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ORIGINAL ARTICLE

The role of E-cadherin and Runx3 in Helicobacter Pylori – Associated gastric carcinoma is achieved through regulating P21waf and P27 expression

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Abstract

Background: We assessed the role of E-cadherin (CDH1), runt-related transcription factor 3, p21waf and p27 promoter methylation (PM) and protein expression in Helicobacter pylori (HP)-associated gastric carcinomas (GCs) and adjacent non-neoplastic tissues (ANNTs).

Patients and methods: 192 cases were assessed for PM and protein expression of CDH1, RUNX3, p21waf and p27 by methylation-specific PCR (MSP) and immunohistochemistry. The CagA gene was also assessed.

Results: In GCs, 66 (34.4%) and 84 (43.8%) cases showed CDH1-PM and reduced expression. It is significantly affected in GCs rather than in non-neoplastic groups ($p < 0.001$). In ANNTs, 108 (56.3%) cases showed CDH1-PM and all cases revealed preserved protein expression. *RUNX3*-PM was detected in 78 GCs (40.6%) and 69 ANNTs (35.9%), whereas reduced protein expression was detected in 99 (51.65%) GC compared to ANNTs 90 (46.9%). p21WAF and p27 showed PM in (48.4% and 45.3%) GCs and ANNTs; respectively. p21waf protein was reduced in 90 (46.9%) cases and 91 ANNTs (47.4%). p27 was reduced in 86 (44.8%) cases and 87 ANNTs (45.3%). CDH1 aberrations correlated with HP in tumors and ANNTs and with diffuse/intestinal tumors ($p = 0.014$, $p = 0.014$ and $p = 0.02$). RUNX3 aberrations associated with HP ($p = 0.04$), high grade ($p = 0.04$), and advanced stage ($p = 032$). Tumor grade associated with RUNX3-PM, CDH, p21 and p27 protein ($p < 0.05$ for all). Tumor stage associated significantly with PM and reduced protein expression of all markers. Positive lymph nodes associated significantly with p27PM ($p < 0.001$).

Conclusions: HP plays an important role in the development and progression of GC through silencing of CDH1, RUNX3, p21WAF and p27 expression.

Keywords Gastric carcinoma, Prognosis, CDH1, RUNX3, p21, p27.

Background

Gastric Carcinoma (GC) is the third most fatal cancer worldwide after lung and hepatocellular carcinoma (HCC). It represents 6.8% of the total cancer cases worldwide and 1.6% of all diagnosed cancer cases in Egypt [1,2] Epidemiological studies show that Helicobacter pylori (HP) infection is a major

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risk factor for GC and its precursor lesions (chronic atrophic gastritis, intestinal metaplasia and dysplasia).Thus, the risk of developing GC is estimated to increase 2–6 times in patients with HP infection**.** The prevalence of HP ranges from 25% in the developed countries to more than 90% in developing regions. HP infection can progress to GC by a process influenced by bacterial virulence [3].

The *CagA* antigen, an HP virulence factor, its gene is found on a genomic region called the *cag* pathogenicity island (PAI). Individuals with *CagA*-positive HP strains usually have a higher risk of peptic ulcers and gastric cancer compared to those harboring *CagA*-negative strains [3]. It has been shown that GC developed through a stepwise progression of several genetic and epigenetic events including *p53* mutations, disruption of E-cadherin/B-catenin-containing adherent's junctions and up-regulation of the anti-apoptotic genes [4].

The E-cadherin (CDH1) gene, located on chromosome 16 encodes a trans-membrane glycoprotein which is responsible for cell to cell adhesion [5]. Inactivation of the CDH1 was reported in 17–51% of GC cases, especially the diffuse type and therefore, CDH1 mutations seem to play a major role in the development and progression of GC. Although the exact mechanism(s) of CDH1 gene inactivation in HP positive GC cases are not fully elucidated yet, some studies demonstrated that reduced CDH1 expression in GC is usually achieved through hypermethylation of the CDH1 gene (PM), which is initiated by HP infection [6].

The Runx3 protein is a member of the human runt-related transcription factors family (RUNX1, RUNX2 and RUNX3). It is located on chromosome 21q22.3, a region which is frequently deleted in several tumor types [4,7]**.** It is involved in the *TGF-β* signal transduction pathway. RUNX3 guides the *TGF-β 1/Smad* signaling pathway and promote apoptosis in normal cells [4]. In addition, RUNX3 acts as a tumor suppressor through regulating a series of cancer-related genes, including p53, cyclin-dependent kinase inhibitor-1A (p21), cyclin-dependent kinase inhibitor-1B (p27), Notch-1, and Caspase-3**.** Loss of RUNX3 contributes to hyperplasia and intestinal metaplasia of the gastric mucosal epithelial cells in animal models [8]. Reduced expression is induced by PM, loss of heterozygosity (LOH), hemizygous deletion or mutations [4]. Recent studies demonstrate that 45–60% of surgically assessed GCs do not express RUNX3 protein [4].

In the current study we assessed the role CDH1, RUNX3, $p21^{waf}$ and p27 genes aberrations (PM and protein expressions) in the development and progression of HP-associated GC cases from Egypt.

Patients and methods

(1) *Patients:* we retrospectively assessed 192 GC patients who were diagnosed and treated at the surgery departments of: (1) the national cancer institute (NCI), Cairo University; (2) Al-Demerdash hospital, Ain Shams University and (3) El-Matareya Teaching Hospital during the period from January 2010 to December 2013. Cases were diagnosed histologically as GC according to Lauran classification and the WHO criteria 2010 and staged according to the TNM classification AJCC, 2017 [9]. All patients had resectable tumors ($T_{1,2}$, $N_{0,1}$, M_0) and accordingly, they underwent partial, subtotal or total gastrectomies with lymph node dissection. Resected surgical specimens of all tumors with safety margins from the adjacent non-neoplastic tissues (ANNTs) were histologically examined. The study included: 192 GC cases [114 (59.37%) in the pylorus and antrum, 30 (15.6%) in the body and 48 (25%) in the fundus]. In addition, 192 tissue samples were obtained from the adjacent non neoplastic tissues (ANNTs) of patients undergoing surgical procedures in the stomach for benign lesions. Sixty normal gastric mucosal tissues (NGMTs) were also included as a control. The relevant clinico-pathological features of all studied patients are illustrated in Table 1. The ethical committees of the faculty of medicine-Ain Shams University and the NCI, Cairo University approved the study protocol, which was in accordance with the 2011 declaration of Helsinki.

Table 1. Relevant Clinico-pathological features of the 192 gastric carcinoma patients.

Variable	Number (%)
Gender	
Male	126 (65.6%)
Female	66 (34.4%)
Age (years)	
≤50	72 (37.5%)
>50	120 (62.5%)
Mean ± SD	53.2 ± 14.1
Tumor site	
Pylorus and antrum	114 (60%)
Body	30 (15%)
Fundus	48 (25%)
Tumor size	
<4 cm	99 (51.5%)
≥4 cm	93 (48.4%)
Histological type	
Diffuse	66 (34.5%)
Intestinal	126 (65.5%)
Grade	
G 1	48 (18.75%)
G 2	84 (43.75%)
G 3	60 (3.12%)
Pathological T stage	
T1	0(0.0%)
T2	72 (37.5%)
T 3	84 (43.7%)
T4	36 (18.8%)
Pathological N stage	
N0	60 (31.3%)
N1	132 (68.7%)
***H. pylori*-ANNT**	
Negative	84 (43.75%)
Positive	108 ((56.25%))

From each tumor, ANNT and NGMT samples, five micron-thick sections were cut onto positive charged slides (6 slides): one slide was stained with hematoxylin and eosin to confirm the diagnosis. The second slide was stained with Giemsa to detect the presence of HP, and the other four slides were used to assess the expression levels of CDH1, RUNX3, p21waf