World Journal of Gastroenterology

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DOI: 10.3748/wjg.v2.i2.82

World J Gastroenterol 1996 June 25; 2(2): 82-85
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Measurements of cell proliferation in esophageal and gastric cardia epithelia of subjects in a high incidence area for esophageal cancer

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Author contributions: All authors contributed equally to the work.

Supported by The Foundation of Chinese Education Commission and Scientific Foundation for Excellent Young Scientists in Henan.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

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Received: April 29, 1996
Revised: May 25, 1996
Accepted: June 10, 1996
Published online: June 25, 1996

Abstract

AIM: To determine the proliferation patterns of normal and different precancerous lesions of esophageal and gastric cardia epithelia by measuring levels of proliferating cell nuclear antigen (PCNA), Ki-67 and bromodeoxyuridine (BudR) incorporation.

METHODS: One-hundred-and-seventy-five esophageal biopsies and 45 gastric cardia biopsies were collected from symptom-free subjects in Huixian. Of these, 24 esophageal biopsies were incubated with BudR. The avidin-biotin-peroxidase complex (ABC) method was used to detect PCNA, Ki-67 and BudR. Quantitative data of the immunostaining results were recorded as the number of positive cells per mm^2 of the biopsied epithelium.

RESULTS: Intense immunostaining for PCNA, Ki-67 and BudR was observed in the cell nuclei of normal tissues and of tissues with different severities of precancerous lesions. For esophageal biopsies, the numbers of both PCNA and Ki-67 increased significantly as the epithelia progressed from normal to basal cell hyperplasia (BCH) and to dysplasia (DYS). The number of PCNA- and Ki-67-positive cells was three times higher than that of BudR in the same category of BCH. For cardia biopsies, the number of Ki-67-positive cells was lower in normal tissues and increased significantly from chronic superficial gastritis (CSG) to chronic atrophic gastritis (CAG) and to DYS.

CONCLUSION: The staining patterns for PCNA and Ki-67 were correlated with the histopathology of the esophagus and gastric cardia. These methods may be useful for screening subjects at high risk for esophageal and gastric cardia cancers and for monitoring the effect of chemoprevention. PCNA is relatively easy to analyze and may prove to be very useful in studies on esophageal cancer.

Key words: Esophageal neoplasms/pathology; Epithelial cells; Stomach neoplasms/pathology; Precancerous conditions

Wang LD, Zhou Q, Gao SS, Li YX, Yang WC. Measurements of cell proliferation in esophageal and gastric cardia epithelia of subjects in a high incidence area for esophageal cancer. *World J Gastroenterol* 1996; 2(2): 82-85 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i2/82.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i2.82

INTRODUCTION

Carcinoma of the esophagus (EC)[1] is a widely occurring disease in Linxian and Huixian of Henan Province in northern China and remains a leading cause of cancer-related deaths[2,3]. Because of poor prognosis of this disease, early diagnosis and prevention are of great importance. An early indicator of abnormality in persons predisposed to EC is the increased proliferation of esophageal epithelial cells, morphologically manifested as basal cell hyperplasia (BCH) and dysplasia (DYS)[1,4-6]. Gastric cardia adenocarcinoma (AC) seems to occur together with EC in many high incidence areas in China and other countries[7,8]. AC of the gastric cardia is an under-studied subject. The pathogenesis and cell proliferation of this disease have not been well characterized. There is evidence, however, that cardiac AC differs from cancer of the rest of the stomach in terms of time trend, risk factors and histopathogenesis[9-11].

Measurements of cell proliferation are being increasingly used to assess the effects of cancer prevention interventions[12-15]. Tritiated thymidine labeling of S phase cells is an established method for identifying proliferating patterns in tissue sections. Using this labeling technique, we found that the esophageal BCH and DYS had higher labeling indices than normal epithelium. These precancerous lesions also showed expansion of proliferating zones toward the

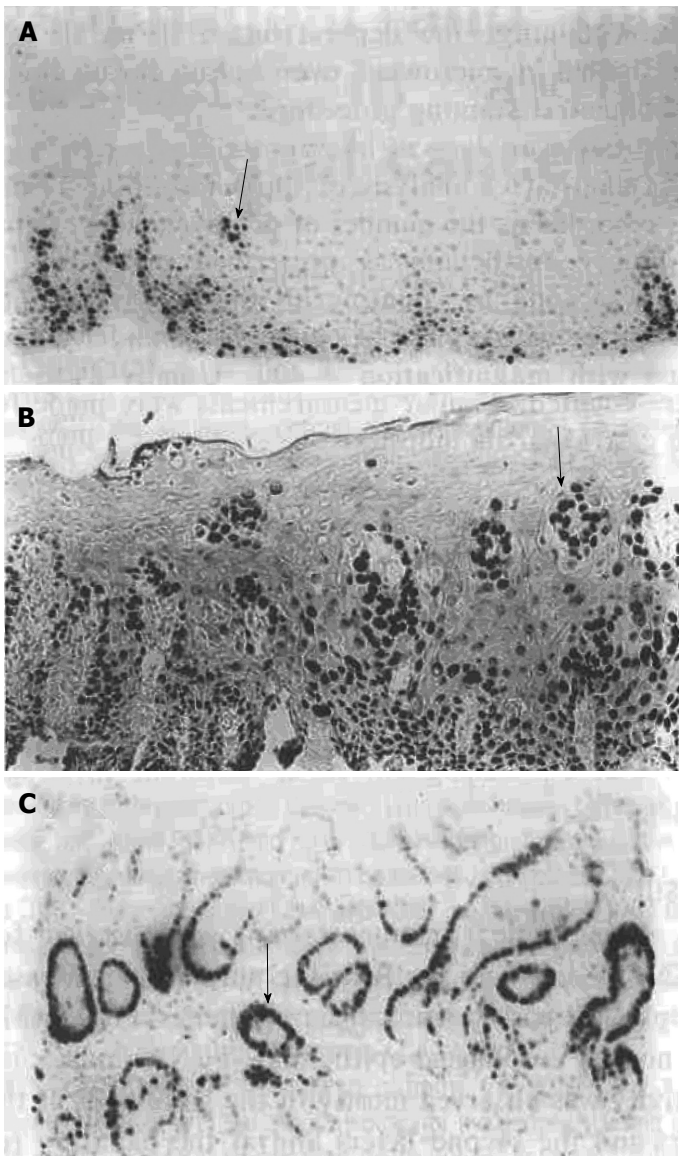

Figure 1 Immunostaining of Proliferating cell nuclear antigen in biopsied samples of esophageal and gastric cardia epithelia. Immunoreactivity is located in the nuclei of basal cells in the papillary region of the normal epithelia of esophagus (A, arrows) and the positive cells expand in the upper region of DYS (B). C: PCNA staining in gastric cardia epithelium with CSG (× 100). PCNA: Proliferating cell nuclear antigen; DYS: Dysplasia; CSG: Chronic superficial gastritis.

esophageal surface[3]. Recently, immunohistochemical identification of cell cycle-related proteins has been used for the assessment of cellular proliferation. The markers used include the proliferating cell nuclear antigen (PCNA), Ki-67 and bromodeoxyuridine (BudR). PCNA is a 36-kDa nuclear protein identified as an auxiliary protein of DNA polymerase δ. Its synthesis is sharply increased in late G1 phase and also increased during S phase, but is declined throughout G2 and M phases[16]. Ki-67, a nuclear antigen, is present in G1, S and G2 phases of the cell cycle[17]. BudR, a pyrimidine analogue of thymidine, is incorporated into DNA during cell replication in S phase and can be detected by anti-BudR antibody[18]. In the present study, we used PCNA, Ki-67 and BudR incorporation labeling index as parameters to characterize the cell proliferation patterns of human esophageal and gastric cardia epithelia with normal and different severities of precancerous lesions from symptom-free individuals in Henan, China.

MATERIALS AND METHODS

Tissue collection and processing

One-hundred-and-seventy-five esophageal biopsies and 41 gastric cardia biopsies were collected from symptom-free subjects in Huixian of Henan Province, China. Of the 175 esophageal biopsies, 24 were immediately incubated with BudR (1.5 mg/100 mL; Sigma) in a 95% basal medium with 10% fetal calf serum, with shaking at 37 ℃ for 1 h. All tissues were fixed with 80% alcohol, embedded in paraffin, and serially sectioned at 5 μm. The sections were mounted onto Histostick-coated slides. Three or 4 adjacent ribbons were collected for histopathological analysis (hematoxylin and eosin (H & E) staining) and immunohistochemical analysis.

Histopathological analysis

Histopathological diagnoses of esophageal epithelia were made according to the previously established criteria[7]. The normal esophageal epithelium contained 1-3 layers of basal cells; the papillae were confined to the lower half of the epithelium. In BCH, the number of proliferating basal cells was increased to more than 3 cell layers. DYS was characterized by partial loss of cell polarity and nuclear atypia. The following histopathological classifications were used for the gastric cardia epithelia: chronic superficial gastritis (CSG; inflammation manifested as a mild lymphocyte and plasma cell infiltration in biopsies from the gastric cardia); chronic atrophic gastritis (CAG; glandular morphology disappeared partially or completely in the mucosa and replaced by connective tissues; interglandular space infiltrated mainly by plasma cells and lymphocytes); and DYS (neoplastic features including nuclear atypia and/or architectural abnormalities confined to the gastric epithelium, without invasion)[19].

Immunohistochemical analysis

The avidin-biotin-peroxidase complex (ABC) method was used for PCNA, Ki-67 and BudR antigen detection. Briefly, after dewaxing, inactivating endogenous peroxidase activity, and blocking cross-reactivity with normal serum, the sections were incubated overnight at 4 ℃ with a diluted solution of the primary antibodies (1:200 for PCNA and Ki-67; 1:30 for BudR). Localization of the primary antibodies was achieved by subsequent application of a biotinylated anti-primary antibody, an avidin-biotin complex conjugated to horseradish peroxidase, and diaminobenzidine (Vectastain Elite Kit). Normal serum blocking and omission of the primary antibody were used as negative controls. Counterstaining with H & E was used only for BudR. For Ki-67 immunostaining, the deparaffinized tissue sections were boiled in a microwave oven before the immunohistochemical staining procedure.

Quantitative analysis of immunostaining results

Quantitative data of immunostaining results were recorded as the number of positive cells per mm^2 of biopsied epithelium, as described previously[20]. This was done by counting all the positive stained cells in the whole biopsied tissue sample under microscope with magnification of × 400. Usually, 24 fields were counted. Similar measurements were made for the gastric cardia specimens.

Statistical analysis

The $\bar{x} \pm s_{\bar{x}}$ of the PCNA, Ki-67 and BudR positive-immunostained cell number/mm^2 in the esophageal and gastric cardia specimens in each histologic category were calculated using univariate analysis. The ANOVA test followed by Fisher's PLSD test was used to assess the significance of differences ($P < 0.05$) among values of different histologic parameters.

RESULTS

Clear nuclear immunostaining was observed for PCNA, Ki-67 and BudR in the normal and diseased esophageal and gastric cardia epithelia (Figures 1-3). In normal esophageal epithelium, PCNA immunoreactivity was observed mostly in the basal cells at the first and second layers and at the papillary regions (Figure 1A). As the esophageal tissue progressed from normal to BCH and DYS, the PCNA positive immunostaining cells increased significantly in number ($P < 0.001$) and expanded to the upper part of the epithelium (Table 1, Figure 1B). A similar pattern of immunoreactivity was observed for Ki-67 (Figure 2). In samples with BCH and DYS, the number of PCNA positive cells appeared to be larger than that of Ki-67. The number of BudR-labeled cells was similar to that of PCNA and Ki-67 in the normal epithelium and appeared to be higher in DYS. However, the numbers of both PCNA and Ki-67 positive immunostaining cells were much larger (3.5-times) than that of BudR in the category of BCH (Table 1). In the gastric cardia, the number of PCNA-positive cells was smaller in

Table 1 Cell proliferation of esophageal and gastric cardia epithelia measured by proliferating cell nuclear antigen, Ki-67 and BudR in symptom-free subjects from high incidence area of northern China

Histological type	PCNA		Ki-67		BudR	
	No. of biopsies examined	Positive cells/mm^2 ($\bar{x} \pm s_{\bar{x}}$)	No. of biopsies examined	Positive cells/mm^2 ($\bar{x} \pm s_{\bar{x}}$)	No. of biopsies examined	Positive cells/mm^2 ($\bar{x} \pm s_{\bar{x}}$)
Esophagus						
Normal	18	144 ± 16	16	145 ± 20	3	112 ± 56
BCH	136	338 ± 21[b]	96	308 ± 25[b]	19	103 ± 9
DYS	21	928 ± 157[b]	15	773 ± 229[b]	1	425
Gastric cardia						
Normal	9	290 ± 43	6	180 ± 41	-[1]	-
CSG	17	356 ± 78	12	195 ± 32	-	-
CAG	11	382 ± 85	6	376 ± 134[a]	-	-
DYS	4	633 ± 111	2	620 ± 143[a]	-	-

[a]Significantly different from adjacent low-grade lesions; $P < 0.05$ by ANOVA test. [b]Significantly different from adjacent low-grade lesions; $P < 0.001$ by ANOVA test. [1]Not applied for BudR incorporation. PCNA: Proliferating cell nuclear antigen; BCH: Basal cell hyperplasia; DYS: Dysplasia; CSG: Chronic superficial gastritis; CAG: Chronic atrophic gastritis.

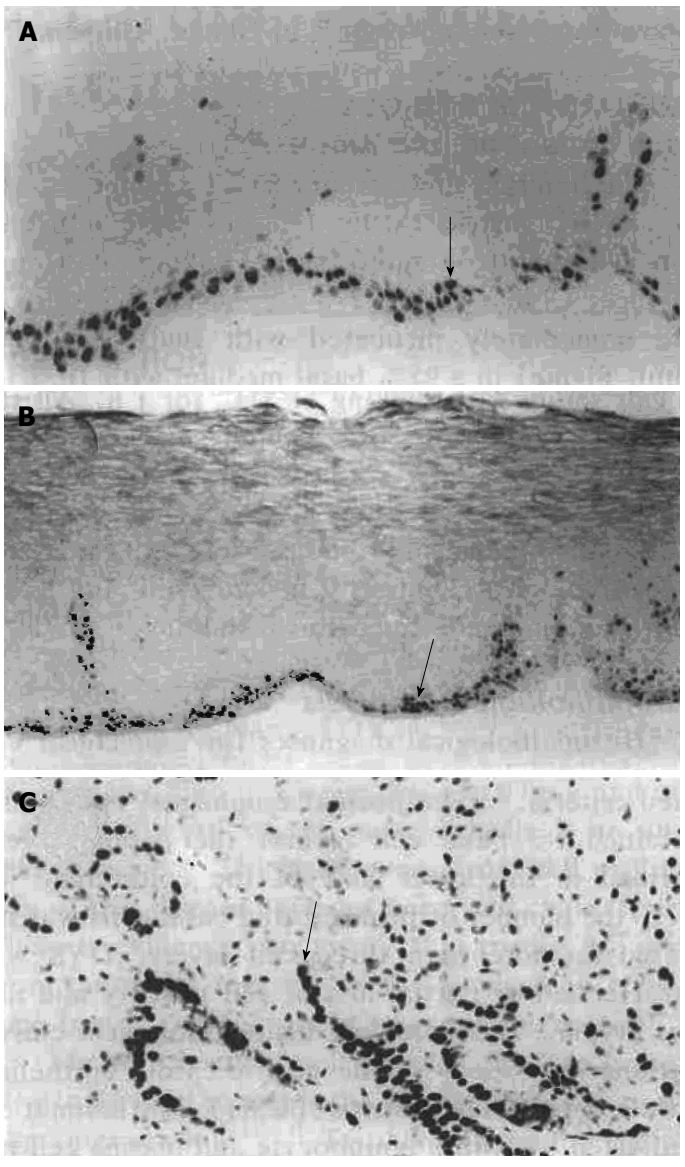

Figure 2 Immunostaining of Ki-67 in biopsied samples of esophageal and gastric cardia epithelia. Immunoreactivity is located in the nuclei of basal cells in the papillary region of the normal epithelia of esophagus (A, arrows) and the positive cells expand in the upper region of DYS (B). C: Ki-67 staining in gastric cardia epithelium with CSG (× 100). Immunoreactivity is located in the nuclei of cells at the basal cells of the crypts and at the deep glands (arrows). DYS: Dysplasia.

the normal tissues and slightly increased as the epithelia progressed from normal to CSG, CAG and DYS (Table 1, Figure 1C), but the difference was not significant. In normal gastric cardia epithelium, Ki-67 immunoreactivity was observed mostly in the basal cells of the crypt (Figure 2). As the gastric cardia tissue progressed from normal to CSG, CAG and DYS, the Ki-67 positive immunostaining cells increased significantly in number ($P < 0.05$) and expanded to the upper part of the crypt (Figure 2).

DISCUSSION

In the present study, the staining patterns for PCNA and Ki-67

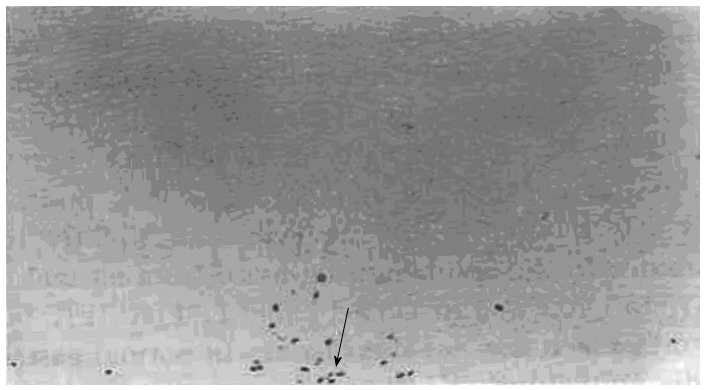

Figure 3 Immunohistochemical studies of BudR-labeled cells in the esophageal epithelia of basal cell hyperplasia (with methyl green counterstaining). Immunoreactivity is located in the cell nuclei (× 200, arrows).

were correlated with the histopathology of esophagus and gastric cardia and may represent useful additional parameters for screening subjects at high risk for esophageal and gastric cardia cancers and monitoring the effect of chemoprevention. The proliferation pattern of esophageal epithelia measured by PCNA was similar to that measured by tritiated thymidine incorporation[1]. In terms of DNA precursor and antigen unmasking pretreatment, PCNA seems to be relatively easy to analyze in a large scale study of humans. This is of special importance in view of the increasing numbers of studies on dietary intervention and chemoprevention. Quantitative analysis of the immunostaining results showed that the positive cell numbers of PCNA and Ki-67 were almost 3.5-times larger than that of BudR in the same category of BCH lesions. It is possible that the present method is not stable enough to detect BudR-labeled cells. This possibility remains to be investigated.

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ISSN 1007-9327