EXPRESSION OF GST-π GENE IN HUMAN ESOPHAGEAL CARCINOGENESIS

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ABSTRACT

Objective: To investigate the possible role of GST-π in esophageal carcinogenesis. Methods: GST-π expression at mRNA level was studied by *in situ* hybridization (ISH) and at protein level by immunohistochemistry (IHC). GST-π expression in normal epithelial cells (NC) of the esophagus, hyperplastic cells (HC), dysplastic cells (DC) from grade I to III, carcinoma *in situ* (CIS) and all the cells in squamous cell carcinomas (SCC) were examined in the same esophageal cancer specimens (n=48) which provided a model reflecting the process of esophageal carcinogenesis. Results: The positive rate of IHC staining was 87. 5% for NC, 95.3% for HC, 55.9% for DC (grade I: 73.9%, grade II: 47.4%, grade III: 41.2%), 36.4% for CIS and 45.8% for SCC. The positive rate of GST-π mRNA expression was 81.2% for NC, 94.4% for HC, 61.9% for DC (grade I: 76.5%, grade II: 61.5%, grade III: 41.7%), 44.4% for CIS and 83.3% for grade I SCC, 30.0% for grade II SCC and 0% for grade III SCC. There was no statistically significant difference in GST-π expression at the mRNA and the protein level. Conclusion: There is a decreasing tendency of GST-π expression from dysplasia to CIS and SCC. The decrease in GST-π expression is an early event in esophageal carcinogenesis.

Key words: Esophageal neoplasms, Squamous cell carcinoma, Gene expression

Glutathione S-transfereases (GSTs) are an important family of isoenzymes involving in the detoxification of xenobiotics including mutagens and carcinogens and thus play a significant role in protecting cells from carcinogenesis and mutagenensis.[1] Extensive attention has been paid to the relationship between the human placental form of the enzyme (GST-π) and a tumor form.[2] The development of human esophageal carcinoma (EC) is a multistage process which shows a natural successive course from hyperplasia, dysplasia, carcinoma *in situ* to invasive carcinoma.[3] In the present study, the state of GST-π expression in EC was investigated by establishment of a model reflecting the progress of esophageal carcinogenesis via assay of immunohistochemistry (IHC) and *in situ* hybridization (ISH) in the same EC specimens.

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MATERIALS AND METHODS

EC Tissue Specimens

Tissue samples from 48 patients with EC were obtained at the time of surgery at Anyang Tumor Hospital (Henan, China). Resected specimens were dissected and stored in liquid nitrogen within 15 to 30 minutes after removal. All patients were permanent residents in a high-risk region of EC and received no treatment before surgery. The specimens were fixed in 4% paraformaldehyde, routinely dehydrated and paraffin-embedded following by sectioning at 4 to 5 um. The above operations were carried out under stringent conditions so as to prevent RNA in the tissue from being degraded by foreign RNase.

Agents

Anti-GST-π polyclonal antibody used in the study was purchased from Dako and was the product of Boehinger Mannheim Corporation (Germany).

Plasmid and Bacteria Strain

pGEM4 (GST-π cDNA, 725bp, EcoR I fragment) and pA2 (β-actin, 300bp, Hind III fragment) and HB101 were all kindly provided by Professor Li Chunhai, Department of Tumor Molecular Biology, China Academy of Military Medical Science.

Establishment of Multistage Model of Esophageal Carcinogenesis

According to the histological diagnosis criteria

of Qiu, et al.[3] each type of the clinicopathological lesions and their number among all specimens, including tumor tissues and adjacent mucosa, were shown as follows: For mucosa: hyperplasia 43, grade I dyplasia 23, grade II dyplasia 12, grade III dyplasia 17, carcinoma *in situ* (CIS) 22. For tumor: squamous cell carcinomas (SCC) grade I there were 21, SCC grade II there were 23, and SCC grade III there were 4. All of 48 samples contain normal epithelia.

IHC and Results

Tissue samples were stained immunohistochemically using LSAB method (procedure omitted) accompanyed by positive, negative and blank controls. In reference to the 1996 China Immunohistochemistry Techniques and Diagnosis Standardizing Symposium, the staining results were divided into four categories: (+) 25% to 50% of cells stained with light yellow fine granulae showing in cells; (++) 50% to 70% of cells stained with dark brown granulae showing in cells; (+++) more than 70% of cells stained with a great amount of dark brown granulae showing in cells; (–) less than 25% of cells stained or the cells staining intensity being consistent with that of the background.

ISH

Gene probe preparation

Recombinant plasmid transformation, amplification, DNA extraction, purification, restriction enzyme digestion and fragment recovering were processed as described in the reference.[4] Purified plasmid DNA and restriction enzyme fragments were identified by gel electrophoresis. Probe labeling and identification was performed according to the direction of the kit used.

Hybridization procedures

The procedures were adopted from those of Sato, et al.[5] The slides were exposed to 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro-blue tetrazolium (NBT) and counterstained with hematoxylin. Control sections were processed by the same procedure. Positive control hybridization was done by replacing GST-π positive placental tissue. As by using a known to negative control, the slides were treated with RNase 100 μg/ml for 1.5 hours before hybridization.

Statistical Analysis

Proportion (relative number, %) was used for result analysis and Chi-square test was selected for statistical analysis.

RESULTS

IHC

Protein expression of GST-π in esophageal carcinogenesis is illustrated in Table 1. Positive GST-π expression rates in normal epithelial cells (NC), hyperplastic cells (HC), total dysplastic cells (DC) and DC from grade I to III and carcinoma *in situ* (CIS) were 87.5%, 95.3%, 55.9%, 73.9%, 47.4%, 41.2% and 36.4% respectively. There was a significant expression difference in DC and CIS compared with NC and HC ($P<0.005$). The GST-π expression, however, showed no marked correlation related to the grade of DC ($P>0.05$). Concerning tumor tissue, positively stained rate of SCC, SCC grade I and II were 45.8%, 81.0% and 30.4% respectively. 4 cases of SCC Grade III were all negatively stained. Comparing the positive rate of total SCC with that of NC and HC, the differences were remarkable, ($P<0.005$). The grade of SCC was correlated to GST-π expression ($P<0.05$) with a decreasing tendency.

Table 1. GST-π protein expression in esophageal carcinogenesis

Histology	Number	GST-π expression				Positive rates(%)
		–	+	++	+++	
Normal Epithelial cells(NC)	48	6	31	10	1	87.5
Hyperplastic cells(HC)	43	2	20	19	2	95.3
Dysplastic cells(DC)	59	26	20	12	1	55.9*
Grade I	23	6	10	7	0	73.9
Grade II	19	10	5	4	0	47.4*
Grade III	17	10	5	1	1	41.2*
Carcinoma *in situ*(CIS)	22	14	4	2	2	36.4*
Squamous cell carcinoma (SCC)	48	26	17	3	2	45.8*
Grade I	21	4	13	2	2	81.0
Grade II	23	16	6	1	0	30.4
Grade III	4	4	0	0	0	0.0

Note:DC:HC, CIS:HC,SCC:HC, $P<0.005$; dysplastic cells $\chi^2 = 5.08$, $P>0.05$; squamous cell carcinoma $\chi^2 = 15.57$, $P<0.05$
*Comparing with normal epithelial cells, $P<0.005$

ISH

The results revealed that GST-π mRNA positive signals were predominant in nuclei and perinuclear cytoplasm. The positive rate of hybridization was 81.2% for NC, 94.4% for HC, 61.9% for DC (grade I: 76.5%, grade II: 61.5%, grade III: 41.7%), 44.4% for CIS and 83.3% for grade I SCC, 30.0% for grade II SCC and 0% for grade III SCC. No statistically significant difference was observed between the result of IHC and that of ISH ($P>0.05$). The distribution of positive hybridization signals gradually varied from basal one-third to the whole layer of the epithelia. In tumor tissue, the signals were exhibited diffusely or patches. On the same slide, positive signals in NC and HC appeared to be equal to or stronger than that DC and CIS in tumor cells. The positive result using β-actin as a probe demonstrated that mRNA in EC specimens was well conserved during sample preparation.

DISCUSSION

GST-π had been first proposed as a marker for preneoplastic lesion in rat liver based on the research of Sato, et al. in 1984,[5] which subsequently acted as a stimulus to the studies of the correlation between GST-π and human carcinogenesis. Instead of generally staging the progress of esophageal carcinogenesis, as almost all previously studies have done so far, we divided the esophageal carcinogenesis into a detailed process as described above. The elevation of GST-π protein expression occurs earlier than the pathological changes in the EC. Thus GST-π can serve as a marker of early esophageal carcinogenesis. The expression changes in all histological lesions from HC, DC (grade I, II, III), CIS to SCC indicate that GST-π plays a role in esophageal carcinogenesis and the low expression tendency suggests that GST-π may be one of the factors leading to EC. However, consistent with the report from Sasano, et al.,[6] GST-π may not necessarily be a marker of developed human EC. With regard to the conformity of GST-expression at protein and mRNA levels, it was indicated that the changes of GST-π protein expression might be the result of changes of GST-π at transcription level. GST-π expression regulation, therefore, might occur at or prior to RNA transcription level and more investigations are needed to clarify its detailed transcription regulation mechanism.

A phenomena which should not be neglected is that equivalent or even higher GST-π mRNA transcription appeared in NC and HC over DC, CIS and tumor tissues. A similar case was also observed by Peters, et al.[7] through quantitative analysis of GST-π in EC. What then might be the biological roles of GST-π in human EC and NC precancer lesions? One common feature of other investigators' and ours is that the "normal" mucosa studied was obtained from the same patients with EC. regarding GST-π protein expression analysis, though those mucosa appeared microscopically normal, it is highly possible that they had undergone some preneoplastic changes in molecular level which are undetectable by using routinely stained slides and it is consistent with the fact of their increased GST-π expression level, an early event of enzymatic changes in human esophageal carcinogenesis. It is known that carcinogens and mutagens are detoxified by GSTs in human bodies, but reversely, some of the carcinogens and mutagens, so far more than 100 kinds,[8] can also have some effects on GSTs expression regulation. It can be inferred that the significance of increased GST-π expression in NC precancer lesions will be further elucidated if the state of GST-π expression in normal esophageal mucosa is examined comparatively between people living in a high-risk region of EC and those living in other regions.

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