03 ± 0.52	3.61	0.000
CYFRA21-1 (ng/mL)	7.25 ± 0.82*	2.18 ± 0.12	6.118	0.000
SCC-Ag (ng/mL)	2.04 ± 0.17*	1.06 ± 0.06	5.49	0.000
CA125 (U/mL)	49.65 ± 5.69*	19.65 ± 2.69	4.76	0.000
CA199 (U/mL)	26.34 ± 3.70*	16.86 ± 1.98	2.26	0.025
CA724 (U/mL)	6.27 ± 0.84	5.43 ± 2.59	0.34	0.736
CA153 (U/mL)	18.27 ± 1.34*	10.58 ± 0.79	4.95	0.000

Note: * represents the difference is statistically significant compared to the benign group.

NSE, CYFRA21-1, CA125, CA199, CA724, SCC-Ag and CA153. Seen from the table, except CA724, all levels of other seven serum tumor markers in malignant SPN group were higher than that in benign SPN group ($P < 0.05$).

3.3. Screening results by Fisher discriminant analysis

According to clinical test results, CEA, NSE, CYFRA21-1, CA125, CA199, CA724, SCC-Ag and CA153 were regarded as variables included in discriminant analysis model. Wilks' lambda method was employed to conduct a stepwise discriminative analysis for these variables with F value being discriminative statistics standard and totally four predictors with statistical significance were screened out which were CYFRA21-1, CA125, SCC-Ag and CA153 (see Tables 2 and 3).

3.4. GO and KEGG analysis of genes correlated to lung cancer

CYFRA21-1, CA125, CA153 and SCC-Ag were searched through databases like NCBI Database, Wanfang Database, VIP Database and CNKI Database, and so on. CYFRA21-1, a soluble fragment of cytokeratin 19 which is the expression product of KRT19 gene, is mainly applied for detection of tumor marker of non-small-cell lung cancer (Liu et al., 2015). CA125 and CA153 both are carbohydrate antigens. CA125 whose gene is MUC16 is the specific markers of ovarian cancer diagnosis and commonly used in lung cancer diagnosis (Homma et al., 2004); CA153 is the specific marker of breast cancer diagnosis but its genetic expression is still unclear based on current reports. SCC-Ag is squamous cell carcinoma antigen which is significant to non-small-cell lung cancer diagnosis, and two genes producing SCCA have been identified nowadays which are SCCA1 and SCCA2 and their homology in terms of animo acid level was 92% (Yan et al., 2011), and SCCA is the expression of SERPINB3 gene. ErbB4 also called HER4 gene is the fourth oncogene encoding human epidermal growth factor receptor and it was reported to be overexpressed in lung cancer tissues and to be correlative to lymph node metastasis, TNM staging and postoperative survival rate (Starr et al., 2006). KRT19, MUC16, SERPINB3 and erbB4 were input in the homepage of Amigo for their annotation in human.

The annotation of KRT19 gene is shown in Table 4 which indicates that KRT19 played an important role in cell component, molecular function and biological process and its protein products distributed in the central filament, the glycoprotein complex and the cell membrane, etc.; erbB4 has function of protein binding and muscle composition and cytoskeleton and is involved in many biological processes like Notch signaling pathway, embryonic cell differentiation, embryonic development and estrogen response. GO analysis results of MUC16 are listed in Table 5, and MUC16 gene mainly participates in cell component and biological process whose protein products can be seen in cell membrane and Golgi apparatus cavity and it plays a crucial role in multiple biological processes including cell adhesion, protein post-translational modifications and protein metabolism in cells, etc. The annotation of SERPINB3 gene is shown in Table 6, similarly, SERPINB3 is involved in cell components, molecular function and biological process. Specifically, the cell components contain the cytoplasm, the nucleus and cytoplasmic vesicle, etc.; molecular function covers activities of virus receptor, binding of protease, activity of serine-type and cysteine-type inhibitor; the biological processes include positive regulation of cell proliferation, regulation of endopeptidase activity, regulation of cell migration and negative regulation of proteolysis; what's more, it participates in autocrine and paracrine process and penetration of virus into a host cell. Additionally, GO analysis outcomes of erbB4 gene are listed in Table 7 showing that erbB4 gene is involved in multiple functions, and its cell component includes nucleus, mitochondria and plasma membrane; its molecular functions contain protein tyrosine kinase activity, activation of protein tyrosine kinase receptor signal, epidermal growth factor receptor and ATP etc.; besides, it also participates in cell proliferation, ras protein signaling transduction, mitogen-activated protein kinases signaling pathway, transmembrane receptor protein tyrosine kinase signaling pathway, endothelial growth factor receptor signaling pathway and insulin receptor signaling pathway.

Inputting the keyword "erbB4" into KEGG signaling pathway database, explicit signaling pathway it participated in were found, and erbB4 was turned out to be involved in the signaling pathway of lung cancer cell escaping from apoptosis through PI3K → PKB/AKt → MDM2 → p53 while nothing about MUC16, SERPINB3 and KRT19 gene was found.

Table 2 Serological variables input /deleted in Fisher discriminant method[a,b,c,d].

Step	Entered	Wilks' lambda							
		Statistic	df1	df2	df3	Exact F			
						Statistic	df1	df2	P
1	CYFRA21-1	.896	1	1	258.000	29.897	1	258.000	0.000
2	SCC-Ag	.811	2	1	258.000	30.039	2	257.000	0.000
3	CA153	.753	3	1	258.000	27.966	3	256.000	0.000
4	CA125	.726	4	1	258.000	24.068	4	255.000	0.000

Note: At each step, the variable that minimizes the overall Wilks' lambda is entered.
[a] Maximum number of steps is 16.
[b] Minimum partial F to enter is 3.84.
[c] Maximum partial F to remove is 2.71.
[d] F level, tolerance, or VIN is insufficient for further computation.

Table 3 Serological variables included in Fisher discriminant method.

Step		Tolerance	*F* to remove	Wilks' lambda
1	CYFRA21-1	1.000	29.897	
2	CYFRA21-1	0.991	32.331	0.912
	SCC-Ag	0.991	27.151	0.896
3	CYFRA21-1	0.989	32.096	0.848
	SCC-Ag	0.991	25.358	0.828
	CA153	0.998	19.497	0.811
4	CYFRA21-1	0.988	29.358	0.810
	SCC-Ag	0.983	27.157	0.803
	CA153	0.931	10.973	0.757
	CA125	0.925	9.565	0.753

Table 4 Results of GO analysis for KRT19 gene.

Gene	Gene/product name	Direct annotation	Ontology	GO number
KRT19	Keratin, type I cytoskeletal 19 (CK19)	Intermediate filament	Cellular_component	0005882
		Dystrophin-associated glycoprotein complex	Cellular_component	0016010
		Sarcolemma	Cellular_component	0042383
		Z disk	Cellular_component	0030018
		Terminal web	Cellular_component	1990357
		Plasma membrane	Cellular_component	0005886
		Costamere	Cellular_component	0043043
		Extracellular exosome	Cellular_component	0070062
		Cell periphery	Cellular_component	0071944
		Protein complex binding	Molecular_function	0032403
		Structural molecule activity	Molecular_function	0005198
		Structural constituent of cytoskeleton	Molecular_function	0005200
		Protein binding	Molecular_function	0005515
		Structural constituent of muscle	Molecular_function	0008307
		Notch signaling pathway	Biological_process	0007219
		Viral process	Biological_process	0016032
		Cell differentiation involved in embryonic placenta development	Biological_process	0060706
		Response to estrogen	Biological_process	0043627
		Sarcomere organization	Biological_process	0045214

And through gene annotation and literature search, these three genes were added to the signaling pathway of lung cancer cell escaping from apoptosis (see Fig. 4). According to the gene annotation, KRT19 is able to act on Notch signaling pathway. Besides, it's reported (Gong, 2013) that MUC16 can enhance transportation of β-catenin which is the key effector molecule in Wnt signaling pathway from cytoplasm to nucleus, and it can activate Wnt signaling pathway and stimulate and enhance the expression of downstream oncogenes through interaction with β-catenin. As a tumor suppressor gene, the activation of p53 can cause cell apoptosis while its deactivation can help tumor's development. Moreover, study by Molès found that p53 can inhibit KRT19's expression (Molès et al., 1994). And SERPINB3 can act on β-catenin and TGFβ, which has influence on the growth and development of tumor (Turato et al., 2014).

4. Discussion

SNP is the unique roundlike solitary lesion whose diameter is less than 30 mm, and 150,000 cases of SNP are detected globally every year, among which 10–70% is malignant which is possible to develop into lung cancer anytime. Serological tumor marker is significant for determination of begin and malignant SPN, and the commonly used markers are CYFRA21-1, CA125, CA153 and SCC-Ag.

CYFRA21-1 known as cytokeratin 19-fragments is a soluble polypeptide and its principle of being a tumor-detection

Table 5 Results of GO analysis for MUC16 gene.

Gene	Gene/product name	Direct annotation	Ontology	GO number
MUC16	Mucin-16 (CA125)	Integral component of membrane	Cellular_component	0016021
		Extracellular space	Cellular_component	0005615
		Plasma membrane	Cellular_component	0005886
		External side of plasma membrane	Cellular_component	0009897
		Golgi lumen	Cellular_component	0005796
		Extrinsic component of membrane	Cellular_component	0019898
		Vesicle	Cellular_component	0031982
		Extracellular exosome	Cellular_component	0070062
		Protein O-linked glycosylation	Biological_process	0006493
		Cell adhesion	Biological_process	0007155
		O-glycan processing	Biological_process	0016266
		Post-translation protein modification	Biological_process	0043687
		Cellular protein metabolic process	Biological_process	0044267

Table 6 Results of GO analysis for SERPINB3 gene.

Gene	Gene/product name	Direct annotation	Ontology	GO number
SERPINB3	SerpinB3 (SCCA)	Extracellular space	Cellular_component	0005615
		Nucleus	Cellular_component	0005634
		Cytoplasm	Cellular_component	0005737
		Cytoplasmic vesicle	Cellular_component	0031410
		Vesicle	Cellular_component	0031982
		Extracellular exosome	Cellular_component	0070062
		Virus receptor activity	Molecular_function	0001618
		Protease binding	Molecular_function	0002020
		Serine-type endopeptidase inhibitor activity	Molecular_function	0004867
		Cysteine-type endopeptidase inhibitor activity	Molecular_function	0004869
		Positive regulation of cell proliferation	Biological_process	0008284
		Negative regulation of peptidase activity	Biological_process	0010466
		Positive regulation of epithelial to mesenchymal transition	Biological_process	0010718
		Positive regulation of endopeptidase activity	Biological_process	0010950
		Negative regulation of endopeptidase activity	Biological_process	0010951
		Positive regulation of cell migration	Biological_process	0030335
		autocrine signaling	Biological_process	0035425
		Paracrine signaling	Biological_process	0038001
		Negative regulation of catalytic activity	Biological_process	0043086
		Negative regulation of JUN kinase activity	Biological_process	0043508
		Negative regulation of proteolysis	Biological_process	0045861
		Viral entry into host cell	Biological_process	0046718

marker is that when apoptosis of tumor cells happens, the increasing of cytokeratins stimulated by activated protease will elevate patients' CYFRA21-1 level in serum (Thomas et al., 2015). Currently, CYFRA21-1 is a top priority in detecting non-small-cell lung cancer. As for CA125, it not only is an ovarian cancer associated antigen but also has a high expression in serum of lung cancer patient, especially in lung adenocarcinoma patient. CA153 also is an important specific marker that can be used in detection of lung cancer (Ghosh et al., 2013). SCC-Ag, a squamous cell carcinoma antigen, exists in cytoplasm of squamous cell carcinoma of the uterus, lungs, cervix and head and neck, and is especially rich in non-keratinizing cancer cells. Chu et al. (2011) studied 805 patients with lung cancer and patients with benign lung diseases, even though the area under ROC of individual detection on lung cancer isn't ideal, 37.3% patients with early stage lung cancer can be detected correctly through detection of SCC combined with other tumor markers, showing potential value of SCC in clinic application.

In this study four serological tumor markers which are significant for malignant and benign SPN detection were screened out through clinical cases, and the four markers are CYFRA21-1, CA125, CA153 and SCC-Ag. Then the corresponding genes of CYFRA21-1, CA125 and SCC-Ag respectively were respectively identified as KRT19, MUC1 and SERPINB3 through literature search except CA153. Our previous research mainly studied the structures and function of erbB4 before and after its mutation and found that erbB4 expressed high in non-small-cell lung cancer (Kurppa et al., 2016) and that it's closely correlated to the metastasis of cancer

Table 7 Results of GO analysis for erbB4 gene.

Gene	Gene/product name	Direct annotation	Ontology	GO number
erbB4	Receptor tyrosine-protein kinase erbB4	Extracellular region	Cellular_component	0005576
		Nucleus	Cellular_component	0005634
		Nucleoplasm	Cellular_component	0005654
		Mitochondrion	Cellular_component	0005739
		Mitochondrial matrix	Cellular_component	0005759
		Cytosol	Cellular_component	0005829
		Plasma membrane	Cellular_component	0005886
		Basolateral plasma membrane	Cellular_component	0016323
		Receptor complex	Cellular_component	0043235
		Integral component of membrane	Cellular_component	0016021
		Protein tyrosine kinase activity	Molecular_function	0004713
		Transmembrane receptor protein tyrosine kinase activity	Molecular_function	0004714
		Epidermal growth factor receptor binding	Molecular_function	0005154
		Protein binding	Molecular_function	0005515
		Protein homodimerization activity	Molecular_function	0042803
		Transcription regulatory region DNA binding	Molecular_function	0044212
		Receptor signaling protein tyrosine kinase activity	Molecular_function	0004716
		ATP binding	Molecular_function	0005524
		MAPK cascade	Biological_process	0000165
		Activation of MAPKK activity	Biological_process	0000186
		Neural crest cell migration	Biological_process	0001755
		Positive regulation of protein phosphorylation	Biological_process	0001934
		Signal transduction	Biological_process	0007165
		Transmembrane receptor protein tyrosine kinase signaling pathway	Biological_process	0007169
		Epidermal growth factor receptor signaling pathway	Biological_process	0007173
		Small GTPase mediated signal transduction	Biological_process	0007264
		Ras protein signal transduction	Biological_process	0007265
		Nervous system development	Biological_process	0007399
		Axon guidance	Biological_process	0007411
		Heart development	Biological_process	0007507
		Lactation	Biological_process	0007595
		Cell proliferation	Biological_process	0008283
		Positive regulation of cell proliferation	Biological_process	0008284
		Negative regulation of cell proliferation	Biological_process	0008285
		Insulin receptor signaling pathway	Biological_process	0008286
		Fibroblast growth factor receptor signaling pathway	Biological_process	0008543
		Embryonic pattern specification	Biological_process	0009880
		Cell migration	Biological_process	0016477
		Peptidyl-tyrosine phosphorylation	Biological_process	0018108
		Central nervous system morphogenesis	Biological_process	0021551
		Olfactory bulb interneuron differentiation	Biological_process	0021889
		Regulation of cell migration	Biological_process	0030334
		Fc-epsilon receptor signaling pathway	Biological_process	0038095
		Positive regulation of tyrosine phosphorylation of Stat5 protein	Biological_process	0042523
		Negative regulation of apoptotic process	Biological_process	0043066
		Positive regulation of phosphatidylinositol 3-kinase activity	Biological_process	0043552
		Mitochondrial fragmentation involved in apoptotic process	Biological_process	0043653
		Innate immune response	Biological_process	0045087
		Positive regulation of transcription DNA-templated	Biological_process	0045893
		Protein autophosphorylation	Biological_process	0046777
		Vascular endothelial growth factor receptor signaling pathway	Biological_process	0048010
		Neurotrophin TRK receptor signaling pathway	Biological_process	0048011
		Phosphatidylinositol-mediated signaling	Biological_process	0048015
		Positive regulation of cardiac muscle cell proliferation	Biological_process	0060045
		Mammary gland epithelial cell differentiation	Biological_process	0060644
		Mammary gland alveolus development	Biological_process	0060749
		Cardiac muscle tissue regeneration	Biological_process	0061026
		Positive regulation of ERK1 and ERK2 cascade	Biological_process	0070374

(*continued on next page*)

Table 7 (*continued*)

Gene	Gene/product name	Direct annotation	Ontology	GO number
		Positive regulation of STAT protein import into nucleus	Biological_process	2000366
		Transcription, DNA-template	Biological_process	0006351
		Positive regulation of phosphatidylinositol 3-kinase signaling	Biological_process	0014068
		Cell fate commitment	Biological_process	0045165
		Positive regulation of protein localization to cell surface	Biological_process	2000010
		Negative regulation of neuron migration	Biological_process	2001223

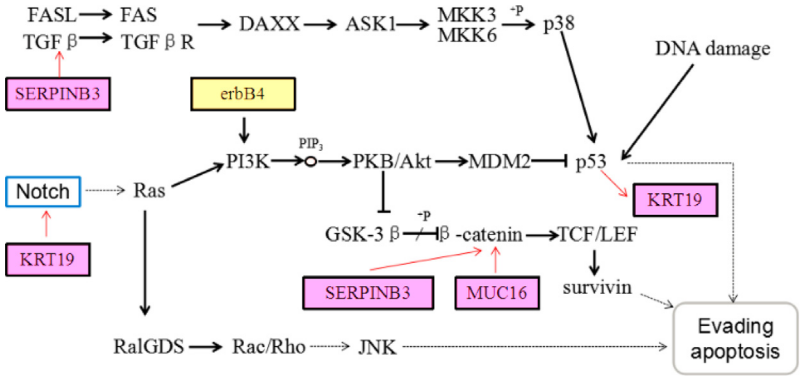


Figure 4 Regulatory network of lung cancer cell escaping from apoptosis.

cells and to patients' prognosis, which indicates that erbB4 is a possible candidate of targeted molecular therapy (Starr et al., 2006). Therefore, this study conducted GO functional analysis and KEGG pathway analysis on KRT19, MUC16, SERPINB3 and erbB4, aiming to deeply explore the growth and development mechanism of lung cancer.

According to the GO analysis of KRT19, MUC16, SERPINB3 and erbB4, these four genes all cover three domains, cell component, molecular function and biological progress. For instance, KRT19 takes part in Notch signaling pathway, MUC16 is involved in protein translational modifications, SERPINB3 participates in cell proliferation and erbB4 takes part in Ras protein signaling transduction, mitogen-activated protein kinase (MAPK) signaling cascades and transmembrane receptor tyrosine kinase signaling pathway, and so on. At present, there are more full-fledged studies on erbB4 and it is known that erbB4 is involved in multiple signaling pathways and has explicit signaling pathway in KEGG pathway data base and it can help tumor cell escape from apoptosis through PI3K-Akt signaling pathway. Based on these current analyses, this study added KRT19, MUC16 and SERPINB3 to KEGG signaling pathway database through gene annotation and literature search and drew a signaling pathway on lung cancer cell escaping from apoptosis. As mentioned, KRT19 is involved in Notch signaling pathway and the cell surface receptor coded by the key gene Notch in this pathway plays an important role in the development of many biological cells. And it was reported that Notch signal has influence on cell proliferation, formation of cell borders, cell apoptosis and multipotent progenitor cell specialization, etc. Notch signaling pathway interacts with other key pathways, having significant influences on tumor's growth and development (Iso et al., 2003). Moreover, it's was reported that MUC16 and SERPINB3 both can act on the key factor β-catenin of Wnt signaling pathway (Gong, 2013; Molès et al., 1994; Turato et al., 2014). Wnt signaling pathway is an evolutionarily conserved signaling transduction pathway which also is important to control embryological development, and it contacts with other pathways through complicated networks. Aberrant activation of Wnt signaling pathway plays a crucial role in cell canceration, tumor growth and tumor invasion, thus any change of Wnt gene itself or its any member may induce tumor. In Wnt pathway, β-catenin plays an important role in it and was considered as the key hub, therefore, if it moves from cytoplasm to nucleus, it means that the signaling pathway has been activated and begins to perform its functions. Nowadays, it's become a hot issue to regard Wnt signaling pathway as the target of gene therapy of tumor in scientific filed (Tai et al., 2015). On the other hand, SERPINB3 was reported to be able to act on TGFβ (Turato et al., 2014) which is a kind of cytokine with multiple biologic activities and it is involved in cell proliferation, differentiation and apoptosis. There has been a study indicating that TGFβ has very complicated influence on tumor. It can inhibit or improve tumor's development and metastasis by tumor microenvironment, for instance, during early stage of tumor TGFβ can inhibit cell proliferation and induce apoptosis, but if the tumor is in developing stage, TGFβ can promote tumor's development and metastasis through multiple mechanisms (Brian and Moses, 2006). p53 is a tumor suppressor gene (Meek, 2015) and was thought to be able to inhibit KRT19 expression in study of Molès et al. (1994). Mutant p53 gene can cause its original

function loss which means it cannot regulate cell proliferation, growth or DNA repair thus it becomes an oncogene. Signaling pathway mediated by P53 gene plays a crucial role in regulating normal cell life activities and it has complicated connection with other signaling pathway inside cell, and p53 has been the most relevant gene to human tumors till now.

5. Conclusions

In this study, four serological tumor markers, CYFRA21-1, CA125, CA153 and SCC-Ag, related to SPN nature were screened out by Fisher discriminant method, and their corresponding genes were identified through literature search. And then correlative genes to lung cancer taking part in the regulatory pathway of lung cancer's growth and development were analyzed by GO and KEGG analysis. Based on signaling pathway of erbB4, several tumor marker genes were supplied to draw a regulatory network of lung cancer cell escaping from apoptosis, which lays a foundation for supplement and improvement of lung cancer signaling pathway and curves out the way for cancer treatment targeting with oncogenes.

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