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Kurashige, Junji

Sawada, Genta

Takahashi, Yusuke

Eguchi, Hidetoshi

他

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Suppression of *MAL* Gene Expression in Gastric Cancer Correlates with Metastasis and Mortality

Junji Kurashige[1)2)], Genta Sawada[1)], Yusuke Takahashi[1)], Hidetoshi Eguchi[1)], Tomoya Sudo[1)], Toru Ikegami[3)], Tomoharu Yoshizumi[3)], Yuji Soejima[3)], Tetsuo Ikeda[3)], Hirofumi Kawanaka[3)], Hideaki Uchiyama[3)], Yo-ichi Yamashita[3)], Masaru Morita[3)], Eiji Oki[3)], Hiroshi Saeki[3)], Keishi Sugimachi[1)], Masayuki Watanabe[2)], Masaki Mori[4)], Hideo Baba[2)] and Koshi Mimori[1)*]

[1)]*Department of Surgery, Kyushu University Beppu Hospital, 4546 Tsurumihara, Beppu, Oita, 874-0838, Japan*

[2)]*Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, Kumamoto, 860-8556, Japan*

[3)]*Department of Surgery and Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan*

[4)]*Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka, 565-0871, Japan*

Abstract

Background. The Myelin and lymphocyte-associated protein gene (*MAL*), which is located on the long arm of chromosome 2, assigned to the region cen-q13 in humans, has been reported as tumor suppressor in several cancers. The aim of this study was to clarify the clinical significance of *MAL* gene in gastric cancer.

Patients and Methods. The expression levels of *MAL* mRNA was examined using 50 resected gastric cancer specimens used by laser microdissected to determine the clinicopathological significance. *MAL* expression was then examined by real-time quantitative PCR assay, and we analyzed the correlation between *MAL* expression and clinicopathological factors.

Results. In clinicopathologic analysis, the low *MAL* expression group showed significantly higher incidence of lymph node metastasis than the high expression group (79% and 46%, respectively, $p < 0.05$). Furthermore, the low *MAL* expression group had a significantly poorer prognosis than the high expression group ($p < 0.05$).

Conclusions. The *MAL* gene repression related with lymph node metastasis and poor prognosis in gastric cancer, suggesting that the *MAL* may be a new candidate node metastasis-suppressor gene for gastric cancer.

Key words : Cancer suppressor gene・ Lymph node metastasis・Prognostic factor・Cell differentiation and apical sorting

Introduction

The Myelin and lymphocyte-associated protein gene (*MAL*) was originally cloned by Alonso and Weissman from T cells in intermediate or late stage of differentiation[1)]. The *MAL* gene is located on the long arm of chromosome 2, assigned to the region cen-q13 in humans. It encodes a 17 kD membranous poteolipid with a hydrophobic pattern[2)]. Expression of the MAL and its homologs (VIP17/MVP17) were detected in central and peripheral myelin, lymphocytes, and various kinds of epithelial cells, e.g., in kidney, stomach, thyroid gland. MAL has been shown to play an essential

*Correspondence to : Koshi Mimori, MD., PhD.
Department of Surgery, Kyushu University Beppu Hospital, 4546 Tsurumihara, Beppu City, Oita, 874-0838, Japan
Phone : 81-977-27-1650 Fax : 81-977-27-1651
E-mail : kmimori@beppu. kyushu-u. ac. jp

role in the apical transport in these epithelial cells[3)~5)].

Previously, we demonstrated that the *MAL* expression was diminished in esophageal cancer cells compared to the corresponding normal epithelial cells. The proper expression of the *MAL* regulated cellular division, and the *MAL* induced apoptosis indicated the anti-tumor effect[6)]. Then, we disclosed that the *MAL* expression was reduced even at the early phase of carcinogenesis in the mice model for esophageal cancer[7)]. Moreover, downregulation of the *MAL* expression was seen in a variety of cancers, so the *MAL* gene is considered to act as a cancer suppressor gene[8)~10)]. The use of MAL protein as a predictive biomarker to benefit for patients undergoing chemotherapy was also statistically analyzed in breast and ovarian cancers[11)12)] in the current study, we investigated the clinicopathologic significance of the loss of *MAL* expression in gastric cancer cases.

Materials and Methods

Gastric cancer samples

Primary gastric cancer tissues and the corresponding normal gastric mucosal tissues were collected from 50 patients in Kyushu University Beppu hospital. The number of male/female patients was 16/34, respectively (Table 1). Regarding histology, there were 28 differentiated cell cancer and 22 undifferentiated cell cancer. Regarding depth of invasion, 37 cases were within submucosal layer and 13 cases were beyond the muscle. Regarding lymph node metastasis, 15

Table 1 Clinicopathological charactersitics of *MAL* mRNA expression in 50 cases of gastric carcinomas.

variables	n	*MAL/GAPDH* expression ratio: High (T/N > 0.25) (n = 13)	*MAL/GAPDH* expression ratio: Low (T/N < 0.25) (n = 37)	*p* value
Sex				
Male	16	5	11	
Female	34	8	26	
Histology				ns*
Differentiated type	28	7	21	
Un differentiated type	22	6	16	
Depth of invasion				ns
T1 within submucosal layer	37	7	30	
T2-T4 beyond the muscle	13	6	7	
Lymph node metastasis				$p < 0.05$
negative	15	7	8	
positive	35	6	29	
Lymph vessel permeation				ns
negative	12	5	7	
positive	38	8	30	
Vascular vessel permeation				ns
negative	39	11	28	
positive	11	2	9	
Clinical stage				ns
I and II	19	7	12	
III and IV	31	6	25	
Clinical Outcome				$p < 0.05$
Alive	21	9	12	
Cancer Death	28	3	25	
Other cause	1	1	0	

There are significant differences between low and high expression of MAL in the incidence of lyphnode metastasis as well as in the clinical out come. ns = not significant.

cases were negative and 35 cases were positive. Regarding lymph vessel permeation, 12 cases were negative and 38 cases were positive. Regarding vascular vessel permeation, 39 cases were negative and 11 cases were positive. Cancer/normal tissues were laser microdissected to obtain corresponding area as precisely as possible. Total RNA was extracted from these samples, and reverse transcribed into cDNA using oligo-dT primers. *MAL* expression was then examined by real-time quantitative PCR assay, and we analyzed the correlation between *MAL* expression and clinicopathological factors.

Real-time reverse transcription (RT) PCR

MAL expression in the cancer/normal tissues of clinical samples was quantified by real-time RT-PCR with a LightCycler (Roche, Tokyo, Japan), using SYBER-Green I dye (Roche) as described previously. *MAL* : forward CAGTGGCTTCTCGGTCTTCAC, reverse GTCTTGCATCGTGATGGTGGC. The length of the amplified product is 307 bp, which spans from exon1 to 3 of the *MAL* open-reading frame. Cycling conditions of PCR was as follows ; initial denaturation at 95℃ for 10 min, followed by 40 cycles of amplification (95℃ for 10 sec, annealing at 62℃ for 10 sec, and extension at 72℃ for 10 sec), and cooling to 40℃ at 0.2℃ / sec under continuous fluorescence monitoring.

Statistical analysis

The relationship between *MAL* mRNA expression and clinicopathological factors was analyzed by Chi-square test and Student's t-test. Overall survival curves were plotted according to the Mantel-Cox method and the generalized log-rank test was applied to compare the survival curves. All tests were analyzed using JMP software (ver. 7, SAS institute, Inc., Cary, NC) and the findings were considered significant when the P-value was < 0.05.

Results and Discussion

MAL expression was normalized by the expression of *GAPDH* as an internal control. *MAL* expression was decreased in cancer tissues compared with normal mucosal tissues ($p < 0.001$, Fig 1A). T/N ratio was defined as tumor/normal tissue ratio of *MAL* expression, and the patients were divided into two groups by the median value (Table 1). There was no significant difference in their backgrounds between the low expression group (T/N ratio < 025, n = 37) and the high expression group (T/N ratio >= 0.25, n = 13). The low expression group showed significantly higher incidence of lymph node metastasis than the high expression group (79% and 46%, respectively, $p < 0.05$). As for 5-year survival rate, 32% of patients

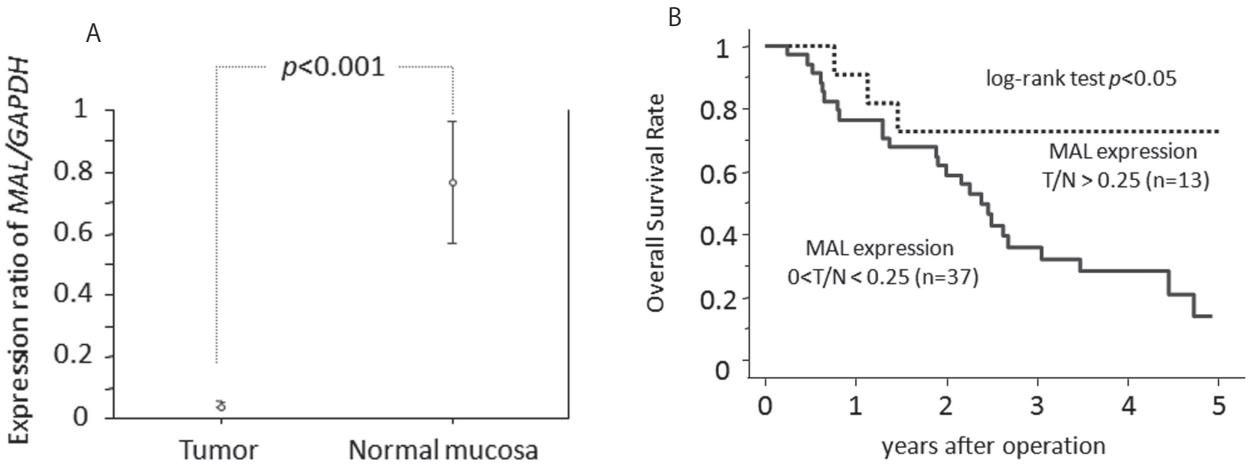

Fig. 1 *MAL* expression in gastric cancers and the correlation with clinicopathological factors
(A) *MAL* expression was decreased in cancer tissues compared with normal mucosal tissues ($p < 0.001$). (B) As for 5-year survival rate, the low expression group is significantly poorer prognosis than the high expression group ($p < 0.05$, Fig 1B).

were alive in the low expression group, whereas 75% in the high expression group ($p < 0.05$, Fig 1B).

In the current study, the *MAL* expression was reduced in primary gastric cancer tissue compared to corresponding normal gastric mucosa. Moreover, the *MAL* repression related with lymph node metastasis and poor prognosis of overall survival rate.

The MAL is a proteolipid which has been known as one of minor myelin components. Outside the nervous system, MAL protein was detected in T-lymphocyte and several kinds of epithelial cells such as kidney, stomach, and esophagus. MAL has been considered to interact with glycosphingolipids in detergent insoluble domains on membranes of these epithelial cells, which indicates some functions of MAL in cell differentiation and apical sorting. More recently, it has been established that *MAL* gene acts as a cancer suppressor gene in esophagus cancer[6)7)], colon cancer[13)], and head and neck squamous carcinoma[8)] and, DNA methylation in the *MAL* promoter represses its expression in non-small lung cancer[14)], head neck squamous carcinoma[15)] and cervix cancer[16)].

Although *MAL* could be an excellent prognostic biomarker, most of the mechanism that *MAL* suppression leads to tomorigenesis remains unknown. We demonstrated that *MAL* gene expression in esophageal cancer induces apoptosis *in vitro* and *in vivo* previously, and speculated that it was through the Fas signaling pathway. *MAL* suppression could associate with tomorigenesis and progression of gastric cancer in the same way, however, further study is required to uncover the role of *MAL*.

In conclusion, our results show that the *MAL* repression related with lymph node metastasis and poor prognosis in gastric cancer, suggesting that the *MAL* may be a new candidate node metastasis-suppressor gene for gastric cancer. Further investigation including in vitro and in vivo experiments needs to be conducted to identify the functional role of the *MAL* gene in the node metastatic and invasive process in gastric cancer.

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（和文抄録）

胃癌における *MAL* 遺伝子抑制の意義

[1)]九州大学病院別府病院外科
[2)]熊本大学大学院　消化器外科
[3)]九州大学　消化器・総合外科
[4)]大阪大学大学院　消化器外科

藏重淳二[1)2)]，澤田元太[1)]，高橋祐典[1)]，江口英利[1)]，主藤朝也[1)]，
池上　徹[3)]，吉住朋晴[3)]，副島雄二[3)]，池田哲夫[3)]，川中博文[3)]，
内山秀昭[3)]，山下洋市[3)]，森田　勝[3)]，沖　英次[3)]，佐伯浩司[3)]，
杉町圭史[1)]，渡邊雅之[2)]，森　正樹[4)]，馬場秀夫[2)]，三森功士[1)]

われわれは癌組織において極めて高頻度に発現が減弱または消失している *MAL* 遺伝子を同定した．その発癌と癌進展にかかわる分子機序を明らかにするために，胃癌の株化細胞と臨床症例を用いた解析を行った．インフォームドコンセントを得た胃癌 50 症例を対象に Real time PCR にて半定量的に MAL 発現量を求め，予後および臨床病理学的因子との関係を調べた．胃癌 50 例の 37 例（74%）は癌部において *MAL* 遺伝子の発現消失，減弱を認めた（低値群）．これを発現が比較的保たれている症例（高値群）と比較すると，高値群は低値群に比し予後良好であった．また低値群はリンパ節転移陽性例が多い傾向を示した．MAL は胃癌において極めて高頻度に発現が減弱，消失していた．本来 MAL はゴルジ体または ER における蛋白輸送によって過集積を防ぐ機能を有する分子として報告されていたが，本研究でその発現喪失が胃癌進展に対しても深く関与することが示された．また，*MAL* 遺伝子が胃癌における癌抑制遺伝子であることを示した．