EXPRESSION OF TUMOUR ANTIGEN MAELSTROM IN OESOPHAGEAL CARCINOMA AND ITS CORRELATION WITH CLINICAL PROGNOSIS

Yu Zeng[1,*], Xuelian Liao[2], Pengcheng Li[2], Yanlin Tan[2]
[1]Department of Oncology, Jintang First People's Hospital, Chengdu, PR China - [2]Jintang First People's Hospital, Chengdu, PR China

ABSTRACT

Objective: *To study the expression of Maelstrom (MAEL) in oesophageal carcinoma and its correlation with clinical prognosis.*

Methods: *68 specimens of oesophageal cancer tissues were collected in our hospital from February 2015 to August 2016, with normal oesophageal mucosa tissues collected as controls. Using the results of immunohistochemistry, the expression rate of the MAEL protein in oesophageal cancer tissues and adjacent tissues was measured and correlations between MAEL protein expression and gender, age, lymph node metastasis, tissue differentiation degree, and TNM stage were analysed. The relationship between MAEL protein expression and patient survival time was observed and recorded.*

Results: *Immunohistochemistry showed that the positive expression rate of MAEL in oesophageal carcinoma was 78.38%, significantly higher than that in adjacent tissues (22.22%) (P>0.05). MAEL positive expression was associated with the degree of differentiation of oesophageal cancer tissues and the TNM stage. The positive expression rate of MAEL in well-differentiated carcinomas was 71.43%, which was significantly lower than that in moderate/low-differentiated carcinomas (80.85%) (P<0.05). The difference between TNM stages was statistically significant (P<0.05). MAEL positive expression was not associated with gender, age, or lymph node metastasis (P>0.05). Survival analysis was performed using the Kaplan-Meier estimator. The results showed that the 3-year survival rate of patients with MAEL-positive expression in oesophageal cancer tissues was 30% (15/53), which was significantly lower than that of patients with negative expression (65%, 9/15) (P<0.05). Through COX multivariate analysis, tissue differentiation, TNM stage, and MAEL expression were all identified as independent risk factors that affected the prognosis of oesophageal cancer patients.*

Conclusion: *MAEL was highly expressed in oesophageal cancer tissues and was associated with tissue differentiation, TNM stage, and survival, and hence could be used as an indicator to evaluate the prognosis of patients with oesophageal cancer.*

Keywords: *MAEL, oesophageal carcinoma, pathological features, prognosis, risk factors.*

DOI: 10.19193/0393-6384_2020_3_202

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Oesophageal cancer is the most common malignant tumour of the digestive tract in China, accounting for more than 90% of oesophageal tumours, with the main pathological type being oesophageal squamous cell carcinoma. It is second only to gastric cancer as a cause of malignant tumour death and has higher incidence and mortality. In recent years, the incidence rate has increased significantly[(1)]. About 20w people worldwide die of oesophageal cancer every year, making it a serious threat to people's life and health[(2)]. Nitrosamines, fungi, trace element and vitamin deficiencies, smoking and alcohol, and genetic factors can lead to the occurrence of oesophageal cancer[(3)]. Oesophageal cancer progresses slowly; early symptoms are not obvious and there are different degrees of dysphagia when swallowing food. The typical symptom is progressive dysphagia. When the patient is gradually losing weight and suffering from dehydration, weakness, and persistent chest pain or back pain, the cancer has reached an advanced stage and the tumour has invaded the oesophageal tissue. Jaundice, ascites, coma, etc. indicate that the liver, brain, or other organs have been affected[(4)]. At present, surgical resection, radiotherapy, and chemotherapy are often used in the treatment of oesophageal cancer, but their effects are not satis-

factory for patients in the middle and advanced stages and the recurrence rate is high. Clinical studies have shown that about 35% of patients have missed the best chance of surgery due to a lack of early diagnosis[5]. Exploring the mechanisms of oesophageal cancer development is the key to early diagnosis and treatment, leading to prolonged survival.

Maelstrom (MAEL) is a general term for a class of tumour antigens, which are usually expressed only in testicular sperm cells. In other normal cells they exhibit little or no expression, whereas they are highly expressed in various tumour tissues[6]. Clinical studies have shown that tumour immunotherapy with MAEL as a target antigen has achieved satisfactory results, and hence MAEL can be used as a molecular marker for tumour diagnosis and prognosis evaluation[7].

Elucidating the expression of MAEL in oesophageal cancer and its role in the development of oesophageal cancer has great significance for the development of new methods to treat oesophageal cancer. Therefore, this study further explores the correlation between expression and clinical prognosis by detecting the expression of MAEL in oesophageal cancer tissues.

Data and methods

General data

68 specimens of oesophageal cancer tissue were collected from February 2015 to March 2016 in our hospital, and 20 cases of normal oesophageal mucosa (distance > 5cm from the lesion and no tumour cell infiltration) were selected as a control group. Specimens were collected from 42 men and 36 women with an average age of 54.35 ± 7.52 years.

Inclusion criteria:

• Oesophageal squamous cell carcinoma was diagnosed by relevant pathological examination;

• None of the patients involved in the selected specimens had undergone radiotherapy and chemotherapy;

• Complete clinical data was available for the selected patients;

• Oesophageal cancer tissue specimens were obtained from surgical resection specimens in our hospital;

• None of the selected patients had other malignant tumours;

• Patients were informed of the study and signed informed consent. This study was approved by the hospital ethics association.

Methods

Immunohistochemistry was used to test MAEL expression in oesophageal cancer tissues and adjacent tissues. The tissue was fixed and embedded in 4 μm serial sections, and the operation was carried out according to the instructions of the real kit. The sections were dewaxed and dehydrated in a 60oC oven for 60 min, and washed three times with PBS buffer (pH 7.4) for 3 times, for three minutes each time. The treated sections were placed in an appropriate amount of citrate buffer that was heated to boiling point. After mid-range microwave treatment for 10 minutes, the samples were taken out, naturally cooled, and washed with distilled water twice. One drop of 3% H_2O_2 was added to each section and they were incubated for 10 min at room temperature to inactivate the endogenous enzyme. The PBS solution was removed, and the sections were incubated with primary antibody (1:100 dilution) at 4oC overnight. One drop of polymer reinforcement was added to each section and they were incubated with the polymerized HRP-labelled secondary antibody at 37oC for 30 min. The sections each had one drop of DAB solution added for DAB colouring; after 5 min they were counterstained with haematoxylin, differentiated with 0.1% HCI, and washed and blued. The sections were observed after dehydrating, drying, and sealing.

Interpretation of immunohistochemistry results

Five fields under the microscope were randomly selected to observe the colour reaction of the slice. The criteria for identifying MAEL were that it was mainly expressed in the nucleus and it was positive in fine brown-yellow particles.

The staining depth was scored as follows:

• 0: completely negative;

• 1 point: weakly positive;

•2 points: moderately positive;

• 3 points: strong positive.

The proportion of stained cells was scored as:

•1 point: <25%;

• 2 points: 26%-50%;

• 3 points: 51-75%;

• 4 points: 76%-100%.

The total score was given by the product of the depth of staining and the proportion of the stained area, with 0-6 points indicating negative expression and 8-12 points corresponding to positive expression.

Observation index

• Using the results of immunohistochemistry, the negative and positive expression rates of MAEL

protein in oesophageal cancer tissues and adjacent tissues were analysed.

• Using the results of immunohistochemistry, the correlations between MAEL protein expression and gender, age, lymph node metastasis, tissue differentiation degree, and TNM stage were analysed.

• The relationship between MAEL protein expression and patient survival time was observed and recorded. Follow-up was mainly in the hospital, although follow-up was also conducted by telephone for some patients. The patients that were lost were considered to have died from the date on which a loss of follow-up occurred.

Statistical methods

The data were processed by SPSS 23.0 and the count data were analysed by the χ^2 test. Survival analysis was performed by the Kaplan-Meier method while the survival rate was analysed by the log-rank test. P<0.05 was considered to be statistically significant.

Results

Expression of MAEL protein in oesophageal cancer tissues and adjacent tissues

The results of immunohistochemistry showed that the positive expression rate of MAEL in oesophageal carcinoma was 78.38%, which was significantly higher than that in adjacent tissues (22.22%) (P>0.05), as illustrated in Figure 1.

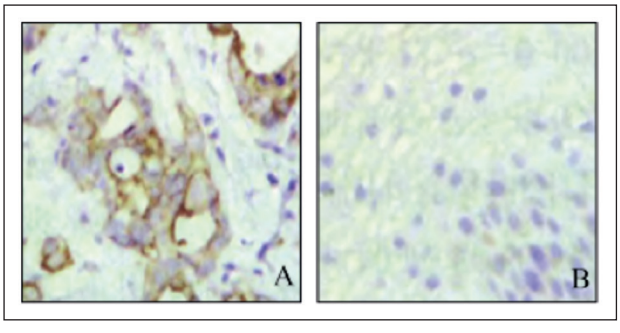

Figure 1: Expression of MAEL protein in oesophageal cancer tissues and adjacent tissues.
A: Positive expression in oesophageal cancer tissue; B: Negative expression in adjacent tissues.

Relationship between positive expression rate of MAEL and clinicopathological features

MAEL positive expression was associated with the degree of differentiation of oesophageal cancer tissues and TNM stage. The positive expression rate of MAEL in well-differentiated carcinoma was 71.43%, which was significantly lower than that in moderate/low-differentiated carcinoma (80.85%) (P<0.05). The difference between TNM stages was statistically significant (P<0.05). MAEL positive expression was not associated with gender, age, or lymph node metastasis (P>0.05). The results are shown in Table 1.

Clinicopathological parameter	n	MAEL		χ^2	*P*
		Positive	Negative		
Gender				0.025	0.873
Male	42	33(62.26)	9(60.00)		
Female	26	20(37.74)	6(40.00)		
Age				0.011	0.916
<60 years old	28	22(41.51)	6(40.00)		
≥60 years old	40	31(58.49)	9(60.00)		
Lymph node metastasis				0.055	0.814
Yes	29	23(43.40)	6(40.00)		
No	39	30(56.60)	9(60.00)		
Degree of differentiation				19.360	<0.001
Well differentiated	21	15(28.30)	6(40.00)		
Moderate/low-differentiated	47	38(71.70)	9(60.00)		
TNM stage				14.52	<0.001
I	21	14(26.42)	7(46.67)		
II	26	20(37.74)	6(40.00)		
III	15	13(24.53)	2(13.33)		
IV	6	6(11.32)	0(0.00)		

Table 1: Relationship between positive expression rate of MAEL and clinicopathological features.

Relationship between MAEL expression and survival time

Survival analysis was performed using the Kaplan-Meier estimator, with the results shown in Figure 2. The 3-year survival rate of patients with MAEL-positive expression in oesophageal cancer tissues was 30% (15/53), which was significantly lower than that of patients with negative expression (65%, 9/15) (P<0.05).

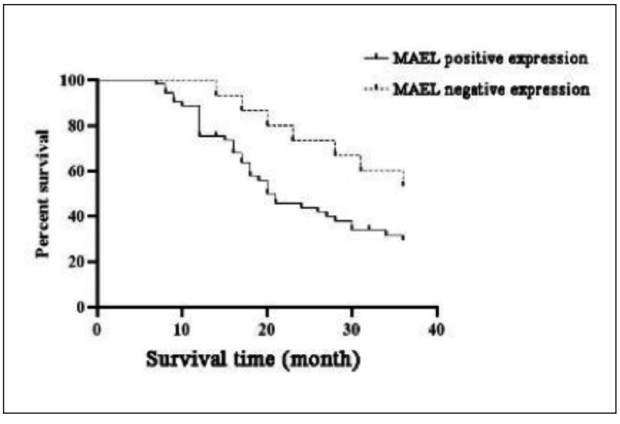

Figure 2: Relationship between MAEL expression and survival time.

Multivariate analysis of COX proportional hazard model in patients with oesophageal cancer

Through COX multivariate analysis, tissue differentiation, TNM stage, and MAEL expression were all shown to be independent risk factors that affected the prognosis of oesophageal cancer patients. Table 2 summarises the results.

Relative factors	*P*	Relative hazard value	95%CI	
			Lower limit	Upper limit
Age	0.609	0.899	0.608	1.319
Gender	0.339	0.931	0.986	1.117
Histological type	0.543	0.909	0.789	1.311
Degree of differentiation	0.038	0.899	0.719	1.003
TNM stage	<0.001	8.525	4.349	16.724
Lymph node metastasis	0.272	0.893	0.657	1.277
MAEL expression	0.035	8.001	4.125	12.975

Table 2: Multivariate analysis of COX proportional hazard model in patients with oesophageal cancer.

Discussion

In recent years, the incidence of distal gastric cancer in many countries and regions has decreased significantly, but the incidence of oesophageal cancer has increased year by year. At present, some biological indicators are valuable in diagnosing the malignant degree of oesophageal cancer in patients, selecting treatment methods, and evaluating prognosis. Detection of these biological indicators can guide clinicians to develop reasonable individualized treatment options and target treatment for patients.

Recent studies have shown that MAEL, which was originally cloned from the Drosophila mutant maelstrom, can be used as a novel cancer antigen of testosterone[8]. Clinical studies suggest that, as a tumour-promoting gene, MAEL exists in the 1q24 position of cancers such as colorectal cancer and liver cancer, playing an important role in promoting tumour cell invasion and migration, and is closely related to the prognosis[9]. Analysis of the human EST database using the electronic differential display method in 2005 showed that this gene can be highly expressed in the human testis but have little or no expression in other normal tissues[10]. Northen hybridisation experiments found that this gene is only specifically expressed in human testes, and not expressed in tissues such as the ovary and liver[11]. In this study, the human MAEL gene was cloned from human testis tissue. The expression of MAEL in oesophageal cancer tissues and adjacent tissues was tested by immunohistochemistry. It was found that MAEL is mainly expressed in oesophageal cancer tissues, while in normal adjacent tissues it has negative expression. The immunohistochemical results showed that the positive expression rate of MAEL in oesophageal cancer tissues was 78.38%, which was significantly higher than that in adjacent tissues (22.22%) (P>0.05), indicating that MAEL is highly expressed in oesophageal cancer tissues. At the same time, this study found that MAEL positive expression was associated with the degree of differentiation of oesophageal cancer tissues and the TNM stage. The positive expression rate of MAEL in well-differentiated carcinoma was 71.43%, which was significantly lower than that in moderate/low-differentiated carcinoma (80.85%) (P<0.05). The difference between TNM stages was statistically significant (P<0.05). The higher the degree of tissue differentiation, the higher the positive expression rate of MAEL protein in the late TNM stages. Clinical studies have shown that when the positive expression rate of MAEL protein is ≥35%, the clinical prognosis of patients is worse[12]. High expression of MAEL protein can affect the histological pathological grade, which in turn affects tumour cell differentiation[13]. MAEL regulates cell differentiation, myometrial invasion, and lymph node infiltration, mainly by regulating epithelial mesenchymal transition and expression of the intercellular adhesion protein E-cadherin, but its mechanisms have not been fully elucidated and need further investigation[14]. Studying the relationship between MAEL protein and tissue differentiation and TNM stage can provide a reference for evaluating clinical efficacy and predicting survival time after surgery[15].

Finally, this study analysed the relationship between MAEL expression and survival time of patients with oesophageal cancer. The results of survival analysis performed by Kaplan-Meier estimation showed that the 3-year survival rate of patients with MAEL positive expression in oesophageal cancer tissues was 30% (15/53), which was significantly lower than those with negative expression (65%, 9/15) (P<0.05). Through COX multivariate analysis, tissue differentiation, TNM stage, and MAEL expression were all identified as independent risk factors for the prognosis of oesophageal cancer patients. Prolonged survival of patients may be related to local tumour cell recurrence and tumour cell invasion controlled by MAEL protein, further confirming that MAEL protein expression is closely related to patient prognosis and affirming the value of MAEL protein in prognosis evaluation.

In summary, MAEL is highly expressed in oesophageal cancer tissues and is associated with tissue differentiation, TNM stage, and survival. It is of great significance for prognosis analysis and postoperative adjuvant therapy in patients with oesophageal cancer, serving as an index for prognosis evaluation.

References

1) Hong S, Dan H, Sinan W, Peng F, Xu G, et al. Baseline Metabolic Risk Score and Postsurgical Esophageal Cancer-Specific Mortality: The Fujian Prospective Investigation of Cancer (FIESTA) Study. J Cancer 2018; 9: 1173-1181.
2) Liao S, Xing S, Ma Y. LncRNA SNHG16 sponges miR-98-5p to regulate cellular processes in osteosarcoma. Cancer Chemother Pharmacol 2019; 83: 1065-1074.
3) Huang X, Zhou X, Hu Q, Sun B, Deng M, et al. Advances in esophageal cancer: A new perspective on pathogenesis associated with long non-coding RNAs. Cancer Lett 2018; 413: 94-101.
4) He H, Wu Q, Wang Z, Zhang Y, Chen N, et al. Short-term outcomes of robot-assisted minimally invasive esophagectomy for esophageal cancer: a propensity score matched analysis. J Cardiothorac Surg 2018; 13: 52-58.
5) Nakatani M, Migita K, Matsumoto S, Wakatsuki K, Ito M, et al. Prognostic significance of the prognostic nutritional index in esophageal cancer patients undergoing neoadjuvant chemotherapy. Dis Esophagus 2017; 30: 1-7.
6) Li Y, Xiao F, Li W, Hu P, Xu R, et al. Overexpression of Opa interacting protein 5 increases the progression of liver cancer via BMPR2/JUN/CHEK1/RAC1 dysregulation. Oncol Rep 2019; 41: 2075-2088.
7) Wakeling LA, Ions LJ, Escolme SM, Cockell SJ, Su T, et al. SIRT1 affects DNA methylation of polycomb group protein target genes, a hotspot of the epigenetic shift observed in ageing. Hum Genomics 2015; 9: 14.
8) Karia B, Zamuner FT, Carlin V, de Oliveira CZ, Carvalho AL, et al. Expression and prognostic relevance of GAGE1 and XAGE1 cancer/testis antigens in Head and Neck Squamous Cell Carcinoma. Curr Mol Med 2018; 51: 878-893.
9) Barda S, Yogev L, Paz G, Yavetz H, Lehavi O, et al. BRDT gene sequence in human testicular pathologies and the implication of its single nucleotide polymorphism (rs3088232) on fertility. Andrology 2014; 2: 641-647.
10) Karan G, Javad P, Maxwell CP. Current Recommendations for the Diagnosis of Acute and Chronic PJI for Hip and Knee-Cell Counts, Alpha-Defensin, Leukocyte Esterase, Next-generation Sequencing. Curr Rev Musculoskelet Med 2018; 15: 98-103.
11) Ding H, Lv F, Wu H, Yan X. Selection and characterization of an improved strain produced by inter-species hybridization between, Pyropia, sp. from India and, Pyropia haitanensis, from China. Aquac 2018; 491: 177-183.
12) Hirayama M, Tomita Y, Yuno A, Tsukamoto H, Senju S, et al. An oncofetal antigen, IMP-3-derived long peptides induce immune responses of both helper T cells and CTLs. Oncoimmunology 2016; 5: 1123368.
13) Lacher MD, Gerhard B, Brian F, Graeve S, Fledderman EL, et al. SV-BR-1-GM, a Clinically Effective GM-CSF-Secreting Breast Cancer Cell Line, Expresses an Immune Signature and Directly Activates CD4+ T Lymphocytes. Front Immunol 2018; 9: 776-782.
14) Wang A, Zhang Y, Cao P. Inhibition of BAP31 expression inhibits cervical cancer progression by suppressing metastasis and inducing intrinsic and extrinsic apoptosis. Biochem Biophys Res Commun 2019; 508: 499-506.
15) Randén-Brady R, Carpén T, Jouhi L, Syrjänen S, Haglund C, et al. In situ hybridization for high-risk HPV E6/E7 mRNA is a superior method for detecting transcriptionally active HPV in oropharyngeal cancer. Hum Pathol 2019; 90: 97-105.

Corresponding Author:
Yu Zeng
Email: m8609h@163.com
(China)