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Immunohistochemical expression and localization of cytokines/chemokines/growth factors in gastric cancer

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ABSTRACT

Our previous studies on gastric cancer tissue and patient plasma samples identified several cytokines/chemokines/growth factors to be differentially expressed, compared to normal samples. In this study our aim was to understand the localization patterns of the markers in gastric tissues. We investigated the expression of PDGFRB, CCL3, MMP3, CXCL8, CXCL10, CCL20, IGFBP3, CXCL9, SPP1, CCL18, TIMP1, CCL15, CXCL5 and CCL4 in gastric tissues using Immunohistochemistry (IHC) on Tissue Microarrays (TMA). The TMA comprised of 25 apparently normal (AN), 87 paired normal (PN) and 134 gastric cancer (T) tissues. The epithelial and stromal expression of markers and their correlation with patient characteristics and outcome were analyzed. Several of the markers [PDGFRB ($p < 0.001$), CCL3 ($p < 0.001$), MMP3 ($p < 0.001$), CXCL8 ($p < 0.001$), CXCL10 ($p < 0.001$), CCL20 ($p < 0.001$), CXCL9 ($p < 0.001$), CCL18 ($p < 0.001$), TIMP1 ($p = 0.025$), CCL15 ($p < 0.001$)] were elevated in the stromal compartment of gastric cancers compared to AN tissues, with some having intermediate levels of expression in PN tissues. Epithelial and stromal PDGFRB ($p = 0.030$, $p = 0.018$) expression was associated with diffuse type gastric cancer. Stromal IGFBP3 ($p = 0.039$), CXCL8 ($p = 0.008$), TIMP1 ($p < 0.001$), CCL4 ($p = 0.003$) and SPP1 ($p = 0.048$) expression was associated with intestinal type gastric cancer. Kaplan-Meier analysis showed higher epithelial PDGFRB ($p = 0.005$ and $p = 0.004$), CXCL8 ($p = 0.009$ and $p = 0.007$) were associated with poor disease free and overall survival. In multivariate analysis, high epithelial PDGFRB ($p = 0.036$ and $p = 0.02$) and SPP1 ($p = 0.003$ and $p < 0.001$) were independent prognostic factors for DFS and OS in patients with gastric cancer. The expression of cytokine/chemokine/growth factor markers is higher in the gastric tumor stroma compared to the normal gastric stroma and PDGFRB and SPP1 may serve as potential prognostic factors in gastric cancer.

1. Introduction

Gastric cancer is one of the most common cancers with an estimated 989,600 new cases and 738,000 deaths in 2008 [1]. In the Madras Metropolitan Ttumor Registry (MMTR) at the Cancer Institute (WIA), India, gastric cancer ranks as the second and fifth most common cancer among men and women respectively [2]. Despite the progress in conventional therapies, the five year survival for gastric cancer remains dismal in developing countries, as more than 90% of the patients are in their advanced stages of the disease at presentation [3]. This is largely due to the lack of early pathognomonic symptoms and the absence of prognostic factors associated with gastric cancer. These problems highlight the need to identify markers that can contribute to improved diagnosis and prognosis in gastric cancer.

Solid tumor tissues are an admixture of tumor cells and the cells that constitute the tumor microenvironment including stromal, immune and vascular cells [4]. A range of secreted proteins like cytokines, chemokines, growth factors, Matrix metalloproteinases (MMPs) and their inhibitors [Tissue inhibitor of metalloproteinases (TIMPs)] are involved in the communication between tumor cells and its microenvironment [5]. These communications play an important role in cancer development, progression and dissemination. Several of these molecules are aberrantly expressing during the tumorigenic process.

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In our previous study, we quantitatively assessed several cytokines/chemokines/growth factors in apparently normal (AN), paired normal (PN) and gastric cancer (T) tissue lysates and showed that they are highly expressed in gastric cancers [3]. However, since these studies were performed on whole tumor/normal tissue lysates and patient/control plasma samples, the cells of origin for these proteins in the tumor milieu could not be ascertained. To further understand their role in gastric cancer it is imperative to study the tissue level expression patterns of these markers in gastric tissues. In the present study our purpose was to analyze the expression levels of these markers in epithelial and the stromal compartments in gastric apparently normal (AN), paired normal (PN) and tumor (T) tissues using immunohistochemistry and correlate their expression with the patient clinico-pathologic variables and survival.

2. Materials and methods

2.1. Patients and clinical samples

The study was approved by the Institutional Ethical committee, Cancer Institute (WIA), a written informed consent was obtained from all the patients participating in the study for the acquisition and use of tissue samples and clinical data. Primary gastric cancer tissues, from patients who underwent surgical gastrectomy at the Cancer Institute (WIA), Chennai, between 2001 and 2008 were used in the study. Formalin fixed paraffin embedded gastric tissues were retrieved from the archived blocks of the Department of Oncopathology, Cancer Institute (WIA). The tissue microarray (TMA) used in the study included 25 apparently normal (AN), 87 paired normal (PN) and 134 gastric cancer tissues. A summary of patient characteristics are given in Table 1. The criterion for selecting AN and PN tissues was based on our previous study [3]. Briefly, AN tissues were obtained from patients with non-gastric cancers (e.g., Hypopharyngeal cancer, upper oesophageal squamous cell carcinoma, peri-ampullary carcinoma, pancreatic cancer) who underwent stomach resection as a part of their primary surgery. PN were the tumor adjacent normal gastric mucosal tissue; both AN and PN were confirmed to be devoid of any premalignant changes. The median age of the patients was 55 years (range 25–78 years). The median follow-up period for the patients in this study was 62 months (range 4–143 months). The complete clinical details of the patient samples included in the TMA are given in Supplementary Table 1.

Table 1
Gastric cancer patient characteristics.

Patient characteristics	
Age (Years)	
Median (Range)	55 (25–78)
⩽45	41 (30.6)
⩾45	93 (69.4)
Gender, n (%)	
Male	97 (72.4)
Female	37 (27.6)
Histologic grade, n (%)	
Grade II	101 (75.37)
Grade III	32 (23.88)
Neuro endocrine differentiation	1 (0.75)
Depth of invasion, n (%)	
pT1	7 (5.2)
pT2	13 (9.7)
pT3	56 (41.8)
pT4	51 (38.1)
missing	7 (5.2)
Nodal status, n (%)	
Positive	38 (28.4)
Negative	96 (71.6)
Histological subtype, n (%)	
Intestinal	81 (60.4)
Diffuse	51 (38.1)
missing	2 (1.5)

2.2. Tissue microarray construction

All gastric tissues were histologically reviewed and the corresponding H&E stained sections of each of the paraffin embedded tissue blocks were reviewed for the representative nature of the tissue samples (Normal or Tumor) before selection of the sample for arraying. 1.0 mm cores from the representative areas (tumor/normal) were carefully selected and embedded into separate virgin paraffin blocks to yield the tissue array. Four micron thick section was cut from the tissue array block and transferred on to APES coated slides. The resulting TMA was stained with H&E to confirm the representative nature of the cores.

2.3. Immunohistochemistry (IHC)

The paraffin embedded tissue sections were deparaffinized using xylene and dehydrated with two changes of ethanol and followed by thorough tap water wash. Antigen retrieval was done using wet autoclaving method by placing the tissue sections in citrate buffer (pH 6.0). Following antigen retrieval the sections were cooled to room temperature, after blocking in 2% BSA-PBS, the sections were incubated overnight in primary antibody prepared at a dilution appropriate for each of the markers in blocking buffer (2% BSA-PBS) in a humidified chamber at room temperature. The sections were stained using Super sensitive™ polymer HRP IHC detection system (Bio Genex, CA, USA) according to the manufacturer's instructions. For each antibody, a positive control and a negative control (primary antibody excluded) were used (Table 2).

2.4. Scoring of IHC

We used a semi-quantitative scoring system to assess the expression of various markers. The scoring of the tissue sections was done by SS and TR independently, in case of discordant scores, a joint review was performed. The investigators were blinded to the clinical outcome of the patients. The scoring was based on percentage of tumor cells immunoreactive (negative – 0; <25% = +1; 25–50% - +2; 51–75% + 3; >75% + 4) and intensity of immunoreactivity (negative – 0; + – 1; ++ – 2; +++ – 3). The samples were graded based on overall degree of immunoreactivity. The immunoreactive score (IRS) for the degree of immunoreactivity was obtained by multiplying the scores for the percentage of cells expressing the marker studied and the intensity of staining. Negative immunoreactivity in a sample was given a score of zero. For each of the markers, the gastric tissues were scored for immunoreactivity in the epithelial compartment (epithelial score) and the stromal compartment (stromal score). The maximal score for epithelial and stromal compartments were 12. The epithelial and stromal scores of the respective markers were dichotomized using the median scores of AN tissues. In this study, the term 'stroma' comprises all non epithelial cells and structures of the tissue microenvironment, including, fibroblasts, macrophages, leukocytes, endothelial cells and extracellular matrix. The number of cases available for scoring for each of the markers differed, as some of the tissue cores were lost during TMA construction, sectioning and the IHC experiments, a well recognized limitation of the procedure [6,7].

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