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Serum ENA78/CXCL5, SDF-1/CXCL12, and their combinations as potential biomarkers for prediction of the presence and distant metastasis of primary gastric cancer

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ABSTRACT

Background: Chemokines play important roles in cancer development and progression. Epithelial-derived neutrophil-activating peptide-78 (ENA78/CXCL5) and stromal cell-derived factor (SDF-1/CXCL12) supposedly contribute to gastric cancer (GC) development and progression. This study aims to evaluate serum levels of ENA78/CXCL5 and SDF-1/CXCL12 along the GC carcinogenesis, and analyze their clinical significance, and diagnostic potentials through human serum samples.

Methods: A total of 300 subjects were enrolled in this study. Serum levels of ENA78/CXCL5 and SDF-1/CXCL12, measured by chemiluminescent immunoassay, were compared among 4 disease groups; normal, high-risk (intestinal metaplasia and adenoma), early GC (EGC), and advanced GC (AGC) groups in both training ($n = 25$ per group) and validation dataset ($n = 70, 30, 50, 50$, respectively) by ANOVA test (post hoc Bonferroni). Correlations between serum ENA78/CXCL5 or SDF-1/CXCL12 levels and clinicopathological parameters of GC patients were evaluated (Spearman's correlation; γ_s). To validate the diagnostic accuracy, receiver operating characteristic (ROC) curve and logistic regression analysis was performed.

Results: Serum ENA78/CXCL5 and SDF-1/CXCL12 levels were significantly higher in AGC groups than EGC, high-risk and normal groups in both training and validation dataset (Bonferroni, from $p < 0.01$ to $p < 0.001$). Clinicopathologically, serum ENA78/CXCL5 was correlated with T-stage ($\gamma_s = 0.231$, $p = 0.021$) and distant metastasis ($\gamma_s = 0.357$, $p < 0.001$), while serum SDF-1/CXCL12 was correlated with lymph node ($\gamma_s = 0.220$, $p = 0.029$) and distant ($\gamma_s = 0.425$, $p < 0.001$) metastasis. ROC curve and logistic regression demonstrated that serum ENA78/CXCL5 and SDF-1/CXCL12 showed higher diagnostic accuracy compared with carcinoembryonic antigen (CEA) in predicting GC. Serum ENA78/CXCL5 could predict both the presence of GC and distant metastasis, while serum SDF-1/CXCL12 could mainly predict its distant metastasis. All combination of serum ENA78/CXCL5, SDF-1/CXCL12, and CEA achieved 92.8% specificity at 75.0% sensitivity to predict distant metastasis of GC.

Conclusions: Combinations of initial serum ENA78/CXCL5, SDF-1/CXCL12, and CEA before any treatment for GC can produce valuable serum biomarker panels to predict the presence and distant metastasis of GC.

1. Introduction

Although the overall incidence of gastric cancer (GC) has decreased over the past few decades [1], GC is still a health problem because this is the second most common cause of cancer-related deaths worldwide [2] and the prognosis advanced GC (AGC) with extensive node invasion and distant metastasis remains poor [3]. Moreover, about 10–20% of AGC patients, who are considered to have curatively resectable cancer, experience the peritoneal seeding at the time of operation, or distant metastasis after complete resection before long [4]. These facts imply that micrometastasis is very frequent in advanced stage of GC and thus, early diagnosis and treatment of GC may contribute to the decrease of GC-related mortality. Appropriate post-operative adjuvant treatment plans are also necessary in potentially far-advanced GCs which have high risks for subsequent lymph node metastasis and distant metastasis as well as already proven far-advanced GC to

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decrease the mortality. Thus, identification of reliable markers to predict the presence of GC and its lymph node or distant metastasis may have the great clinical importance.

Recently, endoscopic examination has often been used for early detection of GC or its premalignant lesions in many countries [5]. However, endoscopy as a routine screening method for GC is somewhat limited owing to its invasive nature and high cost, whereas ideal biomarker screening must be easy to access, safe, and inexpensive. Moreover, repeated endoscopy for monitoring of treatment response or early relapse may not be available. In contrast, serum biomarkers can provide the information about cancer presence or treatment response more conveniently.

Chemokines are small, secreted proteins that act as immune modulators and chemoattractants. They have been suggested to play multiple roles in the development and progression of various tumors [6,7]. Epithelial-derived neutrophil-activating peptide-78 (ENA78), also known as C–X–C motif chemokine 5 (CXCL5), is a strong neutrophil chemoattractant [8]. Recently, it has been recognized as an important angiogenic factor in certain cancers, and considered to be associated with the development and progression of several solid tumors [9–11]. Park et al. reported that ENA78/CXCL5 was overexpressed in the late-staged GC tissues [11], which implies that ENA78/CXCL5 can be used a tissue biomarker to predict the prognosis of GC. However, tissue markers have some limitations despite their high specificity, reproducibility, and reliability, because they require invasive procedures such as endoscopy. Chemokines are secreted soluble proteins. Thus, ENA78/CXCL5 can be measured in serum, which implies that ENA78/CXCL5 can be used as a serologic biomarker for GC. However, there has been little information about the serological levels of ENA78/CXCL5 along the GC carcinogenesis. Although Park et al. showed that serum ENA78/CXCL5 levels were significantly higher in late-staged GC, number of tested serum samples was too small (9 GC patients and 10 benign conditions) [11] to achieve the reliable statistical power.

Stromal cell-derived factor (SDF-1), also known as C–X–C motif chemokine 12 (CXCL12), has been reported to be overexpressed in GC tissue, and closely associated with lymph node and distant metastasis of GC, clinically [12,13]. However, serum levels of SDF-1/CXCL12 in GC, especially according to the gastric carcinogenesis, have rarely reported.

In this study, we measured the serum levels of ENA78/CXCL5 and SDF-1/CXCL12 according to the gastric carcinogenesis (gastritis–dysplasia–carcinoma) [14], and evaluated their clinical significances in GC patients using serum samples obtained from a total of 300 subjects, which were composed of 100 training dataset and 200 following independent validation dataset. Additionally, we validated their diagnostic accuracy to predict the presence of primary GC and its distant metastasis compared with carcinoembryonic antigen (CEA), a pre-existing biomarker for gastrointestinal tumors, as both a single-marker and a part of multiple marker panels.

2. Materials and methods

2.1. Study subjects

A total of 300 subjects, who visited the gastroenterology clinics or health cancer center in the Yonsei University Health System due to their symptoms or health check-up, were enrolled. All subjects received a routine chemistry blood test and an upper gastrointestinal endoscopy (Types XQ-260, Olympus, Tokyo, Japan), and the final diagnosis was made by histological findings. All patients were diagnosed for the first time during the enrollment period, and their blood samples were collected before they received any treatment for their diseases. Subjects were classified into 4 groups according to the 'gastritis–dysplasia–carcinoma' sequence of gastric carcinogenesis [14] based on their histological diagnosis; subjects with normal gastric mucosa or simple gastritis were enrolled as normal control group, subjects with intestinal metaplasia (IM) and dysplasia as high-risk group, subjects with GC confined within submucosal (SM) layer as EGC group, and GC beyond proper muscle (PM) layer as AGC group.

For initial training dataset, the sample size was calculated to be $\geqslant$25 subjects per group using Russ Lenth's interactive power/sample size online calculator because of lack of information about the mean of tested values for each group. This sample size achieved a statistical power >80%, assuming that there were 4 comparison groups, the estimated standard deviation (SD) was 1, and the confidence level was 0.05 (one-way analysis of variance [ANOVA]). For independent validation dataset, 70 subjects were enrolled for normal control group, 30 for high-risk group, 50 for EGC group and 50 for AGC group, respectively. This sample size allowed achievement of statistical power >90% using the Number Cruncher Statistical System Power Analysis and Sample Size (NCSS PASS) program with the results for mean and SD taken from the training dataset.

TNM stage of GC was evaluated according to the 7th International Union Against Cancer (UICC)-TNM stage for GC. *Helicobacter pylori* (*H. pylori*) infection was evaluated by staining of gastric tissue with Giemsa solution (Sigma, Missouri, USA). IM change was diagnosed according to the updated Sydney classification. Histopathological differentiation for GC was diagnosed according to Lauren classification. Subjects suffered from other chronic diseases, other cancers or other gastric neoplasms were excluded. Additionally, patients received any previous treatment for GC or its premalignant lesions were also excluded. Current study was approved by the Institutional Review Board of Yonsei University Health System and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

2.2. Measurement of serum levels of CEA and chemokines

Serum CEA levels were measured by Beckman Access CEA assay (Beckman Coulter Inc. Chaska, USA). Serum levels of ENA78/CXCL5 and SDF-1/CXCL12 were measured by a commercially available MILLIPLEX MAP Human Cytokine/Chemokine Kit (Millipore, Billerica, MA, USA) using a chemiluminescent immunoassay according to the manufacturer's instructions.

2.3. Statistical analysis

IBM SPSS Statistics 20.0 (SPSS Inc, Chicago) was used for all statistical analysis. A p-value of <0.05 was considered statistically significant. All tested values were expressed as a mean with the 25–75% SD. The mean of each group was compared by the one-way ANOVA test with multiple comparisons using the post hoc Bonferroni method. An independent sample t-test was used to compare the means between cancer and non-cancer conditions. To evaluate the correlations between serum levels of tested values and clinicopathological parameters, Pearson's correlation (coefficient, γ_p) and Spearman's correlation (coefficient, γ_s) were performed. Tumor size of GC was classified into three groups: <3 cm; 3–5 cm and >5 cm in order to analyze the relationship between the tested value and primary GC size. The receiver operating characteristic (ROC) curves were generated and the area under the curves (AUC) was calculated to compare the diagnostic accuracy of each value to predict the presence of GC or the presence of distant metastasis of GC, respectively. Logistic regression analysis was performed to obtain the best sensitivity/specificity to predict the presence of GC or the presence of distant metastasis in GC as a single marker or as a multiple marker panel. Each marker was included as a linear term.