Premalignant gastric lesions- histological and immunohistochemical study

Scientific coordinator:
Prof. Univ. Dr. Laurentiu MOGOANTA

Candidate for a doctor’s degree:
Bogdan OPREA

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KEY WORDS: gastric, premalignant, histology, immunohistochemistry, metaplasia, atrophy, dysplasia.
Gastric cancer is one of the most frequent types of neoplasia, being the second death cause due to cancer in the world, after the pulmonary neoplasia.

Gastric carcinogenesis depends on many factors, incompletely known, such as genetic, alimentary, infectious or local factors. Medical literature mentions as risk factors the chronic atrophic gastritis with intestinal metaplasia, the gastric ulcer, polyps, and the infection with Helicobacter Pylori. The least seems to be the most important, being classified by O.M.S. as a class I carcinogen, because it induces chronic atrophic gastritis with intestinal metaplasia which is a premalign gastric lesion. The mechanisms which are the basis of the bacterial involvement in carcinogenesis are not yet well known. Recent molecular studies demonstrate that the bacteria infect the gastric epithelium disturbing the cellular cycle and initiating self-replication thus determining active chronic inflammation which progresses towards other premalign gastric lesions and gastric cancer.

The virulence of *H. Pylori* is influenced by multiple factors. The most important are the presence or urease (which metabolizes the urea into ammonium, which is toxic for the gastric epithelial cells mainly because of the pH changes), of flagella (which give motility to the bacteria) and of the protein products of four different genes.

**CagA** gene („cytotoxin-associated gene”) is a component of the pathogenicity island (PAI). The protein secreted by this gene induces the modification of the human gastric epithelial cells, destroying the cytoarchitecture. It accelerates neutrophiles displacement. The monocyte chemotactic protein brings the monocites in the inflammatory region, infiltrating the mucosa with inflammatory cells.

This explains the predominance of the intens inflammatory reactions in patients with CagA+ strains rather then those with CagA-. Some authors mention that the CagA+ *H. Pylori* inhibits the phagocytes, thus altering the immunologic response of the host. Patients infected with *H. Pylori* CagA+ have 12 times higher risk of developing intestinal metaplasia of the gastro-duodenal mucosa, which has a greater possibility for cancer transformation.

**VacA** gene („vacuolating- associated cytotoxin”) secretes a cyto-toxic protein which destroys the epithelial cells, forms pores in the cell membrane and increases their permeability leading to the formation of vacuoles inside the cell. The bacterial localization in the vicinity of the gastric epithelium is due to the fact that it is capable to produce adhesion molecules that bind to the bacterial wall.

*H. pylori* colonizes the stomach mucosa and determines a series of inflammatory reactions. The colonization of the mucosa is not a disease but a
state that increases the risk of appearance of different gastro-intestinal diseases. Although a number of factors probably influence an individual’s predisposition to gastric cancer and course of progression to gastric cancer, it is clear that chronic inflammation is a feature that links this cancer to many other types of malignancy.

Studies suggested that chronic gastritis was more advanced in individuals with gastric cancer than in individuals with duodenal ulceration. Investigators also recognized that areas of gastric adenocarcinoma were frequently found in areas of chronic inflammation, as well as in settings of atrophic gastritis. Earlier literature, based on studies in several countries, indicated that by the fifth decade of life, more than half the individuals sampled on a random basis had gastritis. This form of gastritis was multifocal, appearing in all areas of the stomach, and was most prevalent in the same population that had an increased risk of developing gastric cancer.

It is now known that *H. pylori*, which infects 50% of the world’s population, is a major factor in both the induction of atrophic gastritis and histological progression to gastric cancer. Less is known about the histological changes that occur during the progression to diffuse-type gastric cancer than about those that occur during the progression to intestinal-type gastric cancer, which evolves through a series of discrete steps known as the Correa pathway. However, most gastric pathologists define atrophic gastritis as the loss of specialized glandular tissue, for example, loss of the oxyntic glands (which contain parietal cells) in the gastric corpus. By contrast, intestinal metaplasia is defined as replacement of original gastric glands with straight tubular crypts lined by alternating absorptive and goblet cells and accompanied by inflammatory infiltrates in the lamina propria. The development of gastric atrophy was recognized as a critical step in the Correa pathway to intestinal-type gastric cancer, and accumulating evidence indicates that gastric atrophy is much more consistently associated with gastric cancer than is intestinal metaplasia. Gastric atrophy therefore appears to be a better indicator of gastric cancer risk than is intestinal metaplasia. Detailed mapping studies of resected stomachs from patients with intestinal-type gastric cancer have shown that atrophic gastritis, but not intestinal metaplasia, is present in every case. Atrophy is generally present as either a multifocal or a diffuse pattern in gastric tissue and is, by definition, associated with the presence of a form of mucous metaplasia that has been termed pseudopyloric metaplasia, also known as spasmolytic polypeptide-expressing metaplasia (SPEM). SPEM is strongly associated with gastric cancer than is intestinal metaplasia and might be the precursor to the cancerous lesion.
OWN CONTRIBUTION

Material and Methods.

The study was performed on a set of 96 stomach samples harvested by biopsy or surgical resection from patients admitted between 2006 and 2008 in the Surgery or Gastroenterology Clinic of the Emergency Regional Hospital of Craiova. All patients had specific digestive symptoms.

The samples were fixated in 10% buffered formalin solution and paraffin-wax embedded using the classical technique. Some 5μm thick samples were obtained using the microtome Microm HM325 which were mounted on histological slides and hematoxylin eosin stained for the histological study.

The immunohistochemical study was performed using the same samples mounted on poly-L-lysine slides, for the following primary antibodies:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Code</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti TAG-72</td>
<td>0.N.561A</td>
<td>T0710-07 USBiological</td>
<td>Citrate buffer pH 6, 11 minutes in the microwave oven (600W)</td>
<td>1:1000</td>
</tr>
<tr>
<td>Anti PCNA</td>
<td>PC10</td>
<td>M 0879 Dako</td>
<td>Citrate buffer pH 6, 11 minutes in the microwave oven (600W)</td>
<td>1:200</td>
</tr>
<tr>
<td>Anti-E-cadherina</td>
<td>NCH-38d</td>
<td>M 3612 DAKO</td>
<td>Citrate buffer pH 6, 11 minutes in the microwave oven (600W)</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti CD34</td>
<td>QBEnd-10</td>
<td>M 7165 Dako</td>
<td>Citrate buffer pH 6, 11 minutes in the microwave oven (600W)</td>
<td>1:50</td>
</tr>
<tr>
<td>Anti factor VIII</td>
<td>F8/86</td>
<td>M 0616</td>
<td>Citrate buffer pH 6, 11 minutes in the microwave oven (600W)</td>
<td>1:50</td>
</tr>
<tr>
<td>Anti Helicobacter Pylori</td>
<td>policlonal Ab7788</td>
<td>Citrate buffer pH 6, 11 minutes in the microwave oven (600W)</td>
<td>1:2000</td>
<td></td>
</tr>
</tbody>
</table>

For the detection we used the Dako EnVision system and the DAB ((3-3' diaminobenzidine tetrahydrochloride) chromogen.

Results and discussions

In most cases, chronic atrophic gastritis was characterized by chronic inflammation and the decrease of gastric glands number as well as by the association of other gastric lesions such as intestinal metaplasia (10 samples) or dysplasia (7 samples).
Mature and immature intestinal metaplasia was observed in 11 cases and was characterized by progressive replacement of the gastric epithelium with an intestinal one, including goblet cells, Paneth cells ciliated cells and different endocrine cells.

Mature type intestinal metaplasia, Col H-E, x400

The epithelial dysplasia was observed in 9 samples, 6 of which were low grade dysplasia. The affected glands were characterized by pseudo-stratification, loss of polarity, elongated and hyper-cromatic nuclei and the presence of intracytoplasmatic mucus.

High grade dysplasia and chronic inflammatory infiltrate, Col. H-E, x200
The immunohistochemical study concluded that high TAG-72 reactivity, low E-Cadherine immunoreaction, along with increases vascular density and intense PCNA reaction could be a pattern for gastric premalignant lesions monitorization. For the blood vessels identification we used a antibody cocktail formed of anti CD34 and Anti FVIII, in order to observe all the vessels (even the smaller ones).

Gastric carcinogenesis is a multi factorial process depending on genetic factors, dietary or infectious ones along with local host dependent factors. Among the host dependent factors the premalignant gastric lesions and the Helicobacter Pylori infection are the most important risk factors.

TAG-72 antigen may be expressed in malignant and dysplastic epithelial cells, as well as in intestinalized epithelium of the stomach which has been closely related to subsequent carcinoma development. Hence, MAb B72.3 may be a useful immunohistochemical adjunct for detecting early foci of adenocarcinomas and premalignant lesions of the stomach.
CURRICULUM VITAE

NAME: Bogdan Oprea
BIRTH DATE: May 28, 1980
CURRENT AFFILIATION: University of Medicine and Pharmacy Craiova, Faculty of Medicine, Histology Department

I graduated the Faculty of Medicine, University of Medicine and Pharmacy of Craiova in 2005. I am a candidate for a doctor’s degree since November 2005 under the coordination of Prof. Univ. Dr. Laurentiu Mogoanta. Thesis title: Premalign gastric lesions- histological and immunohistochemical study. During my doctoral preparation I graduated the doctoral school’s courses and attended a training stage at University Ernst-Moritz-Arndt of Greifswald, Germany in 2006 in immunohistochemistry and real-time PCR domains. Beginning with 2007 I teach Histology to the II\textsuperscript{nd} year medical students. Beginning 2006 until 2009 I was a resident doctor in Family Medicine speciality. Now I am a resident doctor in Radiology and Medical Imagistics speciality and I teach histology and research methodology to medical students.

Working domains:
- tissue immuno-reactivity research
- experimental studies on animal models
- microscopic analysis and computer processing of the obtained images
- beginning 2007 I am part of the editorial board of “Romanian Journal of Morphology and Embriology” as technical secretary.

Grants as project manager and research team member (financial support for this research)
1. Premalign gastric lesions- histological and immunohistochemical study, Project Manager, code CNCSIS TD-515, 2007 competition, finalized
2. Endoscopic, histologic, immunohistochemical and immunologic monitoring of premalign gastric lesions and the characterization of the initial stages of cancer invasion, Project manager: Prof. Univ. Dr. Laurenţiu Mogoantă, CEEX 2006, code 5270.- member, finalized

Papers published regarding this thesis research domain:


B. Oprea, Rodica Dîrnu, G. Cojocaru, D. Mălăescu, L. Mogoanta, New Data Regarding the Colonization of the Gastric Mucosa by Helicobacter Pylori, Analele Universității Constantin Brâncuși, accept de publicare