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The serum immunoglobulin G glycosylation signature of gastric cancer

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ABSTRACT

Biomarkers may facilitate detection of gastric cancer at an earlier stage and reduce mortality. Here we sought to determine if the glycosylation profile of serum immunoglobulin G (IgG) could distinguish patients with non-atrophic gastritis (NAG), duodenal ulcer (DU) and gastric cancer (GC). Serum IgG was released and analyzed using nano-LC–TOF mass spectrometry. Statistically significant false discovery rate (FDR)-adjusted *p*-values were observed for 18 glycans, eight that differed significantly between NAG and GC, three that distinguished NAG from DU, and eight that differed between DU and GC. The IgG glycosylation signature may be useful as a predictive marker for gastric cancer.

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1. Introduction

Gastric cancer (GC) is the fourth most common malignancy, but the second most common cause of cancer-related death worldwide [1–3]. In the industrialized countries, the incidence of gastric cancer has diminished dramatically over the last 50 years, but in less developed countries in Eastern Europe, Latin America and Asia, the disease remains a major cause of morbidity and mortality [4]. Pathologically, there are two histological types of gastric cancer: the diffuse- and the intestinal-type (DGC and IGC, respectively) [5]. While DGC is not well characterized, IGC is known to progress through a series of histologic stages starting with gastritis and progressing over decades to atrophy (loss of glands), intestinal metaplasia, dysplasia, and finally adenocarcinoma [6].

Helicobacter pylori is a bacterium that infects the gastric epithelium of approximately 50% of the world's population, and is designated by the World Health Organization as a Type I (definite) carcinogen. Individuals that are infected with *H. pylori* nearly always develop a non-atrophic gastritis (NAG), which in itself is largely asymptomatic, but in some cases progresses to gastric cancer. Alternatively, *H. pylori* infection may also cause duodenal ulcer (DU), but these patients usually do not develop gastric cancer [7], and so it is thought that these individuals develop a host response to *H. pylori* infection that is different from that in gastric cancer patients.

Mortality from gastric cancer is high because it produces no known specific symptoms in its early stages when it is surgically curable. If gastric cancer is detected at an early stage, the 5-year survival is approximately 90% [8], but most cases present with locally advanced or metastatic disease, which has a median survival of only 24 months and a 5-year survival of less than 15%. Therefore, early detection and preventative strategies are critical to decrease mortality from gastric cancer [3]. The identification of signature molecules for the early detection of gastric cancer would thus be highly valuable. One of the emerging fields for biomarker discovery is protein glycosylation [9]. Several studies have identified altered glycosylation patterns with varying health and disease states, such as liver cirrhosis [10], rheumatoid arthritis [11], pregnancy [12] and aging (e.g. [13–15]), but also various types of cancer (e.g. [16–20]), including gastric cancer [21]. This indicates that protein glycosylation may reflect one's balanced physiological state, and is affected by most disease states.

The majority of glycomic studies performed to date have focused on the global glycomic analysis of plasma or serum [20], including our recent studies on gastric cancer [21]. These approaches rely on the comprehensive release of glycans from proteins, but ignore any protein correlation. It cannot be determined whether altered glycosylation profiles are due to altered protein concentrations or to differential glycan expression. Both site-specific and protein-specific glycosylation information are lost. Protein-specific glycosylation analysis would provide more insight into the actual changes in glycosylation, and would allow the establishment of hypotheses regarding causes and effects of proteins with altered glycosylation. Immunoglobulin G (IgG) is the most abundant glycoprotein in plasma and serum. It is generally believed that glycans on IgG contribute significantly to the global serum glycome.

The large abundance of IgG and its role as a representative protein of the immune system makes it an ideal protein to examine the role of protein-specific glycosylation in cancer. Several recent studies have focused on observing the relationship between health states and IgG glycosylation. Altered glycosylation patterns of the Fc region of IgG have been associated with several physiological states [22], including autoimmune diseases [11,23,24], upon vaccination [25] and with aging [26–28]. However, studies of IgG glycosylation in cancer patients, and thus the role of the humoral immune response in this disease, are scarce. Recently, studies determined altered IgG glycosylation patterns with ovarian [29,30] and gastric [31] cancer. Clearly altered glycosylation patterns were observed in both studies.

Mass spectrometry is often the method of choice for glycosylation studies and our group recently introduced the use of chip-based nano-liquid chromatography with time of flight mass spectrometry (nLC–chip-TOF-MS) on a porous graphitic carbon stationary phase for the analysis of N-glycans [32,33]. This method has been shown to provide good separation, good sensitivity and was shown to be highly stable [34]. In this report, we analyzed the glycosylation pattern of IgG, taking both Fab and Fc glycosylation into account, of a sample cohort consisting of non-atrophic gastritis (NAG), duodenal ulcer (DU), intestinal-type gastric cancer (IGC) and diffuse-type gastric cancer (DGC) patients. Glycans that showed altered levels with the different disease states were identified in this pilot study.

2. Materials and methods

2.1. *Sample collection*

2.1.1. *Patients*

Human sera were obtained from the Gastroenterology Unit of the Mexico General Hospital, Secretaria de Salud and the Oncology Hospital, Instituto Mexicano del Seguro Social, both in Mexico City, from October 1999 to July 2002. All patients were at least 30 years old and presented for endoscopy because of clinical indications. The protocol was approved by the Research and Ethics Committees of each hospital and informed consent letters were signed by all study participants.

2.1.2. *Clinical and histopathology diagnosis*

Gastric biopsies were obtained systematically from six defined locations in the gastric antrum, corpus, and transitional zone and also from the location of a lesion, if one was identified during endoscopy. Biopsies from each location were formalin fixed, paraffin embedded, and stained with hematoxylin and eosin for histopathologic evaluation and classification according to the updated Sydney system by a single experienced pathologist [35]. Final diagnosis was that of the most severe histologic lesion or based on endoscopy findings in the cases of duodenal ulcer.

2.1.3. *Serology*

A 5 mL blood sample was drawn from each patient; serum was obtained and frozen at −80 °C. Serum samples were tested by