ORIGINAL ARTICLES

Upregulation of a disintegrin and metalloprotease 8 is associated with progression and prognosis of patients with gastric cancer

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A disintegrin and metalloprotease 8 (ADAM8) is involved in the tumorigenesis of several types of solid tumors. However, its exact role in gastric cancer (GC) remains unclear. The aim of this study was to evaluate the clinical significance of ADAM8 in GC and to explore its biological effects on gastric carcinogenesis. In this study, quantitative reverse transcription–polymerase chain reaction, Western blotting, and immunohistochemical staining analysis revealed that ADAM8 messenger RNA expression was significantly upregulated in GC tissues compared with noncancerous tissues ($P = 0.004$), and that positive ADAM8 expression is much more common in tumor tissues compared with normal tissues ($P < 0.001$) and is correlated with T stage ($P = 0.036$), N stage ($P = 0.048$), vessel invasion ($P = 0.002$), and a shorter patient overall survival ($P = 0.024$). In vitro assay indicated that ADAM8 overexpression promoted cell growth and increased migration and invasion abilities by decreasing the p-p38/p-extracellular regulated protein kinases (p-ERK) ratio. In conclusion, ADAM8 promotes GC cell proliferation and invasion, and its expression is positively correlated with poor survival, indicating that it might be a promising target in GC therapy. (Translational Research 2015;166:602–613)

Abbreviations: ADAM8 = a disintegrin and metalloprotease 8; GC = gastric cancer; OS = overall survival

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AT A GLANCE COMMENTARY

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Background

A disintegrin and metalloprotease 8 (ADAM8) has been involved in the tumorgenesis of several types of solid tumor. Meanwhile, ADAM8 can be acted as an independent factor for lymph node metastasis in gastric cancer (GC). However, its exact role in GC remains unclear.

Translational Significance

ADAM8 promotes GC cells proliferation and invasion, and correlates with poor survival of patients with GC, indicating that ADAM8 might be a promising target in GC therapy.

INTRODUCTION

Gastric cancer (GC) is the fourth most common alimentary tract malignancy and the second leading cause of cancer-related deaths after lung cancer.[1] Although the incidence of GC has been declining, it is estimated that approximately 400,000 new cases are diagnosed in China annually, comprising approximately 43% of the total cases worldwide.[2] Most GC patients are diagnosed at an advanced stage, with 50%–75% presenting with regional lymph node metastasis.[3] Lymph node status determines the tumor node metastasis (TNM) stage and affects the postoperative prognosis of GC patients. Although preoperative examinations, such as computerized tomography and magnetic resonance imaging, can predict lymph node status, they are not reliable indicators of lymph node metastasis.[4,5] Therefore, there is great interest in identifying biomarkers to predict regional and or distant metastasis. Several genes seem to contribute to lymph node metastasis, including cell proliferation, cell to cell interactions, and cell invasion and migration.[6-9] Of them, members of a disintegrin and metalloproteinases (ADAMs) family are involved in invasion and metastasis in GC.[10-12]

ADAM8, a member of the ADAMs family, was initially reported to play potential roles in inflammatory and allergic processes,[13] and further studies have shown that its overexpression is associated with progression and poor survival in various solid tumors.[12,14,15] ADAM8 has been reported to be an independent indicator of lymph node metastasis in human GC[12]; however, its precise effects on GC progression and prognosis remain unclear. In this study, we focused on the correlation of ADAM8 expression with clinicopathologic features and overall survival (OS); in addition, the mechanisms implicating this protein in GC progression were studied at the molecular level by in vitro assays.

MATERIALS AND METHODS

Patients and tissue samples. Two hundred three consecutive patients undergoing gastrectomy for GC at the Sixth Affiliated Hospital of Sun Yat-Sen University, China, from January 2007 to December 2008 were included in this study. These patients had not received preoperative chemotherapy or radiotherapy. All patients were diagnosed by a clinician, and the diagnosis was confirmed by a pathologist. Tissue samples, including tumor and nontumor tissue samples, were obtained from the resected specimens and were snap-frozen in liquid nitrogen and stored at −80°C until use.

This study was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the research ethics committee of Sun Yat-Sen University, China. Informed consent was obtained from all patients in this study.

Tissue microarrays. Tissue microarrays (TMAs) were performed to assess 203 GC specimens and 20 normal tissues obtained from the Tissue Bank of the Sixth Affiliated Hospital. The TMAs were constructed using an automated TMA instrument (ALPHELYS, Plaisir, France). The method for constructing the TMAs was similar to a previously described method.[16]

Immunohistochemistry and evaluation of immunohistochemical (IHC) staining. IHC staining was performed on the TMA slides using a Polink-2 plus Polymer Horseradish Peroxidase Detection System (GBI, Bothell, WA, USA) according to the manufacturer's instructions, as described previously.[17]

IHC staining was analyzed using Image Pro-Plus (version 6.0; Media Cybernetics, Silver Spring). Briefly, the tumor area was selected as the area of interest (AOI), and the area sum and integrated optical density of the AOI were selected as the measurement parameters. The ADAM8 expression index equaled the quotient between the integrated optical density and the total AOI. Finally, statistical analysis of the mean expression index for each duplicate was performed. Receiver operating characteristic curve analysis was conducted to select cutoff scores for ADAM8. The cutoff value was determined using the log-rank test with respect to OS. The expression of ADAM8 was considered negative if the cutoff value was 4.2 or less and positive if the value was more than 4.2.

Cell culture and establishment of stably transfected cell lines. Five GC cell lines (SGC7901, AGS, MGC803, BGC823, and MKN45) and an immortalized gastric