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ANATOMICAL PATHOLOGY

Tumour infiltrating lymphocytes are predictors of lymph node metastasis in early gastric cancers

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Summary

Lymph node metastasis (LNM) is an important factor for predicting prognosis and selecting appropriate treatment in early gastric cancers (EGCs). We investigated the histopathological and microenvironmental predictors of LNM in EGCs. We retrieved 43 cases of EGC without LNM and 59 cases with LNM. Clinicopathological variables and tumour-infiltrating lymphocytes (TILs), Crohn's-like lymphoid reaction (CLR), tumour stromal percentage (TSP), and FOXA1 expression were evaluated and correlated with LNM. Among the 102 cases, 68 cases (66.7%) had low TILs and 34 cases (33.3%) had high TILs. High TILs were significantly correlated with the absence of LNM ($p < 0.001$), less extent of invasion ($p = 0.004$), absence of LVI ($p = 0.035$), conspicuous CLR ($p < 0.001$), and the absence of TSP ($p = 0.009$). Conspicuous CLR was observed in 47 cases (46.1%) and TSP was present in 17 cases (16.7%) and neither was correlated with LNM. High FOXA1 expression was significantly associated with presence of LNM, low TILs, and submucosal invasion. In multivariate analysis, low TILs ($p = 0.023$), LVI ($p = 0.008$), and submucosal invasion ($p = 0.001$) were independent predictive factors for LNM in EGCs. Evaluation of TILs in biopsied or endoscopically resected EGC specimens may help to predict LNM and select subsequent proper treatment modalities and follow-up.

Key words: Early gastric cancers; lymph node metastasis; tumour-infiltrating lymphocytes; Crohn's-like lymphoid reaction; tumour stromal percentage; FOXA1.

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INTRODUCTION

Early gastric cancer (EGC) is defined as the tumour confined to the mucosa or submucosa, regardless of the presence of lymph node metastasis (LNM).[1] High grade dysplasia (carcinoma *in situ*) is a premalignant lesion which is different from carcinoma in that it is intra-epithelial tumour without invasion of lamina propria.[2] According to the American Joint Committee on Cancer (AJCC) 8th edition, EGC includes T1N0 as stage IA, T1N1 as stage IB, T1N2 as stage IIA, T1N3a as stage IIB, and T1N3b as stage IIIB.[2] The prognosis of EGC is generally excellent, with a 5-year survival rate greater than 90% after curative resection.[3] However, the recurrence rate after resection has been still reported as 1.3–13.8%.[4] LNM is an important prognostic factor for EGC.[5] The 5-year survival rate of EGC patients without LNM is 90–95%, whereas that of patients with LNM is 70–87%.[6,7] Endoscopic dissection is a widely accepted treatment modality for some subsets of EGC patients, and the absence of LNM is an essential prerequisite.[8] Thus, the lymph node status is critical for assessing prognosis and selecting appropriate treatment modalities.

Recently, the tumour microenvironment, including host immune response, has emerged as an important factor in the progression and survival of cancer cells.[9] Tumour-infiltrating lymphocytes (TILs) are a component of host immune response against tumours. TILs have been reported to be a favourable prognostic factor and significant predictor of patient outcome and response to chemotherapy in various malignancies.[10–12] The emerging concept of an 'Immunoscore' represents the significant role of TILs in classification of cancers and prognosis.[13] Other microenvironmental factors include Crohn's-like reaction (CLR) and tumour stromal percentage (TSP), which were first described in colorectal cancer.[14,15] CLR is nodular lymphoid aggregates around carcinoma, which was termed due to its histological resemblance to Crohn's disease showing transmural lymphoid aggregates, and it is a parameter of host immune response to tumours.[14] TSP is defined as the proportion of tumour stroma in invasive area and an increased TSP has been associated with poor survival in some malignancies.[15] However, few studies have evaluated the prognostic and predictive value of microenvironmental factors in EGCs,[16,17] and the association between these microenvironmental factors and clinicopathological factors in EGCs has not been studied.

Forkhead box protein A1 (FOXA1) is a member of the forkhead box gene family and is known to regulate cell growth and differentiation.[18] FOXA1 is expressed in various malignancies with different roles. It has been reported to be a good prognostic marker in breast cancer,[19] have a metastatic role in prostate cancer,[20] and be a negative regulator of the epithelial-mesenchymal transition in pancreatic cancer.[21] However, the predictive role of FOXA1 expression in EGC for LNM has not been studied.

In this study, we evaluated the various clinicopathological factors and tumour microenvironmental factors as predictors for LNM in EGC. In addition, we analysed the correlation between LNM and FOXA1 expression in EGC.

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MATERIALS AND METHODS

Study population and clinicopathological data

This study enrolled 43 cases of EGC without LNM and 59 cases of EGC with LNM which were surgically resected with lymph node dissection at Korea University Anam Hospital, between January 2006 and December 2011. Clinicopathological data including age, sex, tumour size, depth of invasion, histological type, gross type, Lauren classification, and the presence of lymphovascular invasion (LVI), were retrieved from medical records and pathological reports. The Institutional Review Boards of Korea University Anam Hospital approved this study.

Tumour-infiltrating lymphocytes

The histopathological assessment was performed by consensus interpretation of two pathologists (JYK and Y-SC) who were blinded to the clinical information. Representative haematoxylin and eosin (H&E) slides were selected and reviewed and TILs were scored according to the modified TILs scoring recommendations by an International TILs Working Group 2014 in breast cancer,[22] because there is no consensus on scoring TILs in gastric cancer. TILs were defined as percentage of intratumoural stromal tissue occupied by lymphocytes or plasma cells. Granulocytes and other polymorphonuclear cells were not included.[22] All tumour areas were scanned at ×40 magnification and the average percentage of TILs, not focusing on hot spots, was recorded in 5% increments.[22] The cut-off value was determined using the mean value of TILs of all cases and receiver operating characteristic (ROC) curve analysis. All cases were divided into high-TILs and low-TILs using the cut-off value (Fig. 1A,B).[16]

Crohn's-like reaction

CLR was measured according to the Graham–Appelman criteria as follows: absent, no or single lymphoid aggregates in tumour area; mild, occasional lymphoid aggregates with rare germinal centres; or intense, numerous and large lymphoid aggregates with frequent germinal centres.[14,23] Absent and mild grades were grouped as 'inconspicuous' and intense grade was categorised as 'conspicuous' (Fig. 1C,D).[24]

Tumour stromal percentage

TSP was measured in a single ×10 objective field of the most invasive area with tumour cells in all four cardinal sides on microscopic view, and the area of stroma was calculated as a percentage in 5% increments.[15] Necrotic or mucinous areas were excluded in measurement.[15] Over 5% was considered as positive (Fig. 1E,F).[15]

Tissue microarray and immunohistochemistry

All slides were reviewed, and representative sections were selected. Tissue microarray (TMA) was constructed from formalin fixed, paraffin embedded blocks with a tissue microarrayer. Two cores, with a diameter of 3.0 mm, were extracted from representative paraffin blocks of each tumour and rearranged into recipient blocks. From each tissue microarray block, 4 μm-thick sections were cut, and deparaffinised and rehydrated in serial diluted alcohol. For antigen retrieval, sections were heated with citrate buffer for 15 min. To block endogenous peroxidase, slides were incubated in Hydrogen Peroxide Block (Cell Marque, USA) for 10 min. Sections were incubated with primary antibody for FOXA1 (1:100 dilution, SC-101058; Santa Cruz Biotechnology,

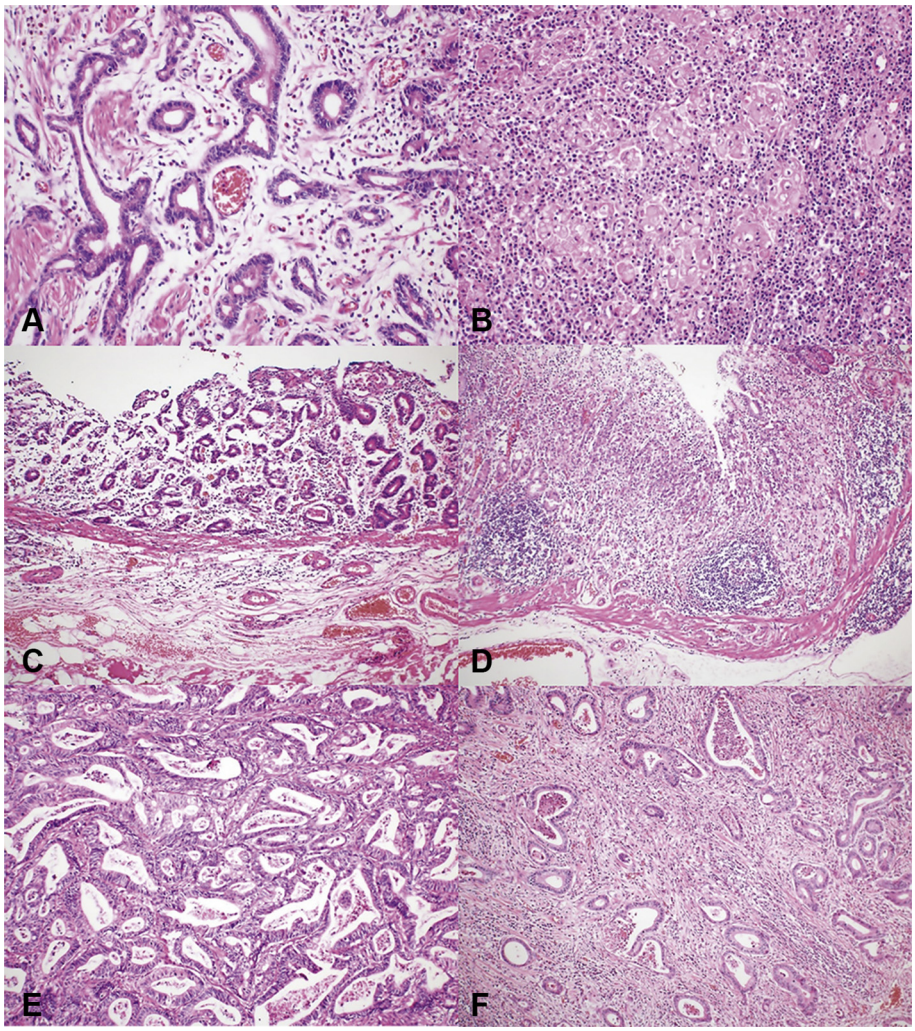

Fig. 1 Representative features of tumours with (A) low and (B) high tumour infiltrating lymphocytes, (C) inconspicuous and (D) conspicuous Crohn's-like lymphoid reactions, and (E) negative and (F) positive for tumour stromal percentage.

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