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Decreased monoamine oxidase (MAO) activity and MAO-A expression as diagnostic indicators of human esophageal cancers

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Abstract

Human esophageal cancer is a common occurring malignancy with high mortality rate partially due to lack of tools for early diagnosis. In this study, we have analysed tumour tissue from 50 cases of primary esophageal cancer. Our studies showed that the activity of monoamine oxidase (MAO) and the expression of MAO-A were strikingly decreased in the tumour tissues of 48 (96%) and 44 (88%) patients, respectively. These results suggest that the activity of MAO and the expression of MAO-A may be used as new diagnostic markers for esophageal cancers.

Keywords: *Cancer biomarkers; detection/diagnosis; esophageal cancer; gene expression; immunology/immunobiology*

Introduction

Monoamine oxidase (MAO) isoenzymes are located on the outer membrane of mitochondria and control the levels of neurotransmitters in the brain and dietary amines in peripheral tissues via oxidative deamination (Mitoma & Ito 1992, Fitzgerald et al. 2007b). There are two isoenzymes of MAO, MAO-A and MAO-B, which oxidize dopamine and tyramine with differential preferences on substrates under normal physiological conditions (Billett 2004). Reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and oxidation products are generated during the oxidation of biogenic amines by MAOs (Pizzinat et al. 1999). MAO-A and MAO-B have 527 and 520 amino acids, respectively, and share 70% sequence identity (Bach et al. 1988). Although MAO-A and MAO-B genes are located on the same X-chromosome and both are composed of 15 exons with identical exon–intron organizations (Grimsby et al. 1991), they are organized in opposite directions and use different promoters. Accordingly, their expression and functions are regulated in distinct mechanisms (Shih & Chen 2004).

Emerging evidences indicate that amine oxidases (AO), especially diamine oxidases (DAO) and polyamine oxidases (PAO), are key regulators of tumour progression (Pietrangeli & Mondovì 2004). MAO-A degrades biogenic amines and generates ROS, which participates in serotonin and tyramine signalling. Although the production of ROS was a byproduct of the catalytic activity of MAO-A, ROS is also recruited by the cell to enhance its apoptotic signalling (Fitzgerald et al. 2007b), suggesting a conceivable relationship between MAO-A and carcinogenesis. A recent report demonstrated that MAO-B activity may be a useful diagnostic tool for differentiating glial tumours as its activity showed significant and selective increase in human gliomas compared with meningiomas or

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non-tumour tissue (Gabilondo et al. 2008). In contrast, our studies showed that decreased MAO activity and MAO-A expression are linked to human esophageal cancers. As such, we have provided a direct clinical evidence for both MAO activity and MAO-A expression as new biomarkers for human esophageal cancers, which can be a helpful tool for diagnostic purpose. Our results also suggest differential roles of MAO-A and MAO-B in carcinogenesis.

Materials and methods

Patients

The esophageal cancer tissue and corresponding peritumour tissue were surgically removed and collected from 50 esophageal cancer patients from May to December of 2007 in the Anyang Hospital, Henan province, China. All patients provided informed consent for their participation in the study, and this study was approved by the Xiamen Ministry of Health Bureau. The clinical pathological materials of the patients are shown in Table 1. Specifically, all tissue samples were removed from patients and immediately frozen in liquid nitrogen until analysis. Cancer stages were categorized based on the TNM system of the American Joint Committee on Cancer (AJCC). All tumours were verified as primary esophageal squamous cell carcinoma with various degrees of invasion and differentiation, and with no distant metastasis. Sections of the peritumour tissues were examined histologically and all were found to be free of tumour cells.

Tissue homogenate preparation

One hundred milligrams of each tissue sample was washed in normal saline solution (NS solution, 0.9% of sodium chloride) and then homogenized in NS solution at 9:1 (v/w) on ice. The homogenate was centrifuged at 3000 rpm for 8 min at 4°C, and the final supernatant was gathered and frozen immediately in liquid nitrogen until use. The total concentration of protein in the supernatant was determined using the Bradford method.

MAO activity analysis

The MAO activity was determined using the MAO kit (Nanjing Jiancheng Bioengineering Institute, China). This kit is based on the mechanism that MAO can use benzylamine as a substrate and generate benzylaldehyde which can be extracted with cyclohexane (Tan & Ramsay 1993). The activity of MAO is calculated through the absorbance of the extracted production at 242 nm. The activity is demonstrated in units per milligram of protein,

Table 1. The clinicopathological characteristics of the patients and the statistic analysis.

Clinicopathological parameters	Number of patients	MAO activity		*p*-Value	MAO-A expression of downregulation (%)
		Tumour tissues	Peritumoral tissues		
Age (years)					
<50	6	3.58 ± 2.54	13.76 ± 1.90	<0.001	100% (6/6)
>50	44	3.58 ± 3.61	10.71 ± 3.50	<0.001	86% (38/44)
p-Value		0.255			
Sex					
Male	30	3.71 ± 3.16	10.98 ± 4.00	<0.001	87% (26/30)
Female	20	3.39 ± 3.99	11.21 ± 2.60	<0.001	90% (18/20)
p-Value		0.937			
T stage					
T1	4	5.08 ± 8.30	11.14 ± 4.33	0.176	100% (4/4)
T2	16	3.40 ± 3.18	11.22 ± 3.31	<0.001	88% (14/16)
T3	29	3.60 ± 2.78	11.18 ± 3.50	<0.001	86% (25/29)
p-Value		0.723			
Metastatic lymph node					
N0	21	3.93 ± 4.43	11.92 ± 2.96	<0.001	90% (19/21)
N1	28	3.44 ± 2.63	10.64 ± 3.69	<0.001	86% (24/28)
p-Value		0.544			
Grade of differentiation					
I	1	0.84	11.15		100% (1/1)
I-II	6	3.74 ± 3.34	11.81 ± 5.82	0.06	67% (4/6)
II	20	3.75 ± 3.10	12.15 ± 2.94	<0.001	90% (18/20)
II-III	16	3.39 ± 4.34	9.60 ± 2.53	<0.001	88% (14/16)
III	6	4.39 ± 3.05	11.59 ± 3.77	<0.001	100% (6/6)
p-Value		0.597			

in which one activity unit (U) is equivalent to the absorbance of 0.01 produced by 1 mg protein at 37°C in 1 h.

Western blot analysis

Western blot was carried out as described previously (Luo et al. 2003). Briefly, 5 μg of total protein extracted from each tissue sample was run in SDS-PAGE and then transferred to a PVDF membrane. After being blocked with 5% bovine serum albumin (BSA), the membrane was incubated with MAO-A (H-70) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (diluted at 1: 1000, v/v) for 1 h to detect the MAO-A expression level in tumour and peritumour tissues. β-Actin antibody (Santa Cruz Biotechnology) (diluted at 1: 2000, v/v) was used to probe the β-actin on the same membrane as the total protein loading control.

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The results of MAO activity were expressed as mean ± standard deviation values. The differences between two groups of samples were evaluated by the paired *t*-test. The Kruskal–Wallis test was used to evaluate groups of samples in each clinical pathological parameter. For both tests, differences with $p < 0.05$ were considered statistically significant.

Results

Decreased MAO activity in esophageal tumour tissues

The tissues were freshly frozen without fixation so that we could detect the physical activity of MAO. To compare the MAO activity between the paired tumour and peritumour tissues from esophageal cancer patients, we performed statistical analysis after determining MAO activity in the samples (Table 1 and Figure 1). Except for patients 2 and 3, MAO activity was decreased in 96% (48 of 50) of the tumour tissue samples compared with their corresponding peritumour tissues. Statistical

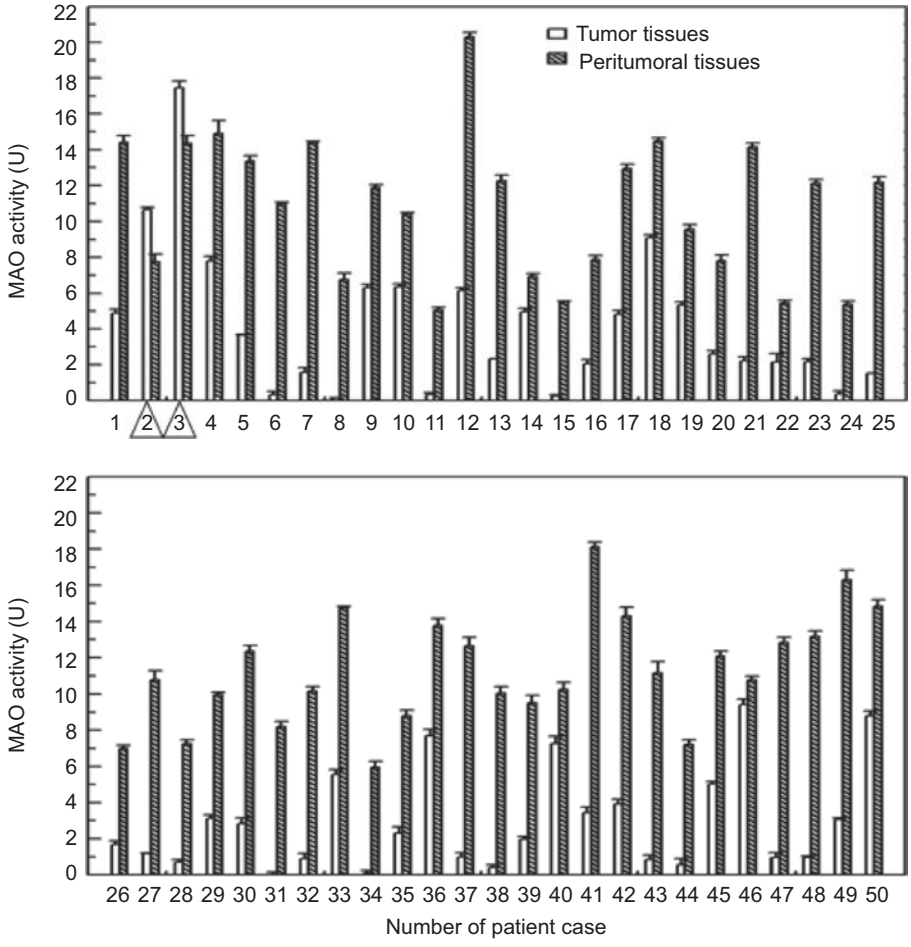

Figure 1. The statistic analysis of monoamine oxidase (MAO) activities in tumour and peritumour tissue. The samples were collected from 50 patients diagnosed with esophageal cancer. The two cases that were not consistent with the majority are indicated by triangles.

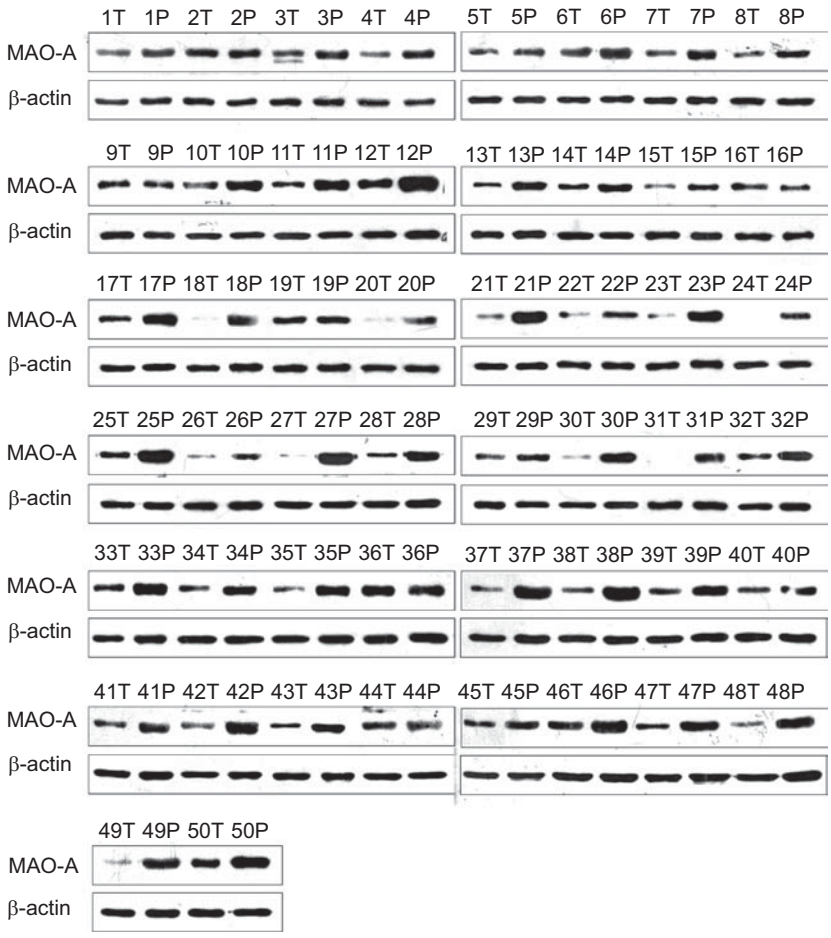

Figure 2. Western blot analysis of monoamine oxidase (MAO)-A expression in 50 pairs of tumour and peritumour tissue samples. Sequence numbers of the patient cases are indicated. T, tumour tissue; P, peritumour tissue. MAO-A, 61 kDa; β-actin (42 kDa) was used as a control for protein loading.

analyses were further performed according to the tumour features including age, sex, T stage (the size of primary squamous cell carcinoma), metastatic lymph node and grade of differentiation. The results indicated that the differences in MAO activity between tumour and peritumour tissues were significant regardless of the age, sex and metastatic lymph node diagnosis of the patients (p <0.001). As for the T stage of the patients, there was no significant difference between the paired tissues in the patients within the T1 stage (p >0.05). However, the differences were significant in patients with tumours in both the T2 and T3 stages (p <0.001). Moreover, no significant differences were observed in tumours within the I–II grade in differentiation (p >0.05), but the differences were significant in all the other grades including II, II–III and III (p <0.001). In addition, the difference of the MAO activities in tumour tissues was not significant within any analysed parameter based on the Kruskal–Wallis test (p >0.05) (Table 1).

Reduced MAO-A expression in esophageal tumour tissues

Although both are expressed in peripheral tissue, MAO-A and MAO-B also show some distinct expression patterns. For example, MAO-A is localized mainly in gastroenteric tissue, liver, kidney, lung and placenta, while only MAO-B can be found in platelets of peripheral tissues (Shih 1991). We analysed the MAO-A expression levels of paired esophageal tumour and peritumour tissue samples by Western blot (Figure 2). Our quantitative data showed reduced MAO-A expression in tumour tissue compared with the corresponding peritumour tissue in a majority of patients (44 of 50, 88%), while no changes in MAO-A expression occurred in the remaining six cases (Table 1, Figures 2 and 3). Taken together, the correlation of decreased MAO activity and MAO-A expression with esophageal cancer suggests that they may provide diagnostic indicators for esophageal cancer.

Discussion

Human esophageal cancer is a malignant tumour with poor prognosis, often detected in esophageal squamous epithelium. It occurs at a high frequency resulting in considerable mortality in China, especially Henan province (Zou et al. 2002). As such, it is imperative to find discriminating biomarker(s) for predicting or diagnosing esophageal cancer. Recently, Rybaczyk et al. found a significant decrease of MAO-A expression in multiple cancer tissues after analysing 10 different organs derived from humans, rodents and fish extracted from the GEO profiles database (Gene Expression Omnibus) (Rybaczyk et al. 2008). The human cancers which they analysed included cutaneous malignant melanoma, small cell lung carcinoma, malignant pleural mesothelioma and breast cancer. They found that MAO-A expression were decreased in 95.4% of human cancer patients and 94.2% of animal cancer cases compared with the non-cancerous controls. In this study, we further provided direct clinical data for MAO as a biomarker for human esophageal cancers. We analysed 50 cases of esophageal cancer and found that the activity of MAO and the expression of MAO-A were clearly decreased in 96% and 88% esophageal cancer patients, respectively.

Interestingly, MAO has been shown to activate and regulate cell death processing in mitochondria. MAO-A has been found to be able to bind to an endogenous dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol, an MAO-A inhibitor, reduce mitochondria membrane potential and induce apoptosis in human neuroblastoma SH-SY5Y cells (Yi et al. 2005, Naoi et al. 2006). Another line of evidence suggested that MAO-A expression and catalytic activities were increased, accompanied by activation of the apoptotic executioner caspase-3 in serum-deprived SH-SY5Y cells, whereas specific inhibition of MAO-A activity resulted in loss of apoptotic cell morphology (Fitzgerald et al. 2007a), and the serum starvation-induced apoptosis was also found reduced in cortical brain cells from MAO-A-deficient mice compared with wild-type (Ou et al. 2006). The relationship between MAO-A and apoptosis can further be supported by the finding that MAO-A inhibitors, pargyline and clorgyline, were able to prevent mitochondrial integrity and its homeostasis, which led to prevention of the induction of apoptosis by

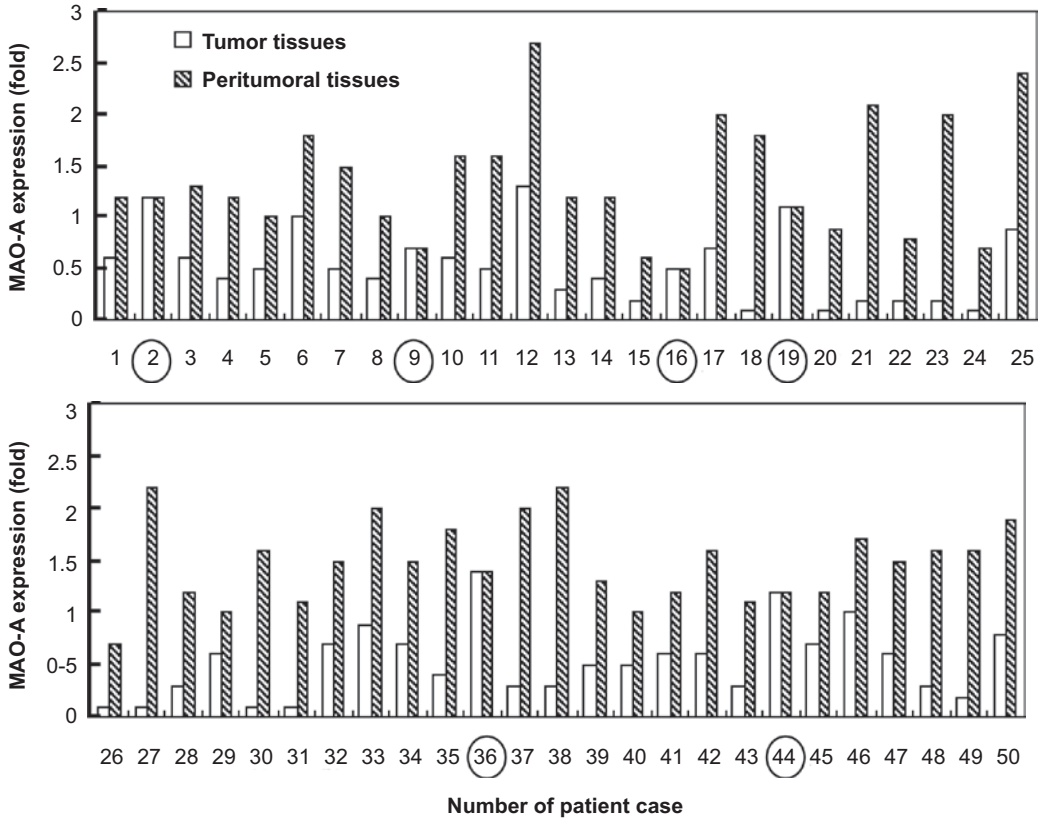

Figure 3. Monoamine oxidase (MAO)-A expression level quantified from the Western blots shown in Figure 2. The relative expression level of MAO was normalized by comparing with the concentration of corresponding β-actin. The six samples that were not consistent with the majority are indicated by circles.

serum starvation in human melanoma (M14) cells; this might be related to a decreased production of aldehydes and hydrogen peroxide (Malorni et al. 1998). Therefore, in our results, that show decreased MAO activity and MAO-A expression in esophageal tumour tissues may suggest a loss of proper apoptotic control in those cancer cells.

Our study also showed an interesting phenomenon in that MAO activity and MAO-A expression did not correlate in some cases. For example, unlike most of the cases analysed, the MAO activity in the tumour tissue of patient 2 was appreciably higher than in normal tissue, while the MAO-A expression level of this patient was similar in both tumour and normal tissues. In patient 3, the activity of MAO was also appreciably higher in tumour tissue, while the MAO-A expression level was much lower in tumour tissue. In cases 9, 16, 19, 36 and 44, the MAO activities were clearly higher in tumour tissues, while the MAO-A expressions were all similar. Generally, the high MAO expression should lead to high activity, which was confirmed by most of our results. However, the results in these cases suggested that there must be some other modifications on the MAO activity after expression. Although the relationships between tumorigenesis and the expression and activity of MAO still need to be further elucidated, our results in this study indicate important diagnostic roles of MAO activity and MAO-A expression as biomarkers in human esophageal cancers.

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