Expression of MDR1mRNA and Its Correlation with Mutant P53 in Esophageal Carcinoma

Dan Su[1], Shenhua Xu[1], Xinghao Ni[2], Gu Zhang[2], Chihong Zhu[1]

[1] Institute of ZheJiang Cancer Research, Hang zhou 310022, zhejiang, china
[2] Department of Pathology ZheJiang Cancer Hospital, Hangzhou, 310022, zhejiang, china

Abstract **Objectives** To understand the clinical significance of multidrug resistance (MDR1) expression and its correlation with mutant P53 in esophageal carcinoma. **Methods** The expression of MDR1 was detected by In Situ hybridization in 57 esophageal carcinomas and the expression of mutant P53 by immunohistochemistry in 44 of 57 esophageal carcinomas, and then evaluated their relationship with clinicopathological factors and their correlation. **Results** The positive expression rate of MDR1mRNA was 35% in 57 esophageal carcinomas. Among them, the positive expression rate were 29% in 28 cases without lymph node metastasis, and were 41% were in 29 cases with lymph node metastasis; Eight positive expression cases (27%) were found in 30 clinical Ⅱ stage cases, and 12 positive expression cases (44%) were found in 27 clinical Ⅲ-Ⅳ stage cases. No significant correlation was found among MDR1mRNA expression and tumor size, position, differentiation degree, infiltration degree. The positive expression rate of mutant P53 was 73% in 44 esophageal carcinomas. Five positive expression cases (45%) were found in 11 cases that tumor cells invaded to muscle, and 27 positive expression cases (41%) were found in 33 cases that tumor cells invaded to serous membrane. Significant difference between them was found ($P<0.01$). No significant correlation was found between mutant P53 expression and tumor size, position, differentiation degree, lymph node metastasis state, clinical stage. Ten MDR1mRNA positive expression cases were found in 32 mutant P53 positive expression cases, no significant correlation between them. **Conclusions** Overexpressions of MDR1mRNA and mutant P53 were significantly associated with esophageal carcinoma progression. The expression of MDR1mRNA might not be regulated by mutant P53.

Key Words Esophageal carcinoma; MDR1; In Situ hybridization; P53

Multidrug resistance (MDR) of malignant tumor cell has aroused widespread interest due to failure of chemotherapy. One of its molecular bases is mdr-1 gene amplification and overexpression of its product, P-gp [1]. Our previous research found overexpression of P-gp by flow cytometry in esophageal carcinoma, and this result showed esophageal tissue originally resist to drug[2]. In order to understand MDR1 gene expression on transcription level and its clinical significance, and to explore possible molecular bases that regulate MDR1 gene expression, we analyzed the expression of MDR1 by In Situ hybridization in 57 esophageal carcinomas and the expression of mutant P53 by immunohistochemistry in 44 of 57 esophageal carcinomas, and then evaluated their relationship with clinicopathological factors and their correlation.

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MATERIALS AND METHODS

Specimens

Fifty-seven specimens were collected from the Surgical Department of Zhe Jiang Cancer Hospital from December 1998 to September 2000. no treatment before operation. Of the 57 cases, 48 were male and 9 were female. Their ages ranged from 39 to 70 (averaging 55) years old. All the samples were squamous cell. The tumor size less than 3cm were 5 samples; 3.1~5.9cm were 32 samples, more than 6cm were 20 samples. According to tumor differentiation degree there were 13 samples were high differentiation, 39 samples were middle differentiation, 5 samples were low differentiation. According to tumor infiltration degree there were 17 samples had muscle infiltration, 40 samples had serous membrane infiltration. According to tumor occurrence position, 8 samples were in upper portion of esophagus, 39 samples were in middle portion of esophagus, 22 samples were in lower por-

tion of esophagus. 29 samples were with lymph node metastasis, 28 samples were without lymph node metastasis. According to clinical TNM stage there were 30 cases were in stage Ⅱ, 27 cases were in stage Ⅲ~Ⅳ.

Reagents

MDR1 In situ hybridization probe and kit were made by Tianjin Haoyang Biological Engineering Company. Mutant P53 mAb was made by Zymed company (supplied by Fuzhou Maixin Biological Engineering Company). Envision immunohistochemistry Reagent Kit was made by DAKO Company (supplied by Shanghai gene Company).

Methods

In situ hybridization of MDR-1mRNA was visualized using Tianjin Haoyang Biological Engineering Company kit following the instructions of the manufacture. Sections were deparaffinized in xylene, and rehydrated in a series of graded alcohol concentrations, than treated with 3% H_2O_2 for 10 mins. Placed in Citrate-buffered saline with 0.01mol/L, PH 6.0 to boil for 10 mins in microwave. After washing by distilled water, sections were added compound digestion liquid for 15 min at 37℃. After washing by PBS, Prehybridization was performed at 42℃ over night. Next morning, Hybridization was performed with 1μg of probe in 25ul of hybridization buffer at 37℃ for 2 hours and then overnight at 4℃. After washing twice by 2× saline sodium citrate (SSC) at PH7.4, the hybridization probe was detected using mouse alkaline phosphatase-coupled anti-Digoxingenin antibody and DAB system. Sections were counterstained with deep blue. Negative control using TBS as primary antibody, positive control using a known positive tissue.

The immunostaining of mutant P53 was visualized using the DAKO EnVision kit (DAKO) following the instructions of the manufacturer. Sections were deparaffinized in xylene, and rehydrated in a series of graded alcohol concentrations, and placed in Citrate-buffered saline with 0.01mol/L, PH 6.0 to boil for 10 mins in microwave, than treated with 3% H_2O_2 for 5 mins to block endogenous peroxidase. Immunohistochemistry was performed with a monoclonal primary antibody against mutant P53 at the concentration of 1:200 and incubated for 30mins at room temperature, than with a second antibody against EnVison incubated for 30 mins. All the slides were counterstained with hematoxylin. Negative control using TBS as primary antibody, positive control using a known positive tissue.

Assessment criteria

Positively expression of MDR1mRNA mainly locates in cell cytoplasm, and staining cells are blue; Positively expression of mutant P53 mainly locates in cell nuclear, and staining cells are brown. Assessment criteria: negative (−): no staining cells or staining cells <5% under the high microscope; positive (+): weak or uncompletely staining cells in 5% ~25% under the high microscope; strong positive (++ or +++): medium-high completely staining cells ⩾25% under the high microscope.

Statistical analysis

SPSS 11.0 x^2 test was used to evaluate the statistics significan of the data, $P<0.05$ represents significant difference.

RESULTS

The positive expression rate of MDR1mRNA was 35% in 57 esophageal carcinomas. Eight posi-

Table 1 Relationship among MDR-1mRNA, P53 expression and pathological factors and clinical stage in esophageal carcinoma

	MDR−1mRNA expression				P53 expression			
	case	+	−	Positive rate (%)	case	+	−	Positive rate (%)
Tumor size								
≤3cm	5	2	3	40	4	2	2	50
3.1~5.9cm	32	11	21	34	23	17	6	74
≥6cm	20	7	13	35	17	13	4	76
Tumor Position								
Upper	8	2	6	25	6	4	2	67
Middle	27	9	18	33	20	13	7	65
Lower	22	9	13	41	18	15	3	83
Differentiation degree								
high	13	6	4	46	8	6	2	75
middle	39	13	26	33	31	22	9	71
low	5	1	4	20	5	4	1	80
Infiltration degree								
Muscle invaded	16	7	9	43	11	5	6	45*
serous membrane invaded	41	13	28	32	33	27	6	82
lymph node metastasis state								
No metastasis	28	8	20	29	20	16	4	80
metastasis	29	12	17	41	24	16	8	67
Clinical stage								
Stage Ⅱ	30	8	22	27	24	18	6	75
Stage Ⅲ−Ⅳ	27	12	15	44	20	14	6	70

* $P<0.01$

Table 2 Relationship between MDR-1mRNA and P53 expression in esophageal carcinoma

P53 expression	MDR-1mRNA expression		Total cases
	+	−	
+	10	10	10
−	3	3	3
Total cases	13	13	13

DISCUSSION

Drug resistance is thought to be the main cause for the failure of tumor chemotherapy, which may occur pre-treatment (intrinsic drug resistance), during treatment or relapse of tumor (acquired drug resistance). Intrinsic drug resistance resulted from intrinsic speciality of tumor cells or pharmacological factors, such as uptake defect of cells, increased drug inactivation, but acquired drug resistance most likely related to the production of P-glycoprotein encoded by MDR1 gene, a trans-membrane drug efflux pump that results in increased drug efflux, and then reduced intracellular drug accumulation[3].

Xu Shenhua[2] detected overexpression of P-gp in esophageal carcinoma tissue before chemotherapy by flow cytometry. This results suggested esophageal tissue had original drug resistance speciality, and MDR1 gene involved in intrinsic drug resistance mechanism[2]. Zhi Jinzhou[4] used RT-PCR detected MDR1 mRNA expression in esophageal carcinoma and got the same conclusion. The high expression of intrinsic MDR1 is one of the possible reasons why no progress has been achieved in the curative of chemotherapy for esophageal carcinoma except for the high proportion of cells in G0 results in an increased non-sensitive cells to chemical drugs acting on cell cycle. So, in the integrated treatment of esophageal carcinoma, surgical and radiotherapy should still be stressed, as well as the selection of patients treated by chemotherapy, carrying out treatment with reverser of MDR and suppressor of P-gp to increase the proportion of chemotherapy in integrated treatment of esophageal carcinoma, ultimately improving curative effect.

Gottesman [5] presented the overexpression of MDR1 not only involved in drug resistance, but also played an important role in cancer cells malignant biological behavior. In this study, no signifi-

cant MDR1 expression difference was found among different clinical stages, but the expression of MDR1 in the cases with lymph node metastasis was higher than that in the cases without lymph node metastasis, and the expression of MDR1 in the clinical Ⅲ-Ⅳ stage cases was higher than that in the clinical Ⅱ stage cases. These results suggest MDR1 expression related to esophageal carcinoma progress. Our previous research [6] found c-erbB-2 and P-gp both positive expression in esophageal carcinoma might suggest unfavorable prognosis.

Mutant P53 as an oncogene promotes cells malignancy. Many researches indicated the over expression of p53 protein may have significance as a prognostic factor for patients with esophageal carcinoma[7]. In this study we found mutant P53 related to esophageal carcinoma infiltration.

Since the view that mutant P53 may up-regulated MDR1 gene expression was firstly clarified by Chin in 1992 [8], many researches have found that MDR1 gene transcription started from down stream promoter 3′ end was activated by mutant P53, and wild P53 negative expression affected MDR1encoded P-gp expression on the transcription level[9].

However, reverse opinions followed. No correlation between MDR1 and mutant P53 expression was found in ovarian cancer[10], hepatocellular carcinoma[11] and colorectal cancer[12]. Recent researches indicated abnormal signal transmission pathway was related to MDR1 gene expression. Activation of NF-kappa B up-regulated MDR1 expression[13]; Activation of c-Jun enhanced MDR1 expression[14]. Activation of phospholipase C Induces the expression of the Multidrug Resistance (MDR1) gene through the Raf-MAPK pathway[15]. In this study, no relationship between MDR1 and mutant P53 expression was found in esophageal carcinoma. It is likely that MDR1 expression is not regulated by mutant P53, but another factors that need to be further confirmed.

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