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ORIGINAL RESEARCH

MAEL Cancer-Testis Antigen as a Diagnostic Marker in Primary Stages of Gastric Cancer with *Helicobacter pylori* Infection

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Abstract

Purpose Gastric cancer (GC) is the third leading cause of cancer related deaths in the world. Cancer testis antigens (CTAs) are involved in tumor progression of various cancers. These markers have not any expression or minimally expression in normal tissues, highlighting them as efficient methods for molecular targeted therapy. In the present study, we assessed the role of MAEL as a CTA in biology of GC and risk of Helicobacter Pylori (H pylori) infection.

Methods Levels of MAEL mRNA expression in 80 GC tumor tissues were compared to their corresponding normal margins using the real-time polymerase chain reaction.

Results There was a significant correlation between MAEL expression and tumor stage ($p = 0.050$). There were also significant correlations between MAEL expression and tumor grade ($p = 0.015$) and depth of invasion ($p = 0.030$) among the H pylori negative cases.

Conclusions MAEL is probably associated with aggressiveness of primary-stage tumors and can be introduced as an efficient marker for the early detection and also H pylori infected tumors in GC patients.

Keywords Gastric cancer · *H. pylori* · MAEL

Introduction

Gastric cancer (GC) is the third leading cause of cancer-related deaths in the world [1]. It is the second and fourth cause of cancer deaths among Iranian men and women, respectively [2]. Regarding the involvement of various environmental and genetic factors in progression of GC, identification of new sensitive markers is required to introduce new methods of targeted therapies. Despite recent advances in GC treatment, there is still a less than 30% of the 5-year survival among GC patients [3]. Lack of efficient therapeutic modalities is one of the main obstacles in GC [4]. To facilitate tumor detection, it is important to introduce novel genes that are essential in proliferation and metastasis of tumor cells. The maelstrom gene (MAEL) was identified in Drosophila, and human MAEL protein contains a DNA-binding domain in its N-terminal [5]. It exerts its role during spermatogenesis via repressing transposable elements [6]. Although MAEL is only normally expressed in testis, its abnormal expression has been reported in cancer patients. MAEL has different roles as oncogene and tumor suppressor in different cancers [6–8]. PIWI protein family is responsible for the genetic stability during spermatogenesis which silence retrotransposons by transcripts degradation or epigenetic regulation [9–11]. MAEL overexpression has been reported in various cell lines such as breast, colon and prostate [12], and several tumor tissues including ovarian [6] and hepatocellular carcinoma [8]. It was shown that the MAEL suppression increases production of reactive oxygen species (ROS) leads to the apoptosis [13]. Regulation of MAEL expression is done by DNA methylation of a CpG island in promoter sequence [12]. Moreover, there is a direct interaction between MAEL and stress granule proteins in tumor cells [14]. MAEL is an activated cancer-testis gene by demethylation in breast cancer [12]. It is contributed to EMT and tumor progression in liver, colon, and bladder cancers [8, 13]. MAEL promotes the EMT

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process in bladder carcinoma cell via down regulation of MTSS1 gene [15]. On the other hand, MAEL functions as a tumor suppressor in ovarian cancer [6]. In the present study, we have assessed the levels of MAEL mRNA expression in gastric cancer patients to clarify the probable role of this CTA in tumor progression and *Helicobacter pylori* (*H. pylori*) infection.

Materials and Methods

Tissue Samples

Fresh tumor and normal margins were obtained from 80 GC patients who were undergone the gastric surgery before receiving any chemo- or radiotherapeutic treatments. All fresh tissues were microscopically examined (hematoxylin and eosin staining) to approve the presence of at least 80% of tumor cells in tumor tissues and lack of tumor cells in normal margins. Fresh tissues were transferred to the RNA later solution (Qiagen, Hilden, Germany) and stored at − 20 °C prior the mRNA extraction. All patients filled and confirmed the informed consent forms which were approved by the ethic committee.

RNA Extraction and Comparative Real-Time RT-PCR

First-strand cDNA was synthesized (Takara, Japan) following the RNA extraction (Takara, Japan) from the normal and tumor tissues. Levels of MAEL mRNA expression were assessed using SYBR® Premix Ex Taq™ II kit (TaKaRa) method in duplicate comparative real-time PCR reactions (LightCycler 96 system, Roche, Germany). All the primer sequences are presented in Table 1. Data were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The thermal cycling conditions included an initial denaturing step at 95 °C for 2 min, 45 cycles at 95 °C each for 30 s, 62 °C for 30 s, and 72 °C for 30 s. Gene expression was analyzed using 2–ΔΔCT algorithm. Over- and underexpression were elucidated for the tumors with more and less than + 2- and − 2-fold changes, respectively. The ranges between + 2- and − 2-folds were considered as normal expression.

Statistical Analysis

All statistical analyses were performed using SPSS 19.0 statistical package (SPSS, Chicago, IL). The associations between gene expressions and clinicopathological features were analyzed by v2/Fisher exact tests. Independent sample *t* test and ANOVA were applied to compare levels of gene expression, between different qualitative data. $P \leq 0.05$ was considered to indicate a significant difference.

Results

Population and MAEL mRNA Expression

Eighty GC patients with age range of 29–86 years old (mean ± SD 63.08 ± 11.23 years old) and tumor sizes 1.5–14 cm (mean size ± SD 6.33 ± 3.10 cm) were enrolled in present study. Majority of tumors were located in non-cardia (47/80, 58.8%), had penetration into the serosa (43/80, 53.8%) and, stage of III (46/80, 57.5%). Sixty-seven out of 80 samples had metastatic lymph nodes (83.8%). Fifty-nine (73.8%) and 21 (26.2%) out of 80 patients were male and female, respectively. Moderately differentiation was also observed in 50 (62.5%) tumors (Table 2). Although there was almost a similar mean age in males and females (63.07 ± 1.47 vs. 63.10 ± 2.48 years old), size of tumors in females were interestingly higher than that in males (7.29 ± 0.75 vs. 5.99 ± 0.38 cm). *H. pylori* were also assessed in 68 patients [16], from whom 36 (52.9%) cases were *H. pylori* positive. A comparative real-time PCR was used to assess the levels of MAEL mRNA expression in tumor compared with normal tissues. MAEL over- and underexpressions were observed in 17 (21.2%) and 28 (35%) out of 80 patients, respectively. There was a huge range of MAEL fold changes among the cases from − 8.90 to 22.38 (mean fold ± SD − 0.18 ± 4.41). MAEL mean fold changes were (− 3.86 ± 0.27, mean fold ± SD) and (6.13 ± 1.20, mean fold ± SD) among the under- and over-expressed tumors, respectively. All fold changes are illustrated in a scatter plot (Fig. 1).

Association of MAEL Expression with Clinicopathological Features of Patients

To assess the probable role of MAEL in GC progression, we performed a correlation study between the levels of MALE mRNA expression and clinicopathological features of patients. Although there was no any significant correlation between age and levels of MAEL mRNA expression, mean age of over-expressed tumors were interestingly lower than that in the under-expressed cases (59.35 ± 2.94 vs. 62.86 ± 2.09 years old). There was no significant correlation between tumor size and MAEL expression; however, there was observed a noticeable increase in tumor sizes of MAEL overexpressed cases in

Table 1 Primer sequences used for quantitative real-time RT-PCR

	Forward primer sequence	Reverse primer sequence
MAEL	5-CCCTCAGAGAAGCAGAAACC -3	5-CTTGGAGAGAATACTTAACACAGC -3
GAPDH	5-GGAAGGTGAAGGTCGGAGTCA-3	5-GTCATTGATGGCAACAATATCCACT-3

Table 2 Correlation between level of MAEL mRNA expression and clinicopathological features of GC patients

	Total	MAEL overexpression	MAEL underexpression	MAEL normal expression	*p value*
Patients	80	17 (21.2%)	28 (35%)	35 (43.8%)	
Mean age (years, mean ± SD)	63.08 ± 11.23	59.35 ± 2.94	62.86 ± 2.02	65.06 ± 1.87	0.124
Size (cm, mean ± SD)	6.33 ± 3.10	6.47 ± 0.77	5.89 ± 0.59	6.61 ± 0.52	0.648
Sex					0.379
Male	59 (73.8%)	13 (76.5%)	19 (67.9%)	27 (77.1%)	
Female	21 (26.2%)	4 (23.5%)	9 (32.1%)	8 (22.9%)	
Location					0.422
Cardia	33 (41.2%)	8 (47.1%)	12 (42.9%)	13 (37.1%)	
Non-cardia	47 (58.8%)	9 (52.9%)	16 (57.1%)	22 (62.9%)	
Grde					0.625
Well differentiated	10 (12.5%)	3 (17.6%)	3 (10.7%)	4 (11.4%)	
Moderately differentiated	50 (62.5%)	11 (64.7%)	17 (60.7%)	22 (62.9%)	
Poorly differentiated	20 (25%)	3 (17.6%)	8 (28.6%)	9 (25.7%)	
Lymph node metastasis					0.379
Yes	67 (83.8%)	13 (76.5%)	24 (85.7%)	30 (85.7%)	
No	13 (16.2%)	4 (23.5%)	4 (14.3%)	5 (14.3%)	
Stage					*0.050*
I/II	19 (23.8%)	7 (41.2%)	4 (14.3%)	8 (22.9%)	
III/IV	61 (76.2%)	10 (58.8%)	24 (85.7%)	27 (77.1%)	
Depth of tumor invasion (T)					0.083
T2	20 (25%)	7 (41.2%)	5 (17.9%)	8 (22.9%)	
T3	43 (53.8%)	6 (35.3%)	17 (60.7%)	20 (57.1%)	
T4	17 (21.2%)	4 (23.5%)	6 (21.4%)	7 (20%)	
Type					0.556
Intestinal	55 (68.8%)	12 (70.6%)	20 (71.4%)	23 (65.7%)	
Diffuse	21 (26.2%)	5 (29.4%)	7 (25%)	9 (25.7%)	
Mixed	4 (5%)	–	1 (3.6%)	3 (8.6%)	
Helicobacter pylori					*0.191*
Positive	36 (52.9%)	9 (69.23%)	11 (50%)	16 (48.48%)	
Negative	32 (47.1%)	4 (30.77%)	11 (50%)	17 (51.52%)	

comparison with the under-expressed cases (6.47 ± 0.77 vs. 5.89 ± 0.59 cm). We observed that the tumors in cardia had higher levels of MAEL mRNA expression in comparison with the non-cardia (0.29 ± 1.00 vs. − 0.52 ± 0.46-fold changes). The poorly and well-differentiated tumors had the lowest and highest levels of MAEL mRNA expression with (− 0.5 ± 0.88-fold changes) and (0.13 ± 1.38-fold changes), respectively. Although, there was no any significant correlation between lymph node metastasis and MAEL expression, majority of over-expressed cases had metastatic lymph nodes (13/17, 76.5%). In the case of tumor stage, there was a significant correlation between levels of MAEL mRNA expression and tumor stages in which the advanced stage tumors (III/IV) had lower MAEL expression compared with primary-stage tumors (I/II) (− 0.57 ± 0.59 vs. 1.04 ± 1.15-fold changes) ($p = 0.050$). Consistently, majority of the under-expressed tumors (24/28, 85.7%) were in advanced stages of tumor (III/IV). It was observed that the tumors with more depths of invasion (T3/4) had lower levels of MAEL expression in comparison with the T1/2 cases (− 0.54 ± 0.56 vs. 0.89 ± 1.02-fold changes). There was not any significant correlation between tumor type and MAEL expression; however, majority of over- (12/17, 70.6%) and under-expressed (20/28, 71.4%) cases were intestinal. In the case of *H. pylori*, we had only the results of *H. pylori* status for 68 cases. Despite the lack of significant correlation between *H. pylori* and MAEL expression, majority of over-expressed cases (9/13, 69.23%) were *H. pylori* positive. Moreover, the levels of MAEL expression in *H. pylori*-positive tumors were noticeably higher than that in the *H. pylori*-negative tumors (0.49 ± 0.89 vs. − 0.91 ± 0.50-fold changes). Among the *H. pylori*-positive cases, we also observed a significant correlation between the tumor stage and levels of MAEL mRNA expression, in which the tumors with stage II had the highest levels of MALE mRNA expression

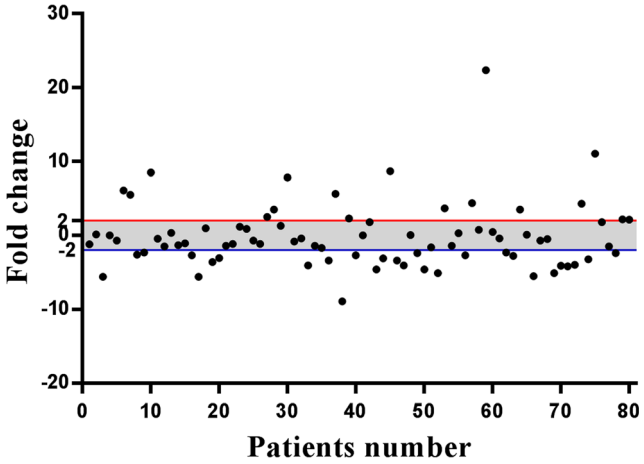

Fig. 1 Descriptive analysis of relative gene expression of MAEL in GC patients. The thresholds for the over- and under-expressed cases are shown by the red and blue lines, respectively. The gray area mentions to the cases with normal levels of MAEL mRNA expression

compared with the other stages ($p = 0.044$). There was also a significant correlation between MAEL expression and grade in *H. pylori*-negative cases, in which there was a rising trend from poorly to well-differentiation state (-1.51 ± 1.26 to 2.25 ± 2.07-fold changes) ($p = 0.015$). Interestingly, the levels of MAEL mRNA expression in *H. pylori*-negative tumors with T1/2 was significantly higher than that in the tumors with T3/4 depths of tumor invasion (1.56 ± 1.38 vs. -1.60 ± 0.45-fold changes) ($p = 0.030$).

Discussion

EMT is known as one of the primary processes of tumor metastasis which increases the therapeutic resistance beside the heterogeneity in primary cancer [17–21]. It has been shown that the MAEL has overexpression in colorectal cancer patients and it functions as a regulator of EMT and stemness in such cases. MAEL promotes and inhibits the Snail and E-cadherin respectively [22]. MAEL preserves genome stability in tumor cells and also inhibits Ras-induced senescence. Moreover, it is involved in protection against genotoxic stress during transformation process of tumor cells [13]. The MAEL has been reported both as an oncogene in liver, bladder and colorectal cancers [8, 15, 22], and tumor suppressor in ovarian cancer [6]. MAEL silencing in GC cells inhibited the cell proliferation, migration, and xenograft tumor growth suggesting the MAEL as an oncogene in GC [23]. Regarding the different reports, MAEL exerts its roles during the tumor progression through different mechanisms in various cancers. In hepatic cancer, MAEL activates the phosphorylation of Akt and GSK-3b [8]; while in colorectal cancer, MAEL inhibits the E-cadherin expression via Snail [22]; in GC, MAEL is also contributed to the ILKAP. Moreover, rescuing ILKAP can counteract the influence of MAEL overexpression on cell proliferation, colony formation, and invasion [23]. MAEL upregulates the self-renewal, cancer stem cell, and drug resistance markers in hepatocellular carcinoma [8]. It was reported that the MAEL overexpression in bladder cancer is correlated with advanced clinical stages. MAEL probably regulates the cell aggressiveness via MTSS1 and miR-186 is a key suppressor of MAEL in bladder cancer [15]. Mouse MAEL is associated with SNF5 and SIN3B chromatin remodelers in testis [24]. Therefore, MAEL exerts its roles in cancer cells through epigenetic regulation processes such as chromatin remodeling and transcription suppression. It was observed that the MAEL has not a uniform pattern of expression in hepatocellular tumor tissues in which tumor edges or invaded tumor cells had higher MAEL expression. MiR-7 is negatively associated with MAEL expression, in which MAEL probably activates the AKT through the miR-7 inhibition [8]. In the present study, we have observed an aggressive behavior of MAEL in GC patients. The tumor progression was initiated in lower ages and it seems that the MAEL expression is directly involved in tumor sizes of GC. Regarding the critical role of angiogenesis in tumor growth and size, therefore activation of AKT signaling through the MAEL can be probably resulted in VEGF activation via the mTOR and HIF1a in GC patients. AKT activation also inhibits the

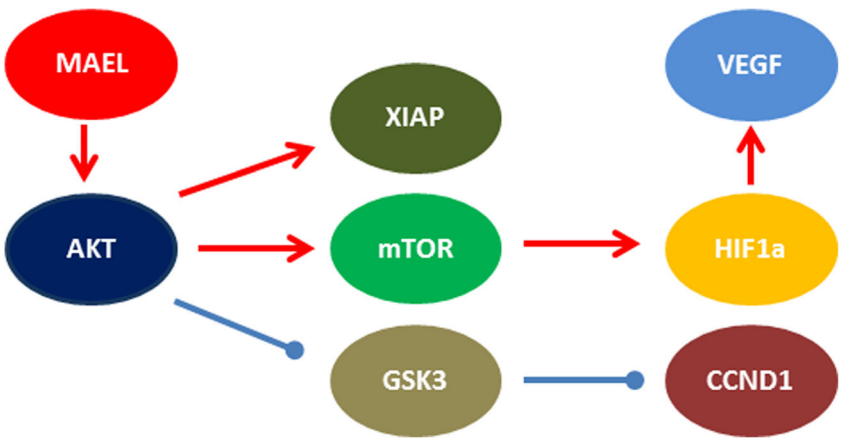

Fig. 2 Probable role of MAEL in gastric tumor size through the activation of AKT signaling pathway

apoptosis via the XIAP assisting the tumor bulks for the bigger sizes. On the other hand, AKT induces the cell proliferation in association with CCND1 (Fig. 2). Moreover, there was a direct correlation between MAEL expression and *H. pylori*, in which the *H. pylori*-positive tumors had higher levels of MAEL expression compared with *H. pylori*-negative tissues. It has been reported that there are significant correlations between cancer-testis antigens (CTAs) such as KK-LC-1 and MAGEA3 and risk of GC [25, 26]. Regarding the higher levels of MAEL expression in primary-stage tumors with fewer depths of invasion, it can be concluded that the MAEL can be introduced as an efficient marker for the primary GC. Although we have observed that the MAEL has the highest activation in primary stages and depths of invasion, its overexpression is directly related to the tumor invasion to the marginal lymph nodes. Therefore, it will be expected to see primary GC tumors with higher metastatic ability in MAEL over-expressed tumors. It seems that the MAEL has also a self-renewal role in GC cases and suppresses the tumor differentiation and can be introduced as a cancer stem cell marker in GC.

In conclusion, these data have shown for the first time a probable correlation between MAEL and *H. pylori* involvement in gastric cancer. Therefore, this marker can be introduced for diagnosis of GC tumors involving *H. pylori* infection. CTAs are fascinating markers for the targeted therapy because of low or lack of expression in normal tissues. Moreover, we have shown that the MAEL is an efficient diagnostic marker for the early detection of GC.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

This project was approved by the ethics committee of Mashhad University of Medical Sciences.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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