Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Low junctional adhesion molecule A expression correlates with poor prognosis in gastric cancer

Jin-yu Huang, MD,[a] *Ying-ying Xu, MD, PhD,*[b,*] *Zhe Sun, MD, PhD,*[a] *Zhen-ning Wang, MD, PhD,*[a] *Zhi Zhu, MD, PhD,*[a] *Yong-xi Song, MD,*[a] *Yang Luo, MD, PhD,*[c] *Xue Zhang, MD, PhD,*[c] *and Hui-mian Xu, MD, PhD*[a]

[a] *Department of Surgical Oncology, First Affiliated Hospital of China Medical University, Shenyang, China*
[b] *Department of Breast Surgery, First Affiliated Hospital of China Medical University, Shenyang, China*
[c] *The Research Center for Medical Genomics and MOH Key Laboratory of Cell Biology, China Medical University, Shenyang City, China*

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form
7 June 2014
Accepted 13 June 2014
Available online 18 June 2014

Keywords:
Gastric cancer
JAM-A
Migration
Invasion
Junctional adhesion molecule A
Prognosis

ABSTRACT

Background: The aberrant expression of junctional adhesion molecule A (JAM-A), which has a close correlation with the development, progression, metastasis, and prognosis of cancer, has been frequently reported. However, neither JAM-A expression nor its correlation with clinicopathologic variables and patient survival has been defined in gastric cancers. Moreover, little is known about the role of JAM-A in gastric cancer progression. We carried out the present study to investigate the prognostic value of JAM-A expression in gastric cancer patients. Furthermore, the biological roles of JAM-A in gastric cancer progression were also investigated.

Methods: We determined JAM-A expression in 167 primary gastric cancer tissues and 94 matched adjacent non-tumor tissues by immunohistochemistry. Transwell migration assays and matrigel invasion assays were used to explore the role of JAM-A in gastric cancer cells migration and invasion. CCK-8 assays were used to examine the effect of JAM-A on the proliferation of gastric cancer cells.

Results: JAM-A was downregulated in gastric cancer tissues. Low JAM-A expression was significantly associated with tumor size, lymphatic vessel invasion, lymph node metastasis, and TNM stage. Low JAM-A expression was also significantly associated with poor disease-specific survival in gastric cancer patients. Multivariate analysis demonstrated low JAM-A expression as an independent factor predicting poor survival. In addition, JAM-A had the effect on inhibition of gastric cancer cells migration and invasion. However, JAM-A had no significant effects on proliferation of gastric cancer cells.

Conclusions: Low JAM-A expression correlates with poor clinical outcome and promotes cell migration and invasion in gastric cancer.

1. Introduction

Gastric cancer is the fourth most prevalent malignancy worldwide and has recently been identified as the second leading cause of cancer-related death. About 1,000,000 new cases of gastric cancer occur in the world each year [1]. Despite recent advances in the diagnosis and medical treatment of gastric cancer, most patients are diagnosed with advanced

* *Corresponding author*. Department of Breast Surgery, First Affiliated Hospital of China Medical University, Shenyang, Liaoning 110001, China. Tel.: +86 13940521129; fax: +86 24 22834060.
E-mail address: lovecmustar@163.com (Y.-y. Xu).
0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2014.06.025

gastric cancer and the survival rate remains unsatisfactory. Thus, to identify molecules predicting tumor progression and patients' survival is important.

Junctional adhesion molecule A (JAM-A), also known as F11R, JAM-1, or CD321, is a type 1 transmembrane IgG glycoproteins, which predominantly expressed at epithelial cells tight junctions and intercellular borders of endothelial cells, and on the surface of platelets and some leukocytes [2–4]. It has been reported that JAM-A is important for a variety of cellular processes, such as intercellular junction assembly, leukocyte migration, platelet activation, angiogenesis, cell morphology, and reovirus binding [2–4]. In recent years, JAM-A expression has been reported to play an important role in the prognosis of patients suffering from various tumor types, such as breast cancer, pancreatic cancer, lung cancer, and endometrial carcinoma [5–8]. However, the prognostic value of JAM-A expression in different cancers is highly inconsistent. In pancreatic cancer and endometrial carcinoma, low JAM-A expression has been showed to correlate with poor prognosis [7,8]. On the contrary, in lung cancer and breast cancer, a positive correlation between high JAM-A expression and poor survival has been reported [5,6]. This indicates that the prognostic value of JAM-A expression in cancers may be organ specific. Therefore, the role of JAM-A expression in the prognosis of gastric cancer patients is highly uncertain. Unfortunately, there has thus far been no study that has explored this issue. In addition, it has been shown that several tight junction proteins, such as claudin-18 and claudin-4, suppress gastric cancer progression by inhibiting tumor cells proliferation, migration, or invasion [9,10]. Nevertheless, little is known about the role of JAM-A in gastric cancer progression.

Therefore, we carried out the present study to investigate the prognostic value of JAM-A expression in gastric cancer patients. Furthermore, the biological roles of JAM-A in gastric cancer progression were also investigated.

2. Materials and methods

2.1. Ethics statement

Ethical approval for the present study was obtained from the Research Ethics Committee of China Medical University, China. All patients providing tumor tissue and adjacent non-tumor tissues had signed a consent form before surgery to allow for this research to be undertaken.

2.2. Tissue samples and follow-up

A total of 167 patients who were treated by total or subtotal gastrectomy with lymphadenectomy for primary gastric cancer between January 2006 and December 2009 at the First Affiliated Hospital of China Medical University were selected for this study. No patients had received neoadjuvant therapy. The group was composed of 123 men and 44 women with a mean age of 65 ± 15 (range, 22–95) years. All patient-derived specimens were collected and archived under protocols approved by the Institutional Review Boards of the First affiliated Hospital China Medical University. The diagnosis was confirmed by at least two pathologists and staging was based on pathologic findings according to the seventh American Joint Committee on Cancer guidelines. Follow-up of the 167 patients was conducted until death or the cutoff date (June 29, 2012) by means of outpatient clinic consultation and/or communication with patients through telephone or letter. The median and mean duration of follow-up was 42 and 39.47 (range, 1–73) months, respectively. There were no patients who died in the postoperative period. Disease-specific survival was defined as the interval from the initial surgery to the death for cancer-related causes.

2.3. Tissue microarray construction and immunohistochemistry

Hematoxylin and eosin-stained slides were screened for optimal tumor tissues and matched adjacent non-tumor tissues (at least 2 cm from the tumor) and the tissue microarray slides were constructed with a tissue manual arraying instrument. Two cores were collected from each formalin-fixed, paraffin-embedded gastric cancer tissue sample and from each adjacent non-tumor tissue sample using a 1.0-mm diameter punch instrument. The tissue microarray consisted of 167 primary gastric cancer tissues and 94 matched adjacent non-tumor tissues.

Immunostaining was performed using the streptavidin biotin complex method. The primary antibody against JAM-A (H00050848-M01; Abnova, Taiwan, China) was used in a dilution of 1:500. The secondary antibody (Boshide, Wuhan, China) and the streptavidin–biotin conjugated with horseradish peroxidase (Boshide) were used according to the manufacturer's instructions. Afterward, the sections were incubated with diaminobenzidine (Boshide) and stained with hematoxylin.

2.4. Evaluation of immunohistochemical staining

Immunoreactivity was evaluated independently by two researchers who were blinded to patient outcomes. Antigen expression was defined as the presence of specific staining on surface membranes of tumor cells [7]. The evaluation was based on the staining intensity and extent of staining. Staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Staining extent was scored as 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%), depending on the percentage of positive-stained cells. The product of the staining intensity and the staining extent scores was used as the final staining score. The total score ranged from 0–12. Membranous JAM-A staining was scored low or high. Low JAM-A expression was defined as a total score of ≤ 4 [7].

2.5. Cell culture and transfection

Normal gastric epithelial cell line (GES)-1 and three human gastric cancer cell lines (SGC-7901, MGC-803, and BGC-823) were purchased from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). GES-1 was cultured in Dulbecco's modified eagle medium with 10% fetal bovine serum (FBS). SGC-7901, MGC-803, and BGC-823 were cultured in recommended Roswell Park Memorial Institute (RPMI) 1640 medium with 10% FBS. Cell lines were cultured at 37°C in a humidified atmosphere of 5% CO_2.