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Full Length Article

Expression of cyclooxygenase 2 and vascular endothelial growth factor in gastric carcinoma: Relationship with clinicopathological parameters

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KEYWORDS

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Abstract *Background:* Gastric cancer is one of the most common cancers and the second most common cause of cancer-related death worldwide. Identification of specific prognostic indicators might allow a better prognostic stratification and more effective therapy.
Aim: To assess the expression and relationship between COX-2 and VEGF protein in gastric adenocarcinoma and whether these markers are useful in predicting clinicopathological prognostic parameters.
Materials and methods: The study included 83 formalin-fixed paraffin embedded tissue samples of excised gastric adenocarcinoma and 20 non tumorous tissue controls. The slides were subjected to COX-2 and VEGF immunohistochemical staining using a streptavidin–biotinperoxidase according to the manufacturer's protocol. The results were assessed independently by two pathologists. The relationships among COX-2 and VEGF expression and clinicopathological parameters were statistically analyzed.
Results: COX-2 and VEGF expressions were obviously higher in carcinoma tissues compared to normal mucosae ($p < 0.001$). The expression rate of COX-2 was 54.2% and of VEGF was 68.7%. COX-2 positive tumors were significantly correlated with Lauren classification, tumor depth and *Helicobacter pylori* infection ($p < 0.001$, $p = 0.008$, $p = 0.035$). VEGF was significantly associated with lymph node metastasis and tumor depth ($p < 0.001$). There was a positive association between VEGF and COX-2 expression in gastric adenocarcinoma (Kappa value = 0.55).
Conclusion: In gastric adenocarcinoma, COX-2 expression might serve as a powerful indicator for intestinal type carcinoma, locally advanced disease and *H. pylori* infection, while VEGF was related to loco-regional progression. COX-2 might be involved in the development of angiogenesis in gastric carcinoma through VEGF upregulation.

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Introduction

Gastric cancer (GC) is one of the most common cancers worldwide with a relative frequency of 7.8% of all cancers [1,2]. More than 90% of gastric cancers are adenocarcinomas. In the latter half of the twentieth century, GC was the second most common cause of cancer-related deaths after lung carcinoma accounting to 11.3% of all cancer deaths [3]. In Egypt, GC represented 1.6% of all cancers and 2.2% of all cancer mortality [4]. According to the registry of the Egyptian National Cancer Institute, GC formed 2.12% of total malignancy and 10.3% of gastrointestinal cancers [5].

Despite advances in diagnosis and treatment, the prognosis of patients with GC has remained unsatisfactory. Nearly one-third of the patients (29.9%) experienced recurrence after gastric surgery. It is the main cause of cancer related death [6]. One major difficulty in the therapy of GC is the presence of only few prognostic indicators that can predict its clinical behavior. Therefore, identification of other specific prognostic markers might allow a better prognostic stratification and thus more effective therapy [3].

Cyclooxygenase (COX) is a key enzyme in prostaglandin synthesis from arachidonic acid. There are two enzyme forms, COX-1 isoform, a component of the normal cells that has been connected to physiological functions, and the COX-2 isoenzyme that is frequently undetectable in most normal tissues [6]. Overexpression of COX-2 protein has been detected in some tumors including GC. It was reported that its overexpression is associated with poor prognosis and reduced survival [7]. The mechanism by which COX-2 induces carcinogenesis is not known until now. COX-2 enzyme may stimulate cell proliferation, inhibit apoptosis, increase invasiveness and induce angiogenesis by elaborating some angiogenic factors such as vascular endothelial growth factor (VEGF) [8].

Angiogenesis plays a critical role in tumor progression and metastasis. In the vast majority of malignancies, including gastrointestinal neoplasms, angiogenesis has been associated with poor prognosis and relapse of the tumor. The best known and the most efficient angiogenic growth factor is VEGF [9]. VEGF is known to accelerate endothelial cellular proliferation, vascular permeability, and endothelial cell migration, and inhibit apoptosis, whereas inhibition of VEGF results in suppression of tumor growth [10].

The aim of the current study is to assess: COX-2 and VEGF immunohistochemical (IHC) expression in GC cases, whether these markers are useful in predicting clinicopathological prognostic parameters and whether there is an association between the expression of COX-2 and VEGF.

Materials and methods

The present retrospective study included 83 patients with histopathologically proven gastric adenocarcinoma who underwent curative surgical resections that were retrieved from the files of Pathology Department, National Cancer Institute, Cairo University between June 2008 and December 2014. The control group included 20 cases with non neoplastic gastric tissues that underwent endoscopic biopsy during the same period. All specimens were taken from the archives of the Pathology Department.

Clinicpathological data including age, sex, location, histological type, grade, depth of invasion, nodal status and *Helicobacter pylori* (*H. pylori*) infection in the non neoplastic adjacent mucosa were determined from the pathology reports. The eligibility criteria included: histopathologically proven gastric adenocarcinoma classified according to the World Health Organization classification [11], no neoadjuvant chemotherapy and/or radiotherapy and availability of complete clinicalpathological data.

Immunohistochemical method

The archival histopathological slides of all studied cases were reviewed to confirm the diagnosis, to detect *H. pylori* in the adjacent mucosa in some cases and to choose the appropriate paraffin embedded tissue blocks for sectioning and IHC staining. For each case; two serial sections, of 4 μm thickness were cut by the microtome then mounted onto positively charged slides.

The slides were subjected to IHC staining using a streptavidin–biotin-peroxidase according to the manufacturer's protocol using BenchMark XT automated slide stainer (a product of Ventana Medical Systems). All sections were deparaffinized by xylene, rehydrated by a graded series of ethanol, and treated with 0.3% H_2O_2 for 5 min at room temperature to block endogenous peroxidase activity. Heat-based antigen retrieval was performed to obtain optimal results. Sections were treated with 5% bovine serum albumin to block non-specific staining. The slides were incubated with the primary antibody, anti-COX-2 antibody (monoclonal rabbit anti-human, clone SP2, in a dilution of 1:100, Thermo Scientific, USA) and anti-human VEGF antibody (monoclonal mouse, clone VG1, M7273, DakoCytomation, Denmark, at a 1:50 dilution). Diaminobenzidine was used as a chromogen and hematoxylin as a counterstain.

Appropriate positive and negative controls were included in each IHC run. Negative controls were prepared by replacing the primary antibody with Phosphate Buffered Saline (PBS). Positive staining controls for COX-2 included sections of colonic carcinoma. Positive staining controls for VEGF included sections of hemangioma tissue

Evaluation of immunohistochemical staining

The expression of COX-2 and VEGF were assessed independently by two pathologists who were blinded to the clinicopathological parameters of the patients. COX-2 and VEGF immunoreactivity was detected in the cytoplasm of the cells. The IHC score was calculated by adding the percentage of positively stained cells to the staining intensity. The percentage of positive cells ranged between 0 and 3, i.e. 0, if less than 10% of tumor cells were stained; 1, if 10–25% of tumor cells were stained; 2, if 25–50% were positive; and 3, if $>50\%$ were positive. The staining intensity was scored as: 0, negative immunoreaction; 1, weak intensity; 2, moderate intensity; and 3, strong intensity. The sum of the two parameters varied between 0 and 6. In our study, we considered: a negative immunoreaction (−), for scores between 0 and 2; a weakly positive immunoreaction (+), for scores 3 and 4; a strongly positive immunoreaction (++), for scores 5 and 6. Cases with scores equal to or higher than 3, were considered as positive [8,12].