Proliferating cell nuclear antigen and nucleolar organizer region for differential diagnosis of dysplasia and adenocarcinoma in gastric biopsies

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Introduction: The value of proliferating cell nuclear antigen (PCNA) and nucleolar organizer region (AgNOR) for differential diagnosis of normal mucosa, dysplasia and adenocarcinoma in gastric endoscopic biopsies, and correlation between these two methods were evaluated. Methods: 15 gastric endoscopic biopsy samples from normal mucosa, 15 from areas of dysplasia, and 15 from low grade adenocarcinoma were studied. AgNOR and PCNA immunostaining were applied to paraffin sections. Results: Mean AgNOR value and PCNA-labeling index were the lowest in normal mucosa and the highest in adenocarcinoma. Mean (SD) AgNOR numbers were 2.9 (0.3) in normal mucosa, 5.9 (1.7) in dysplasia and 15.7 (2.8) in adenocarcinoma. PCNA-labeling index was 2.4 (1.1) in normal mucosa, 27.5 (4.6) in dysplasia and 42.1 (5.3) in adenocarcinoma. The differences between normal mucosa and dysplasia, and between dysplasia and adenocarcinoma were significant (p<0.001). Overlapping values were observed in AgNOR counts between normal mucosa and dysplasia, and in PCNA-labeling indices between dysplasia and adenocarcinoma. No correlation was found between AgNOR and PCNA. Conclusion: Though mean AgNOR values and PCNA indices were significantly different between normal mucosa, dysplasia and adenocarcinoma, these could not be used in differential diagnosis because of overlapping values between groups. [Indian J Gastroenterol 2000;19:57-60]

Key words: Carcinoma stomach

Gastric cancer is one of the most common cancers in Turkey. The poor prognosis of advanced gastric cancer makes early diagnosis important. This can be achieved by identification of at-risk groups, followed by endoscopic surveillance and biopsy. It is sometimes difficult to differentiate between dysplasia, carcinoma and normal mucosa on endoscopic biopsy material using hematoxylin-eosin stains.

Recent developments in markers of cell differentiation and cell kinetics have revealed a variety of preneoplastic phenotypic changes in gastric epithelium, which may provide sensitive indicators of cancer risk. In a variety of malignant neoplasms, correlation has been noted between proliferative activity and metastatic potential, recurrence and overall prognosis.

One method used to measure and correlate proliferative activity and clinical pathological parameters is proliferating cell nuclear antigen (PCNA) immunostaining. PCNA is an auxiliary protein for DNA polymerase B and plays an important role in DNA synthesis; it is believed to be synthesized in nuclei, particularly during the proliferative period of late G1 and S phases. PC10, a monoclonal antibody that can be used on fixed and paraffin-embedded tissues, recognizes PCNA and can be used for immunostaining.

The nucleolar organizer region (NOR) technique has also recently been applied to several tissues and lesions. The silver staining technique of NOR (AgNOR) is based on argentophilia of NOR-associated proteins and can be performed at room temperature on paraffin-embedded tissues. AgNOR count can help distinguish benign from malignant lesions. NORs are loops of DNA that code for rRNA. Their number appears to relate to transcriptional activity and cell proliferation. They are located on five acrocentric chromosomes — 13, 14, 15, 21 and 22 — and can be readily visualized as black dots in the nuclei by a silver staining technique.

We investigated the value of PCNA and AgNOR for differentiating between normal mucosa, dysplasia and adenocarcinoma in gastric endoscopic biopsies.

Methods

Forty-five endoscopic gastric biopsies were selected at random from the records of Pathology Department at Firat University Medical Center. These included low grade gastric adenocarcinoma (n=15), dysplasia (n=15) and normal gastric mucosa (n=15). Tissues had been fixed in 10% formalin and processed by routine methods. Paraffin sections 3 μm in thickness were cut. Hematoxylin and eosin-stained sections from each case were reviewed by two histologists.

The AgNOR staining solution was prepared as described by Ploton. Briefly, it consisted of 2% gelatin in 1% aqueous formic acid added to 50% aqueous silver nitrate solution in a 1:2 ratio. The staining solution was immediately poured over the slides and the reaction allowed to proceed in the dark for 35 min. After the colloid solution was washed off with deionized water, the slides
were dehydrated, cleared and mounted in DPX.

AgNor dots seen as distinct silver-positive intranuclear dots inside and outside the nucleolus were counted. Careful focusing was found to be critical in identifying all dots within nuclei. Counts were performed by two histologists. An oil immersion lens with a maximum magnification of 1000x was used to count consecutive epithelial cells along glands. Fields were selected at random; 100 cells were examined in each sample and mean number of AgNORs per cell was calculated.

Immunohistochemistry

Paraffin sections, 3 μm thick, were mounted on poly-L-lysine-coated glass slides and air-dried overnight at room temperature. Immunohistochemical study was performed using the streptavidin-biotin method. Sections were dewaxed in xylene, rehydrated through alcohol, and then immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. Sections were subsequently washed in phosphate-buffered saline (PBS). Blocking reagent was applied for 10 min to reduce nonspecific antibody binding. The tissue sections were incubated for 1 hour with PC10, a mouse monoclonal antibody (DAKO, Denmark, code no M879) diluted 1:50 in PBS and were washed thrice with PBS. Biotinylated rabbit anti-mouse immunoglobulin was applied (dilution 1:100) for 10 min. After further washing, sections were incubated with streptavidine-biotin-peroxidase complex at a dilution of 1:100 for 10 min and washed with PBS thrice. AEC (DAKO, code no K696) was used as chromogen. Finally, the sections were counterstained with Mayer’s hematoxylin for 5 min and mounted. Normal human tonsil was used as positive controls. For negative controls, the primary antibody was replaced by PBS.

All sections were examined without prior knowledge of clinical features and histological diagnosis. Five hundred cells were counted in each case, and the PCNA index was calculated as percentage of cells containing PCNA-positive cell nuclei.

PCNA immunostaining was confined to the nucleus and consisted of granular and diffuse patterns. As there was some variation in the intensity of nuclear staining around strongly positive cells, weakly PCNA-stained nuclei were scored negative. The nuclei in human tonsillar germinal centers always showed intense positivity for PCNA. Negative control sections always tested negative.

Statistical analysis

Data were expressed as mean (SD). Student’s t test and Spearman’s correlation were used for statistical analysis.

Results

Mean PCNA-labeling indices were mean 2.4 (SD 1.1; range 1.1-4.7) in normal mucosa, 27.5 (4.6; 19-35) in dysplasia and 42.1 (5.3; 33-48) in adenocarcinoma. Mean AgNor numbers were 2.9 (0.3; 2.5-3.4) in normal mucosa, 5.9 (1.7; 3.1-9.2) in dysplasia and 15.7 (2.8; 11.7-19.3) in adenocarcinoma. Mean AgNor value and PCNA-labeling index were the lowest in normal mucosa and the highest in adenocarcinoma (Figs 1-4). There were significant differences in AgNor counts between normal mucosa and dysplasia (p<0.001) and between dysplasia and adenocarcinoma (p<0.001). PCNA-labeling indices were significantly higher in patients with dysplasia than in normal mucosa (p<0.001) and in adenocarcinoma than dysplasia (p<0.001). Overlapping values were observed in AgNor counts between normal mucosa and dysplasia, and in PCNA-labeling indices between dysplasia and adenocarcinoma. No correlation was found between AgNor and PCNA.

Discussion

Most gastric carcinomas appear to evolve through chronic atrophic gastritis to intestinal metaplasia, dysplasia and...
Finally, carcinoma of intestinal type. The other pathway, suggested for the diffuse type of carcinoma, involves maturation arrest of the neck epithelial cells, increasing atypia and dysplasia of non-metaplastic glandular epithelium. Knowledge of morphological precursors of gastric carcinoma may facilitate early diagnosis.

Cell proliferation is directly associated with the rate of tumor growth, and may determine tumor behavior. In this study, using PCNA immunostaining and AgNOR technique, we found that PCNA indices showed a gradual increase as disease progressed from normal mucosa through dysplasia to adenocarcinoma. There was a significant difference in PCNA indices between normal mucosa and dysplasia, and between dysplasia and adenocarcinoma. However, we found some overlap of values in PCNA indices between dysplasia and adenocarcinoma.

Yonemura et al. reported a significant correlation between PCNA-labeling index and S-phase fractions in gastric cancer. PCNA has received attention as one of the parameters for cell proliferation kinetics in recent years. Robbins et al. studied PCNA expression in various cancerous tissues and concluded that PCNA-labeling index can be used as an indicator of malignant potential. Jain et al. also reported on the significance of PCNA-labeling indices as a prognostic factor.

The AgNOR technique has recently been applied to a number of tissues and lesions. It has already been shown that AgNOR counts can, in certain situations, help to distinguish benign from malignant lesions. One study on the use of AgNOR technique in gastric lesions showed that it may be possible to differentiate carcinoma from regenerative lesions and normal controls. Mamaev et al. found that the content of silver granules in the nuclei of tumor cells was significantly higher in 30 patients with esophageal and gastric carcinoma than in those of normal controls. Rosa et al. suggested that AgNOR technique may help in predicting the evolution of ‘borderline’ lesions.
Irazusta et al. studied normal, inflammatory, dysplastic and neoplastic gastric mucosa groups and showed that the highest values of PCNA indices and AgNOR counts were in the carcinoma group but there was considerable overlap between the four groups.

According to these reports it seems possible to differentiate carcinoma from normal tissue with AgNOR technique. In our study, AgNOR counts showed a gradual increase from normal mucosa through dysplasia to adenocarcinoma. We found significant differences in AgNOR counts between normal mucosa and dysplasia, and between dysplasia and adenocarcinoma (p<0.001). However, we noticed overlap of values in AgNOR counts between normal mucosa and dysplasia. We failed to demonstrate correlation between results obtained by PCNA immunostaining method and AgNOR technique.

In conclusion, our study suggests that PCNA staining method may help in the differentiation of dysplasia from normal mucosa, and AgNOR technique may help in the differentiation of dysplasia from adenocarcinoma in gastric endoscopic biopsies.

References

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