Chronic Diseases Prevention Review 2 (2017) 27-31

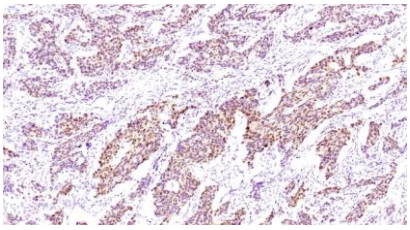
Available at http:// www.cancercellresearch.org ISSN 2158-0820

Expression of PES1 and EGFR in gastric cancer and their clinical significance

Jun Liu[1], Weina Jiang[2], Hua Chen[2,*]

[1]Department of Pathology, Medical College of Qingdao University, Qingdao, 266003, China

[2]Department of Pathology, Oingdao Municipal Hospital, Qingdao, 266071, China



Abstract: **To investigate the expression of PES1 and EGFR in gastric cancer, and then to explore the relationship between their expression and some clinicopathologic parameters. The expression of PES1 and EGFR was detected by immunohistochemistry in 60 cases of gastric cancer. The positive expression of PES1 and EGFR protein in gastric cancer were significantly higher than those in adjacent tissues (P<0.05). The high expression of PES1 is related to tumor invasive depth and clinical stage (P<0.05, respectively), and the high expression of EGFR was closely related with the depth of invasion, clinical stage and lymph node metastasis (P<0.05, respectively). PES1 and EGFR may play an important role in gastric cancer development, which may be potential targets for the treatment and prognostic evaluation of gastric cancer.**

Keywords: PES1; EGFR; Gastric cancer; Immunohistochemistry

Received 16 February 2017, Revised 23 April 2017, Accepted 25 April 2017

*Corresponding Author: Hua Chen, chenhua62@163.com

1. Introduction

Pescadillo gene is initially discovered and identified in a study on the development of zebra fish embryo, and it remains highly conservative in many organisms. The PES1 protein encoded by pescadillo gene is a very important nucleolus protein [1]. It plays an important role in normal embryonic development, production of ribosome, DNA replication, maintenance of chromosome stability, cell cycle control and other vital processes. Some research results indicated in recent years that, PES1 might play an important part in the genesis and development of gastric cancer [2], PES1 might be a potential target for treatment of gastric cancer.

EGFR belongs to the family of tyrosine kinase I receptor, its gene is located at the short arm of No.7 chromosome of human, containing 28 exons. EGFR is distributed over the cell membrane of a variety of epithelial cells, EGFR binds to the ligand by stimulating it and then induces a series of cellular effect through many signal transduction pathways, including the cellular proliferation, migration, adhesion and other links. A high expression of EGFR usually coincides with an occurrence of tumor in organism. An over-expression of EGFR promotes the proliferation of tumor cell, formation of vessels, adhesion, invasion and migration and inhibits the apoptosis of tumor cells. In recent years [3], there have been reports that, EGFR had a high expression in gastric cancer tissue.

Using immunohistochemical staining, this study observed the expression of PES1 and EGFR protein in the gastric cancer tissue and para-carcinoma tissue, in order to explore the correlation between the both and clinical pathological features of gastric cancer and the correlation between the both, and to clarify the significance of expression of PES1 and EGFR in gastric cancer, so as to provide a new thought and basis for the treatment of gastric cancer and prognostic evaluation.

2. Materials and methods

2.1. Object of study

60 cases of specimen of patients with primary gastric cancer who were treated by a surgical treatment in LinYi Central Hospital from Jan, 2015 to Oct, 2016 had been collected, among which there were 45 males and 15 females, aging from 27 to 74 years-old; 9 cases of highly and moderately differentiated adenocarcinoma, 51 cases of lowly differentiated adenocarcinoma including mucinous adenocarcinoma and signet-ring cell carcinoma; 43 cases had serosa invasion, 17 cases didn't have serosa invasion; 46 cases had lymphatic metastasis, 14 cases didn't; there were 22 cases whose clinical staging was I to II, 38 cases whose clinical staging was III to IV. Each case was sampled by cancer tissue and para-carcinoma tissue (more than 5cm away from the cancer tissue) after surgery. None of 60 cases of patient had a family history of tumor, and nor had they received a chemotherapy or radiotherapy or other anti-cancer therapies before surgery, all the cases were definitely diagnosed by a pathological diagnosis.

2.2. Research methods

All the specimens were fixed by 10% neutral

buffered formalin and embedded by paraffin, followed by a conventional pothological reading of section with HE staining before a typical paraffin block was selected for immnohistochemical staining. Rabbit-anti-human polyclonal PES1 antibody was purchased from Abcam company, the antibody titer was diluted to 1:200 using a special antibody diluent, ready-to-use rabbit-anti-human monoclonal EGFR antibody was purchased from the genetic science and technology Company. Enzyme-labeled goat-anti-mouse/rabbit IgG polymer (universal) second antibody was purchased from genetic science and technology Company. The immunohistochemistry was done by the manual two-step S-P method. The known positive sections of breast cancer and lung squamous cell carcinoma were taken as positive controls for PES1 and EGFR, respectively.

2.3. Judgment criterion

PES1 protein positive staining was located at the cell nucleus, EGFR positive staining was located at the cell membrane and cytoplasm, the section was read by more than two senior pathologists. Judgment criterion for immunohistochemical result: the whole section was browsed under a low-power microscope in order to observe the staining in the target cells (tumor parenchymal cells) and distribution of positive cells, 10 high power field (HPF) were randomly chosen and observed in the highly-expressed region, firstly the staining intensity was graded: 0 score signifies colorless, 1 score signifies light yellow, 2 scores signifies brownish yellow, 3 scores signifies tan yellow; then the positive cells were graded in percentage: 0 score signifies negative, 1 score signifies positive cells ≤10%, 2 scores signifies 11%-50%, 3 scores signifies 51%-75%, 4 scores signifies >75%. It is judged as positive when product of staining intensity and percentage of positive cells is more than 3 scores, it is judged as negative when the product is equal to or less than 3 scores.

2.4. Statistical analysis

This study used SPSS17.0 statistical software for the treatment of statistical analysis on data, the difference in protein expression between cancer tissue and para-carcinoma tissue as well as the correlation between them and clinical pathological parameters were tested by chi-square test. The difference is considered to be statistically significant when $P < 0.05$.

3. Results

3.1. The expression of PES1 protein in the gastric cancer and para-carcinoma tissue

PES1 is largely located at the cell nucleus. The result revealed that, the positive expression rate of PES1 protein in gastric cancer tissue was clearly higher than in para-carcinoma tissue, the difference was statistically significant ($P<0.05$, Table 1, Figure 1).

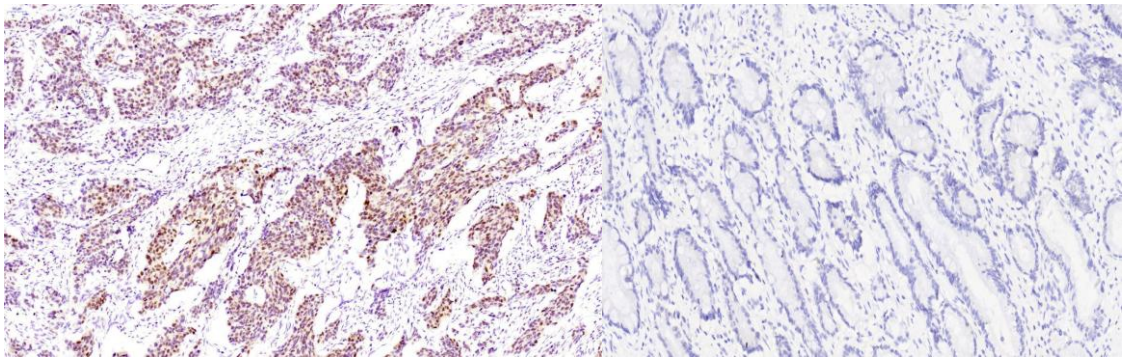


Figure 1. The expression of PES1 in the gastric cancer(left) and the adjacent tissues(right) (200x).

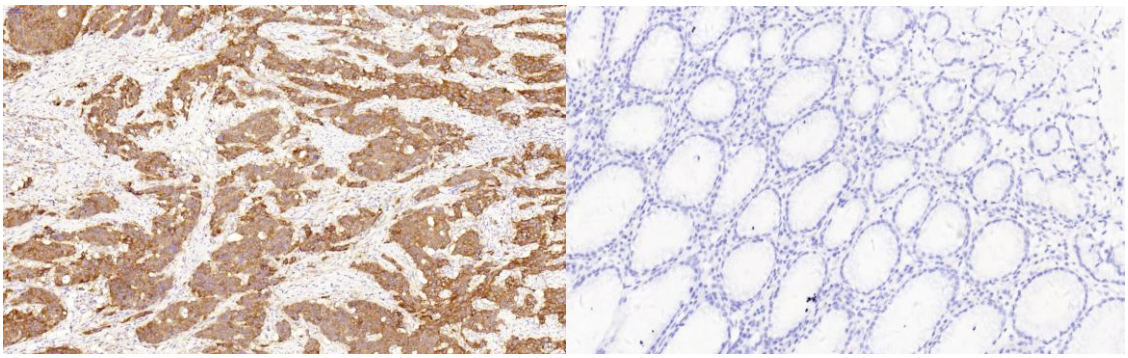


Figure 2. The expression of EGFR in the gastric cancer(left) and the adjacent tissues(right) (200x).

Table 1 The expression of PES1 and EGFR in the gastric cancer and para-carcinoma tissue

Group	n	PES1			EGFR		
		positive(%)	x^2	*P*	positive(%)	x^2	*P*
Gastric cancer tissue	60	19(31.67)	16.681	0.000	45(75.00)	51.867	0.000
Para-cancer tissue	60	2(3.33)			6(10.00)		

3.2. Expression of EGFR protein in the gastric cancer tissue and para-carcinoma tissue

EGFR is largely located at the cell membrane, and it is also stained in the cytoplasm. The positive expression rate of EGFR in gastric cancer tissue was clearly higher than in para-carcinoma tissue, the difference was statistically significant (P<0.05, Table1, Figure 2).

3.3. Correlation between PES1 and EGFR

Since both the expression of PES1 and EGFR in gastric cancer tissue were significantly higher than in para-carcinoma tissue, we further analyzed whether there was a correlation between the both or not. The result showed that, the expression of PES1 was not evidently correlated with that of EGFR (P>0.05, Table2).

Table 2 Study on correlation between PES1 and EGFR

		PES1		*r*	*P*
		positive（n）	negative（n）		
EGFR	Positive(n)	15	30	0.062	0.873
	Negative(n)	4	11		

3.4. Relationship between expression of PES1 and EGFR in gastric cancer tissue and clinical pathological parameters

The expression of PES1 was closely related to the depth of invasion of gastric cancer and clinical staging (P<0.05, respectively); the differences between it and patient's gender, age, degree of tumor differentiation and lymphatic metastasis were not statistically significant (P>0.05, respectively); the expression of EGFR was related to the depth of invasion of gastric cancer, clinical staging and lymphatic metastasis (P<0.05, respectively), and was not related to the patient's gender, age and degree of tumor differentiation (P>0.05, Table 3, respectively).

4. Discussion

Human's pescadillo gene is located at the chromosome 22q12.1 [4], its encoding nucleolus protein PES1 contains 588 amino acids, molecular weight: 69KD. The protein structure contains a BRCT (BRCA1 C-terminal) structural domain, 2 acidic amino acid structural clusters in C terminal, 3 nuclear localization signal sequences, multiple predicted tyrosine/serine/theronine phosphorylation sites and multiple SUMO (small ubiquitin modification)modification sites. PES1 is necessary for the biological synthesis of ribosome. It promotes the growth and proliferation of tumor cells by involving in the production of ribosome RNA.

The role of PES1 in the genesis and development of human tumor attracts more and more attentions, it has been found by many studies that [5-7], the expression of PES1 is up-regulated in many human malignant tumors, such as neuroblastoma, breast cancer, ovarian cancer and colon cancer. There was also literature reporting that [8], the rate of positive expression of PES1 in gastric cancer tissue was 40.7%, the counterpart in para-carcinoma tissue is 0%. In this study, the rate of positive expression of PES1 in gastric cancer tissue (31.67%) was obviously higher than in the corresponding para-carcinoma tissue (3.33%), which was approximate to the data reported in literature.

EGFR is a transmembrane receptor, which promotes the mitosis and proliferation of cell after binding to ligand. A high expression of EGFR was found in many malignant tumors including the digestive system tumor, and it was closely related to the genesis and development, proliferation and metastasis of tumor and to the formation of tumor vessels.

EGFR was overly expressed in gastric cancer, some scholars believe that [9], its high expression was associated with a poor prognosis, the tumor with a positive EGFR may have a stronger activity of mitosis and proliferation and stronger invasiveness. This study found that, the rate of positive expression of EGFR in gastric cancer tissue (75%) was evidently higher in para-carcinoma tissue (10%). And the high expression of PES1 and EGFR were both related to the depth of invasion and clinical staging of gastric cancer; the expression of EGFR was also closely related to the lymphatic metastasis of gastric cancer, indicating that, PES1 and EGFR maybe important indexes for the

prognostic evaluation of patients with gastric cancer.

Table 3 Relationship between expression of PES1 and EGFR in gastric cancer and clinical pathological parameters (case, %)

clinical pathological parameters	n	PES1 positive	x^2	P	EGFR positive	x^2	P
age/years-old							
<60	26	9(34.62)	0.184	0.439	22(84.62)	2.262	0.114
≥60	34	10(29.41)			23(67.65)		
Gender							
Male	45	15(33.33)	0.026	0.445	33(73.33)	0.030	0.444
Female	15	4(26.67)			12(80.00)		
Depth of invasion							
Invading to serosal layer	43	18(41.86)	7.288	0.005	39(90.70)	17.100	0.000
Not invading to serosal layer	17	1(5.88)			6(35.29)		
Tumor differentiation							
High/moderate	9	4(44.44)	0.255	0.299	6(66.67)	0.044	0.399
Low	51	15(29.41)			39(76.47)		
Clinical staging							
Ⅰ-Ⅱ	22	2(9.09)	8.182	0.004	8(36.36)	27.656	0.000
Ⅲ-Ⅳ	38	17(44.74)			37(97.37)		
Lymphatic metastasis							
Yes	46	13(28.26)	0.490	0.239	43(93.48)	31.801	0.000
No	14	6(42.86)			2(14.29)		

Besides, this study also found that, in the gastric cancer tissue, the expression of PES1 was not evidently correlated with that of EGFR (P>0.05), which may be associated with the difference between the signal pathway of both, the expression of PES1 in gastrointestinal tract cancer is subject to the regulation by the intranuclear transcriptional factor c-Jun [10], the member of b-ZIP family. While EGFR induces [11] a stream of cellular effects through RAS-RAF-ErK and other signal transduction pathways after binding to ligand by stimulating the ligand. The specific mechanism awaits a further experimental confirmation.

In view of the over-expression of EGFR in gastric cancer tissue, the EGFR target therapy drugs (e.g., Cetuximab) have been accordingly developed, Cetuximab has achieved a good effect in both the stage-I and II clinical tests during the study on treatment of gastric cancer [12,13]; there are also literatures reporting that [14], PES1 can regulate the sensitivity of colon cancer cell to anticancer drugs, demonstrating the effect of PES1 on guiding the treatment of intestinal malignant tumor, but its effect on guiding the treatment of gastric cancer remains unclear, in need of a further study.

To sum up, this study confirmed that PES1 and EGFR were overexpressin in gastric cancer than those in adjacent tissues, and the higher expression of PES1 was closely related to the depth of tumor invasion and clinical staging, while the higher expression of EGFR was closely related to the depth of tumor invasion, clinical staging and lymphatic metastasis. These results indicate that, PES1 and EGFR protein may be closely related to the genesis and development of gastric cancer, providing a new thought for the treatment of gastric cancer and prognostic evaluation.

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