Alterations of multiple biomarkers in esophageal carcinoma and precancerous lesions from same patient

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Abstract

Background. Esophageal carcinoma (EC) is regarded as a multistage and progressive process. The molecular mechanism of EC is still not clear. *Objective.* To investigate the alterations and significance of p53, $p21^{waf1}$, PCNA and BrdU expressions in the esophageal squamous cell carcinoma (SCC) and the precancerous lesions from same individuals. *Methods.* The 37 surgically resected SCC specimens with tissue adjacent to cancer (normal 7, BCH 29, DYS 23) were collected, fixed with 85% ethanol, embedded with paraffin and sectioned. Immunohistochemistry ABC method was used to detect the PCNA, p53, $p21^{waf1}$ and BrdU. *Results.* From normal (NOR), basal cell hyperplasia (BCH), dysplasia (DYS) to squamous cell carcinom (SCC), p53, PCNA and BrdU were expressed increasingly, but the expression of $p21^{waf1}$ decreased. The expressions of p53, $p21^{waf1}$ and PCNA were not related to the age and gender, but they were related to the lymphatic metastasis and differential level of the cells respectively. *Conclusions.* $p21^{waf1}$ might be one of important protective factors in carcinogenesis of esophageal carcinoma (EC). The alterations of $p21^{waf1}$ and p53 reflect the functional status of esophageal epithelium. p53 and PCNA may be predictive biomarkers for lymph node metastasis and differentiation of EC. [Life Science Journal. 2007; 4(4): 29 – 32] (ISSN: 1097 – 8135).

Keywords: esophageal neoplasm; carcinoma; p53; p21; PCNA; BrdU

1 Introduction

Esophageal carcinoma (EC) is regarded as a multistage and progressive process. The early characteristic for the subject predisposed to EC is the abnormal proliferation of epithelial cells, morphologically manifested as basal cell hyperplasia (BCH), dysplasia (DYS) and carcinoma *in situ* (CIS), which could be considered as precancerous lesions of EC. The precancerous lesions are unstable i.e, it can develop to cancer, or stay couple of years without any changes, even return to normal. To characterize the molecular changes in carcinogenesis of EC could elucidate the mechanism of EC and establish the biomarkers for early diagnosis and biotherapy.

The molecular mechanism of EC is still not clear. Studies in our department and other laboratories indicate that protein P53 accumulation and gene mutation are very early events in esophageal carcinogenisis. The molecular basis for P53 protein accumulation is not clear. And we can not distinguish effectively the wild and mutated type of p53 at the level of immunobiochemistry. Over expression of wild p53 could induce $p21^{waf1}$ arrest the cell at S phase. Thus, to determine the relationship between p53, $p21^{waf1}$ and S phase cell index shed light on the molecular mechanism of p53 protein accumulation.

In this study, we analyzed the changes and rules of p53 accumulation and expression of $p21^{waf1}$ in the esophageal precancerous and cancerous lesions from the same patient at Linzhou and Anyang, the high incidence areas for EC, in Henan, Northern China. The immunopositive cell number of p53, $p21^{waf1}$ was correlated with S phase cells as labeled by bromodeoxyuridine (BrdU) to provide further information for the molecular basis of P53 protein accumulation. We also detected the expression of proliferating

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cell nuclear antigen (PCNA).

2 Materials and Methods

2.1 Samples collection

37 surgically resected EC specimens were obtained from the patients in Linzhou, Anyang and nearby counties, the high incidence area for EC in Northern China. Each specimen from one patient was cut into two segments, the cancer tissue and its adjacent tissue were collected, embedded in the same wax fixed frame and serially sectioned. Thus we got the tumor tissues (37), tissues adjacent to cancer (BCH 29, DYS 23) and normal mocusa (NOR 7) samples. Out of the 37 cases, the relationship between the clinical charcters and the expression of P53, $P21^{waf1}$, PCNA and BrdU were analysed in 32 cases with perfect pathological and clinical data.

2.2 Samples measurement

All the specimens were fixed with 85% ethanol, embedded with paraffin and serially sectioned. Immunohistrchemistry ABC method was used to detect the PCNA, p53, BrdU, $p21^{waf1}$ (Ongogene Science, Inc, Manhasset, NY). Quantitative analysis for immunoreactivity was applied on the samples with Olympus microscope and HPLAS-100 colorful pathological analysis system.

2.3 Statistical analysis

All the results were expressed as $\bar{X} \pm$ SD. Chi-square, Wilcoxon and *t*-test and SAS system were performed for statistical analysis in this study.

3 Results

3.1 Expression of p53, $p21^{Waf1}$, PCNA and BrdU in different types of esophageal cancer and normal tissues

Immunoreactivity of p53, $p21^{waf1}$, PCNA was located in the cell nuclei. The expression of these three targes are showed in Table 1. Immunostaining positive cells of p53, PCNA were located mostly in the first and the second layers of epithelial of basal cells, but $p21^{waf1}$ identified in the third and fourth layers of epithelial of basal cells. With the lesions developed from NOR to BCH, DYS and SCC the p53 positive cells increased ($P < 0.001$). The number of $p21^{waf1}$ positive cells per mililiter were increased slightly from NOR (406 ± 422) to BCH (635 ± 522), and decreased in DYS (394 ± 374) and squamous cell carcinom (SCC) (321 ± 282). The difference was significant ($P < 0.05$).

The number of PCNA positive cells per mililiter was also swelled from NOR (648 ± 230), BCH (746 ± 376), DYS (911 ± 381) to SCC (1428 ± 498). There were notable differences in most groups except NOR *vs.* BCH and BCH *vs.* DYS. The expressions of tumor suppressor gene p53 was related to lymph node metastasis and poor differentiation of EC. The difference was significant ($P < 0.05$). And the number of BrdU positive cells was also expanded from NOR (63 ± 35) to BCH (85 ± 43) to DYS (114 ± 60) and SCC (165 ± 75). There were notable differences in most groups except the group "NOR to BCH".

3.2 Relation of clinical features and p53, $p21^{waf1}$, PCNA and BrdU

Table 2 showed the relationship between the clinical data and the expression of p53, $p21^{waf1}$, PCNA and BrdU. The protein P53 differed greatly in the lymph node metastasis and poorly differentiation of EC. The difference was significant ($P < 0.05$). As to the $p21^{waf1}$ and PCNA, they were bound up with the differentiation level of the cancer cells. The difference was significant. And there was no marked difference between the clinical data and the expression of BrdU.

4 Discussion

Esophageal cancer is still one of the most widespread diseases, and surgery for esophageal carcinoma is hard for patients. The p53 protein is well-known for its function of tumour suppression. The p53 gene encodes a protein of 53 kDa (P53). This gene, first identified as a tumor suppressor gene in 1979, is located on chromosome 17p[1]. Wild p53 regulates diverse cell functions, including cell cycle progression, senescence, differentiation, and apoptosis. Recently, the role of p53 in DNA repair after genotoxic insult has also been reported[2]. The most common responses to oncogenic stress induced by p53 are cell cycle arrest. If the cell can not go into the normal cell cycle, p53 will induce cell apoptosis[3]. The p53 gene product is normally "off" and is "activated" by post-translational modifications, such as phosphorylation, in response to cellular stress or damage. In this research, we found that with the cancer developed, the p53 positive cells increased. This indicated that p53 protein expression might be related to the esophageal cancer progression. Multiple stresses lead to p53 activation. These cellular stresses lead to increased p53 protein expression. Some researches take that p53 mutation may increase the protein half-life, leading to increased levels of protein expression. In this study, we

Table 1. Expression of p53, $p21^{Waf1}$, PCNA and BrdU in esophageal cancer and precancerous lesions (positive cells/ml)

Groups	*n*	p53	$p21^{waf1}$	PCNA	BrdU
NOR	7	81 ± 79	406 ± 422	648 ± 230*	63 ± 35
BCH	29	131 ± 92$^{\#}$	635 ± 522	746 ± 376*	84 ± 43
DYS	23	309 ± 197$^{*\#}$	394 ± 374*	911 ± 381*	114 ± 60**
SCC	37	926 ± 553$^{\#}$	321 ± 282	1428 ± 498	165 ± 75**

$^{\#}$: $P < 0.05$ between every two groups; *: $P < 0.05$, compare with SCC; **: $P < 0.05$, compare with NOR.

Table 2. Relationship between the clinical charcters and the expression of p53, $p21^{waf1}$, PCNA and BrdU

Clinical data		Cases (*n*)	p53 (positive cells/ml)	$p21^{waf1}$ (positive cells/ml)	PCNA (positive cells/ml)	BrdU (positive cells/ml)
Gender	M	23	938 ± 644	270 ± 217	1408 ± 409	161 ± 90
	F	9	1018 ± 474	257 ± 304	1585 ± 749	149 ± 70
Age (years)	≥ 60	18	885 ± 686	285 ± 261	1375 ± 560	133 ± 60
	< 60	14	1046 ± 477	243 ± 220	1564 ± 461	1564 ± 461
Lymphatic metastasis	+	18	1557 ± 439$^{\#}$	218 ± 180	1571 ± 272$^{\#}$	171 ± 92
	–	14	470 ± 149	325 ± 294	912 ± 260	912 ± 260
Cell differentiation	I, II	21	926 ± 628*	325 ± 282	1424 ± 476	148 ± 76
	III	11	1045 ± 517	238 ± 220	1887 ± 361	1887 ± 361
Cell infiltration	intermucous	17	757 ± 421	261 ± 213	1357 ± 368	144 ± 68
	serosa	15	1120 ± 661	271 ± 274	1547 ± 622	1547 ± 622

$^{\#}$: $P < 0.05$ compare with the group without lymphatic metastasis; *: $P < 0.05$ compare with the group with differentiation of degree I and II.

also found that the expression of p53 is related to the lymphatic metastasis which indicates p53 may be a promising biomarker for lymph node metastasis.

$p21^{waf1}$ is a potent, tight-binding inhibitor of Cdks which is induced by the wild-type p53 gene. The $p21^{waf1}$ level was lower in tumor tissues than in the corresponding nontumor tissues. These results suggested that the $p21^{waf1}$ expression level, which might be an index of cancer, was suppressed in human tumor tissues compared with the corresponding nontumor tissues[4]. Experimental data and clinical observations indicate that an increased expression of oncogenes or their point mutations play an essential role in the process of carcinogenesis. It is important to find out that environmental and occupational carcinogens activate cellular oncogenes and contribute to increased amounts or occurrence of oncoproteins[5,6]. The researches are mainly concerned about oncoprotein coded by the ras oncogene, called p21 protein. In our study, the expression of p21 markedly decreased, which indicated the p21 can suppress the cancer cells to some distance. As shown in Table 2, the $p21^{waf1}$ was related to the differential level of the cells.

Proliferating-cell nuclear antigen (PCNA) plays an essential role in nucleic-acid metabolism in all cells[7]. The PCNA protein interacts with a large number of proteins. These proteins can be divided into two groups: one group contains proteins that have a known enzymatic activity; the other group contains regulatory proteins that are involved in cell-cycle progression, checkpoint control and cellular differentiation. PCNA in the cell cycle control is recognised on the basis of the interaction with cyclins, cyclin-dependent kinases (cdks) and the cdk-inhibitor $p21^{waf1}$ protein. From the Table 1, we can find that PCNA is markedly increased from NOR to BCH, DYS, and SCC, which is related to the cell proliferating and cell differentiation.

Bromodeoxyuridine (BrdU) is a thymidine analog that incorporates DNA of dividing cells during the S-phase of the cell cycle. BrdU is widely used for labeling dividing cells to determine their fate In our study, we found that the BrdU positive cells increased more and more from NOR to SCC, which indicates that BrdU can evaluate the proliferation of the esophageal epithelial cells. And this results is similar with the study of Wang[8].

5 Conclusion

With the lesions progressed from NOR → BCH → DYS → SCC, the number of immunostaining positive cells for p53, PCNA increased significantly, especially from BCH → DYS; the similar result was observed for BrdU labeled cells. In contrast, $p21^{waf1}$ decreased after BCH, indicating that $p21^{waf1}$ may be one of important protective factors in EC, and that alterations of $p21^{waf1}$ and p53 reflect the functional status of esophageal epithelium. p53 and PCNA may be promising biomarkers for lymph node metastasis and poorly differentiation of EC.

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