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THE EXPRESSION OF KI-67 ANTIGEN AT THE ULTRASTRUCTURAL LEVEL IN PATIENTS WITH LARYNGEAL CARCINOMA

OBECNOŚĆ ANTYGENU KI-67 NA POZIOMIE ULTRASTRUKTURALNYM U PACJENTÓW Z RAKIEM KRTANI

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ABSTRACT: In the present study we localized the Ki-67 antigen at the ultrastructural level in 15 patients with laryngeal squamous cell carcinoma. Ki-67 labeling was detected in 8 of 15 (53.3 %) samples. We used gold streptavidin method to study intracellular localization of Ki-67 antigen. The Ki-67 antigen was localized in the nucleus and was rather associated with matrix of nucleus and it was seen in nucleoli in all 8 cases. Controls of incubation with normal mouse serum showed no labeling with gold streptavidin particles.
KEY WORDS: larynx, gold streptavidin method, immunoelectron microscopy, Ki-67 antigen, laryngeal carcinoma

Introduction

Antibody Ki-67 recognizes a nuclear antigen which is present only in proliferating cells. The production of the monoclonal antibody Ki-67 has been described by Gerdes et al. (6). Recent investigation suggest that this antigen is a component of the nuclear matrix (13, 14). Ki-67 recognizes cells in all phases

of the cell cycle except Go and early G1 (12). Sasaki et al.(12) showed that the antigenic expression increase with cell cycle progression, rising during the latter half of the S phase and reaching a peak in the G2 and M phase. Guillad et al. (7) also demonstrated the increase in antigen expression with cell progression through the cell cycle in both a normal and malignant cell line. Ki-67 immunostaining study can be used to identify the proliferative cell population from a tumor. The cellular proliferation may be of value in predicting the clinical outcome in patients with neoplasm. Some reports related Ki-67 antigen with histological grade of the tumor for example in breast cancer Ki-67 correlate with poorly differentiated tumors (1, 10) whereas in others carcinomas relation was not find (2, 11). In the present study we wanted to asses the Ki-67 antigen at the ultrastructural level in patients with laryngeal squamous cell carcinoma using gold method.

Materials and methods

Tissue samples obtain from 15 patients with laryngeal squamous cell carcinoma were fixed in 4% paraformaldehyde in 0.1M cacodylate buffer pH 7.4 for 3 hours at 4°C. The fixed tissue were then washed overnight at 4°C in 0.1M cacodylate buffer pH 7.4. Dehydration was performed in an ascending series ethanol and embedded in Epon 812.For showing Ki-67 antigen postembedding streptavidin gold method was used. Sections were cut at 60 nm and placed on nickel grids. The grids were floated on nonimmune rabbit serum (DAKO A/S Denmark) for 20 minutes and transferred onto drops 1:50 dilution of mouse anti human Ki-67 antigen (MIB-1, Immunotech/France). After incubation with primary antiserum for 30 minutes, followed by washing in PBS the grids were exposed to biotinylated rabbit anti mouse immunoglobulins (DAKO A/S Denmark) diluted 1:50 and washed in PBS as well. After that, the grids were transferred onto drops of 1:20 dilution of 5 nm streptavidin gold particles for 30 minutes. The grids were then washed in PBS and dried. All incubations steps of the staining procedures were performed at room temperatures. The preparations were examined by electron microscope JEM 100 CX. Controls specimens were incubated with nonimmune antiserum (normal mouse serum, DAKO A/S Denmark)

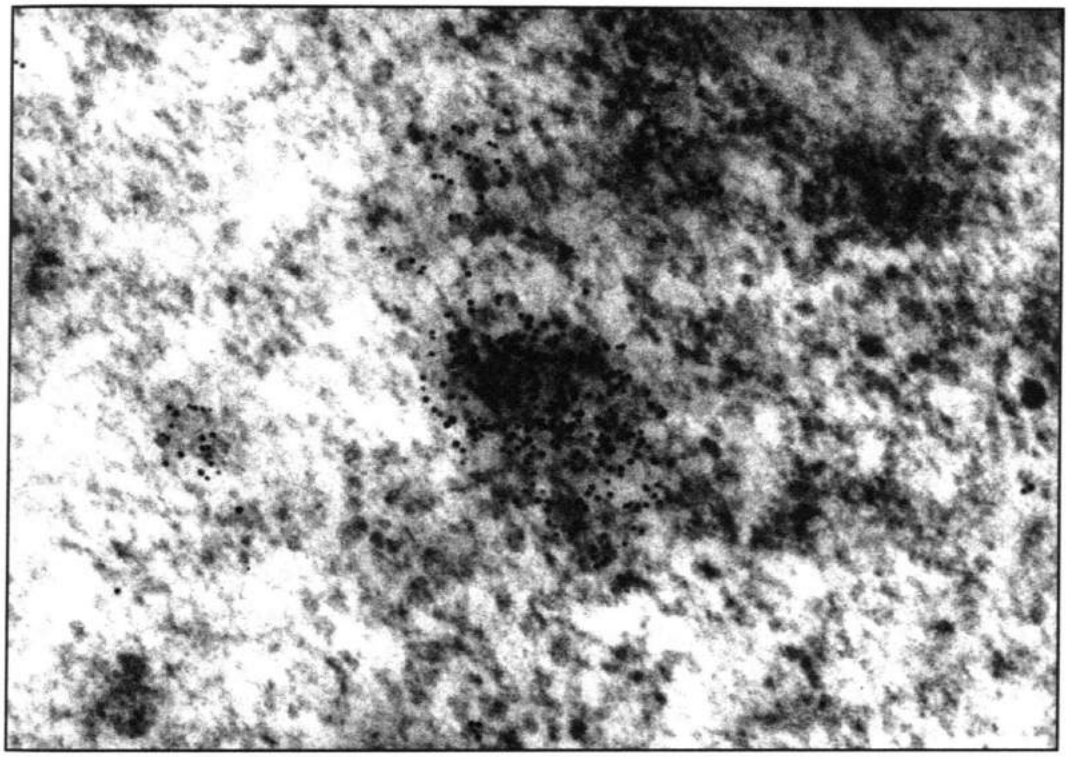

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FIG. 1. Localization of Ki-67 antigen by using gold method in tissue section obtain from laryngeal squamous cell carcinoma. The gold particles are seen scattered througout the nucleus. Staining reaction with Ki-67 antigen is seen in nucleoli as well. X 100 000

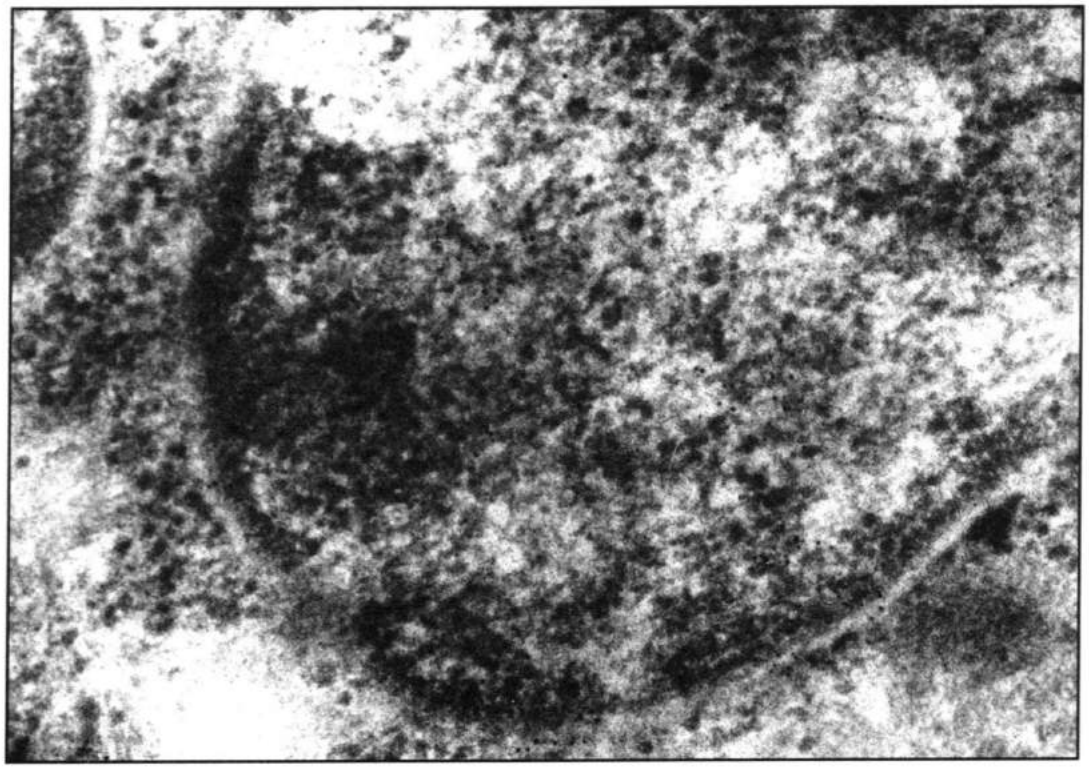

FIG. 2. Electron micrograph of laryngeal squamous cell carcinoma containing positive reaction for Ki-67 antigen is seen in nucleus, nucleoli and in cytoplasm. X 65 000

Results

Fifteen laryngeal squamous cell carcinoma were analyzed at the ultrastructural level in our studies using gold method. We tried to assess Ki-67 antigen in cells of laryngeal squamous cell carcinoma. The Ki-67 labeling was detected in 8 of 15 samples (53.3%). In our studies this antigen was seen predominantly in nucleus. The gold particles were scattered throughout the nucleus and they were seen to be associated with matrix of the nucleus rather then with heterochromatin. In all 8 cases staining reaction with Ki-67 antigen was seen in nucleoli (fig.1). There were granular material of gold particles in cytoplasm as well. Cytoplasmic staining occurred only in two cases and was found scattered throughout the cytoplasm (fig. 2). We did not find the positive labeling in control samples.

Discussion

In our studies of laryngeal squamous cell carcinoma we wanted to assess the localization of Ki-67 antigen at the ultrastructural level. Ki-67 antigen is nonhistone nuclear protein that function in the active phase of the cell cycle, its expression is considered as a measure of tumour proliferative activity (4). The different immunohistochemical methods evaluate cell proliferation in human tumours, including bromodeoxyuridine labeling (3), DNA polymerase staining (16), PCNA staining (9) and Ki-67 staining as well (2, 13). The use of Ki-67 antigen for assessing cell proliferation has been described in wide range of neoplasms (8). Previous studies showed that Ki-67 antigen can be detected by immunocytochemical assay predominantly in nucleus. These studies suggested that the Ki-67 antigen may be a component of the nuclear matrix (14, 15). The application of the gold labeling technique in our studies showed Ki-67 antigen predominantly in nucleus as well. Ki-67 antigen visualized by gold particles were seen scattered throughout the nucleus and in nucleoli of the cells of laryngeal squamous cell carcinoma. Gold particles were associated with matrix of the nucleus in our studies, our date are in agreement with studies Verheijen et al. (15). They found Ki-67 antigen using immunoperoxidase method in association with the nuclear matrix and Ki-67 antigen was seen in nucleoli particularly at its periphery and in the dense fibrillar components of cultured cells. In summary, the results of this study confirm a previously reported expression of Ki-67 antigen at the ultrastructural level and have a significance in evaluating this proliferative marker at the level of single cell.

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Streszczenie

W pracy przedstawiono lokalizację antygenu Ki-67 na poziomie ultrastrukturalnym u 15 pacjentów z rakiem krtani. Antygen Ki-67 został wykryty w 8 z 15 próbek pobranych od pacjentów z rakiem krtani, co stanowiło 53.3% przebadanych przypadków. W badaniach zastosowano metodę złota koloidalnego do wykrycia antygenu Ki-67. Antygen Ki-67 został wykryty w jądrze i był związany z matrix jądrowym, jak rówież umiejscowiony był w jąderku we wszystkich 8 dodatnich przypadkach. W próbkach kontrolnych nie stwierdzono dodatniej reakcji immunocytochemicznej.