Analysis on the Expression and Significance of Circadian Clock Gene Per2 in Non-small-cell Lung Cancer

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Abstract. *Purpose:* The purpose of this subject is to explore the expression and significance of clock gene Per2 in non-small-cell lung cancer patient. *Method:* taking the normal lung tissues of 40 cases of patients received pulmonary cyst and pulmonary bulla surgery of one domestic hospital during April 2015 to August 2017 as control group, perform immunohistochemically staining with the lesion tissue of 40 cases of non-small-cell lung cancer patients of the same period so as to determine the expression of clock gene Per2. *Result:* the negative expression of clock gene Per2 in non-small-cell lung cancer patients are mainly related to the factors such as differentiation grade, TNM staging, etc($P<0.05$), while the Per2 positive expression rate 72.5% is lower than that of normal lung tissue, which is 95%($P<0.05$). And the 1 year survival rate, 2 year survival rate and 3 year survival rate are 85%, 62.5%, and 52.5% respectively. Meanwhile, the 3 year survival rate shows correlation ($P<0.05$)mainly with factors including differentiation grade, TNM staging, lymphatic metastasis, and Per2 positive expression and so on. These 4 pathological indexes, namely differentiation grade, TNM staging, lymphatic metastasis, and Per2 positive expression, are not independent risk factors($P>0.05$), but they are influence factors ($P<0.05$)for the increase of lung cancer death. *Conclusion:* the expression of clock gene Per2 in non-small-cell lung cancer patient lowers appreciably, and the negative expression plays an important role in the occurrence and development of non-small-cell lung cancer, which has a great influence on prognosis.

Introduction

The organism in living nature has universal circadian clock in aspects such as biochemistry, physiology and behavior, while disorder appears in circadian changes may easily cause tumor [1]. Some scholars have found in epidemiological studies that incidence of breast cancer in females with long-term night shift is obviously higher that in women working normally, and meanwhile, the prostate cancer happens more often in male night shift workers whose circadian clocks are disordered than those having regular working time [2]. As important gene components of circadian clock, clock gene Per2 is closely related to the development and incurrence of tumor; Common malignant tumors including neuroglioma, pancreatic cancer also has the expressions [3] of deficiency and reduction of Per 2. In this paper, 40 cases of non-small-cell lung cancer patient

are chosen to test their cancer tissues Per2 expression with immunohistochemically stain method and in-depth research on the role of Per2 in the incurrence, development and metastasis of non-small-cell lung cancer, as well as the relationship between Per2 and prognosis of patients is conducted.

Materials and methods

All medical records of 40 cases of non-small-cell lung cancer patients received by a domestic hospital during April 2015 to August 2017 (2015-04 to 2017-08) are collected. In which, 27 cases are male patients, 13 cases are female patients; aging from 37~76, averagely(62.38±8.36)years old, 16 cases ≤60 years ole, while 24 cases >60 years old; 14 cases without history of smoking, while 26 cases with history of smoking. 25 cases are peripheral, 15 cases are of central type; 21 cases of glandular cancer, 19 cases of squamous cell carcinoma; as of differentiation grade, 23 cases are medium and high, while 17 cases are of low differentiation; as of TNM staging, 8 cases are stage I, 19 cases are stage II, and 13 cases are stage III; 23 cases have no lymphatic metastasis, while the other 17 case have lymphatic metastasis. 40 cases of patients received pulmonary cyst and pulmonary bulla surgery of the same period are chosen as control group, of which the normal lung tissues removed during surgery are made into specimen and submitted for censorship. Follow-up period is calculated since the date of surgery. And the cut-off time shall be the death time of patients or August 27, 2017.

Immunohistochemistry SP Kit and Mouse Anti Human Per2 Monoclonal Antibody are purchased from Beijing Beiruida Medicine Technology Co., Ltd. and Shanghai Tiancheng Technology Co., Ltd. respectively. After completing related operation according to instructions, slice up the paraffin block of tissues according to testing requirements, apply xylene for dewaxing to water, perform antigen retrieval, and then replace the primary antibody with phosphate buffer as negative control. Take ten views of high power lens and separately count one hundred cells, the counting principle is as follows: 0 point refers to no positive cell; 1 point refers to that 1%~25% are positive cells; 2 points refers to that 26%~50% are positive cells; 3 points refers to that 51%~75% are positive cells; and 4 refers to that 75% above are positive cells. At the same time, colorless cell is 0 point; pale yellow cell is 1 point; yellow cell is 2 points; and brown cell is 3 points. The positive staining standard of circadian clock gene Per2 is that brown (or yellow) particles appear in cytoplasm or nucleus.

Statistical analysis is performed with statistical software SPSS22.0. Enumeration data applies Chi-square test, while the measurement data for paired design applies paired t-test. Enumeration data is described with (%) while measurement data with (±s). $P<0.05$ means having statistical significance.

Result

The negative expression of circadian clock gene Per2 has correlation ($P<0.05$) with the TMN staging, differentiation grade of non- small cell lung cancer patients, and meanwhile, smoking history, age, gender, gross type, lymphatic metastasis has non-correlation with factors such as pathological type. The positive expression rate of circadian clock gene Per2 in non-small cell lung cancer tissues is only 72.5%(29/40), which is obviously lower than the positive expression rate of circadian clock gene Per2 in normal lung tissues, 95% (38/40). Statistical difference ($P<0.05$) exists between these two Per2 positive expression rate. Please see table 1 for details.

Table 1 Circadian clock gene Per2's expression and pathology feature in non-small cell lung *cancer tissues*

Pathology feature	No. of cases (n)	Circadian clock gene Per2		x^2	P
		(+)	(-)		
Normal lung tissue	40	38	2		
Non small cell lung cancer tissues	40	29	11	5.8783	0.0153
Differentiation grade					
Medium and high differentiation	23	19	4	7.3758	0.0066
Low differentiation	17	10	7		
TMN staging					
Stage I	8	7	1		
Stage II	19	14	5	6.1573	0.0104
Stage III	13	8	5		

The 1-year survival rate, 2-year survival rate and 3-year survival rate of patients with non small cell lung cancer are 85% (34/40), 62.5% (25/40), and 52.5% (21/40) respectively. It's found in Kaplan-Meier Single Factor Analysis that the 3-year survival rate of patients with non small cell lung cancer is related to factors such as TNM staging, differentiation grade, positive expression of circadian clock gene Per2, lymphatic metastasis, etc ($P<0.05$), and meanwhile, it has non-correlation with factors including pathological type, gender, gross type, age, history of smocking, etc ($P>0.05$).

Including the 4 pathological indexes (namely lymphatic metastasis, differentiation grade, positive expression of circadian clock gene Per2, and TNM staging) screened out via Kaplan-Meier Single Factor Analysis into COX proportional hazards model to perform multi-factor analysis, it is prompted that the reduction of differentiation grade of non small cell lung cancer and the appearance of lymphatic metastasis, the expression of Per2 is negative, and the risk ratio of lung cancer death after TNM progress is obviously increased ($P<0.05$). But none of these 4 pathological indexes is the independent risk factor which influences the prognosis of non-small cell lung cancer ($P>0.05$). As shown in table 2:

Table 2 Multi-factor analysis on 4 pathological indexes of non small cell lung cancer

	SE	B	df	Wald	Sig.	Exp
Differentiation grade	0.394	0.573	1	2.115	0.143	1.776
TNM staging	0.336	0.364	1	1.172	0.279	1.442
Lymphatic metastasis	0.491	-0.187	1	0.143	0.703	0.828
Per2 negative	0.408	-0.563	1	1.892	0.167	0.568

Discussion

Circadian clock gene was firstly discovered in the SCN of mammal (hypothalamic suprachiasmatic nucleus), and then its expression was also found in peripheral tissue. So far, scholars have found 10 genes, including Bmal1, clock and Per1, etc having the effect of circadian rhythm regulation, in mammalian body [4]. Under the action of mechanism, Clock / Bmal1 heterodimers combining with R e-Erba, Cry and Per gene can start-up the e-box on partition to activate its gene transcription. Meanwhile, Cry and Per proteins directly affect Clock / Bmal1 heterodimers, and the suppressor gene transcription can also be clearly feedbacked. Body clock is of negative feedback loop constitution.

Circadian clock gene Per2 can make great difference in regulation of biological cycle circadian. In case of circadian clock gene Per2 deletion or mutation, circadian system may then disorder. Expert Chi Chuang found in his research on hereditary and familial breast cancer that the Per2 gene in tumor tissue reduced significantly, which promoted that cause of disease of patient with breast cancer especially familial breast cancer is related to the disorder of circadian clock gene [5].

Yang Pei et al [6]suggested that the expression of circadian clock gene Per2 in colorectal cancer; is relatively lower than that of normal person, while the negative expression of Per2 is closely related to the TMN staging, age, invasion depth and histological grade of patient with colorectal cancer. Through analysis on nonsmall-cell lung cancer patients, it is found in this study that Per2 protein has expressions in both normal lung tissue and nonsmall-cell lung cancer tissue, but the positive expression rate of the later is obviously lower than that of the former ($P<0.05$). Per2 protein is related to the TMN staging, differentiation grade of nonsmall-cell lung cancer, then the positive expression rate may also reduce when the differentiation grade of tumor is relatively low ($P<0.05$). Therefore, close relation with the occurrence and development of disease may be observed in the abnormal expression of circadian clock gene Per2 protein of nonsmall-cell lung cancer tissue.

After regulation of tumor suppressor genes and proto-oncogenes, it is found that circadian clock gene plays a role in tumor progression. Lin Xiaoming et al[7] discovered that, thanks to the agonist activity of vascular endothelial growth factor caused due to tissue hypoxia, the Per2

protein in transplanted tumor cell is restrained, and there is also limitation to the angiogenesis of tumor. Research findings show that, circadian gene mPer2 has obvious effect when tumor occurs to mice, and the tumor incidence of wild-type mice is obviously lower than that of mPer2 mutant mice. There is also study suggesting that over-expression of Per2 may cause reduction of cell differentiation and rapid cell apoptosis, and corresponding changes may happen at the meantime of expression of apoptosis associated gene.

In summary, circadian clock gene Per 2 plays an important role in the occurrence and development of nonsmall-cell lung cancer, so it can offer a help to the early treatment and prevention of such cancer.

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