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THE CORRELATION OF p53 PROTEIN WITH PCNA AT THE ULTRASTRUCTURAL LEVEL IN PATIENTS WITH LARYNGEAL SQUAMOUS CELL CARCINOMA ESTIMATED BY GOLD METHOD

KORELACJA BIAŁKA p53 I PCNA NA POZIOMIE ULTRASTRUKTURALNYM U PACJENTÓW Z PŁASKONABŁONKOWYM RAKIEM KRTANI OCENIANYM Z ZASTOSOWANIEM METODY ZŁOTA KOLOIDALNEGO

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ABSTRACT: Two proteins PCNA (proliferating cell nuclear antigen) and p53 estimated at the ultrastructural level in patients with laryngeal squamous cell carcinoma were correlated in this studies. PCNA and p53 protein were localized ultrastructurally with 5 nm gold colloidal particles. Postembadding technique to study PCNA and p53 protein was used. Immunoelectron staining for PCNA was seen in 9 of 15 cases . It was 60% of all observed cases. Five of 15 samples from patients with laryngeal squamous cell carcinoma showed positive labeling for p53 (33.3% of all examined samples). Proliferating cell nuclear antigen visualized by colloidal gold particles was found in these same five samples where the positive product for p53 protein was observed.
KEY WORDS: PCNA, p53 protein, gold method, laryngeal squamous cell carcinoma.

Introduction

It is known that p53 protein is critical to normal regulation of cell growth and is a suppressor of tumour cell proliferation. Inactivation of p53 gene may occur by a number of mechanisms, these include genomic damage like missense mutations, viral interaction, radiation and others point mutations of the p53 gene. It is also associated with a variety of tumours and results in the alteration of the p53 which can be detected immunohistochemically. Expression of immunohistochemically detectable p53 protein has been found in 22-76% of cases of malignancies in colon, stomach, bladder, breast, lung, testis, melanomas and soft-tissue sarcomas (1, 4, 6, 9, 10, 13, 14, 18, 21, 25). Proliferating cell nuclear antigen (PCNA) is defined as an intranuclear polypeptide whose synthesis reaches its maximum during S-phase of the cell cycle. It was identified as the polymerase δ accessory protein in both S phase and also in DNA synthesis associated with DNA repair (5, 8, 12). Antibodies recognize PCNA may allow the identification of proliferation. Assessing cell proliferation may have prognostic value, but there are small size of these kind of studies and these are not any connections with laryngeal carcinoma. There is good evidence that in breast and lymphoma pathology assessing proliferating state provides prognostic information (7, 13, 16). In this study we would like to assess whether is any relationship between overexpression of the p53 protein and PCNA in patients with laryngeal squamous cell carcinoma estimated by using immunogold method at the ultrastructural level.

Materials and methods

In this studies p53 protein and PCNA demonstrated on fifteen specimens of laryngeal squamous cell carcinoma were correlated. The tissue segments to be studed were cut into small fragments and fixed in 4% paraformaldehyde in 0.1 M. cacodylate buffer pH 7.4. Dehydration was performed in a ascending series of ethanol and embedded in Epon 812. Showing p53 protein and PCNA the postembadding streptavidin gold method was used according to a previously described (22). For showing p53 protein mouse anti human p53 antibody (DAKO A/S, Denmark) and for PCNA mouse anti human PCNA antigen (PC 10, DAKO A/S, Denmark) were used of 1:50 dilution.

Results

In this study we tried to correlate the p53 protein and PCNA. We visualized both proteins with colloidal gold at the ultrastructural level. The p53 protein was detected in five of fifteen cases of laryngeal squamous cell carcinoma. It was 33.3% of all studied samples. Proliferating cell nuclear antigen was found in nine of fifteen cases. It was 60% of all studied cases of laryngeal squamous cell carcinoma. The p53 protein was detected in nucleus and in three of five positive cases it was seen in cytoplasm as well (ryc. 1). PCNA was seen not only in nucleus but also in cytoplasm in four of nine positive cases (ryc. 2). In both cases p53 protein and PCNA visualized by colloidal gold particles were seen on endoplasmic reticulum and were scattered throughout the cytoplasm cells. Immunocytochemical staining for PCNA was found in this same five samples where the positive product for p53 protein was observed. There were not positive immunoreactivity for p53 staining in four positive PCNA samples. These results showed correlation between the presence of p53 protein and PCNA in samples from patients with laryngeal squamous cell carcinoma studied by gold method at the ultrastructural level.

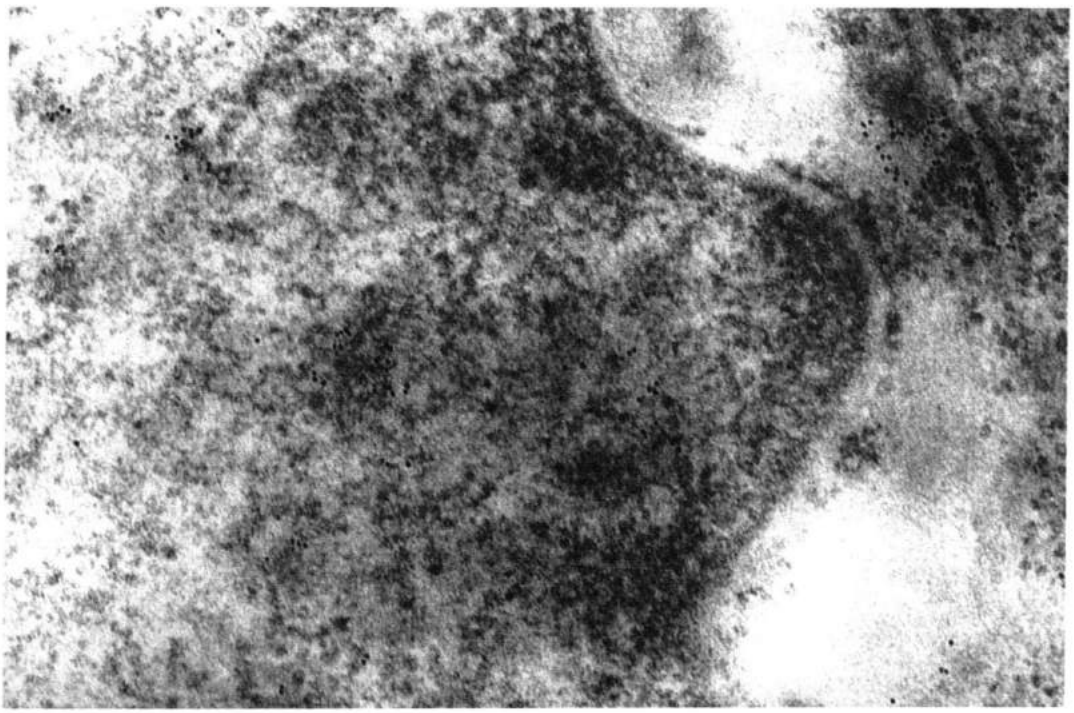

FIG. 1. Electron micrograph of laryngeal squamous cell carcinoma containing positive reaction for p53 protein in nucleus and on the endoplasmic reticulum. X 90 000

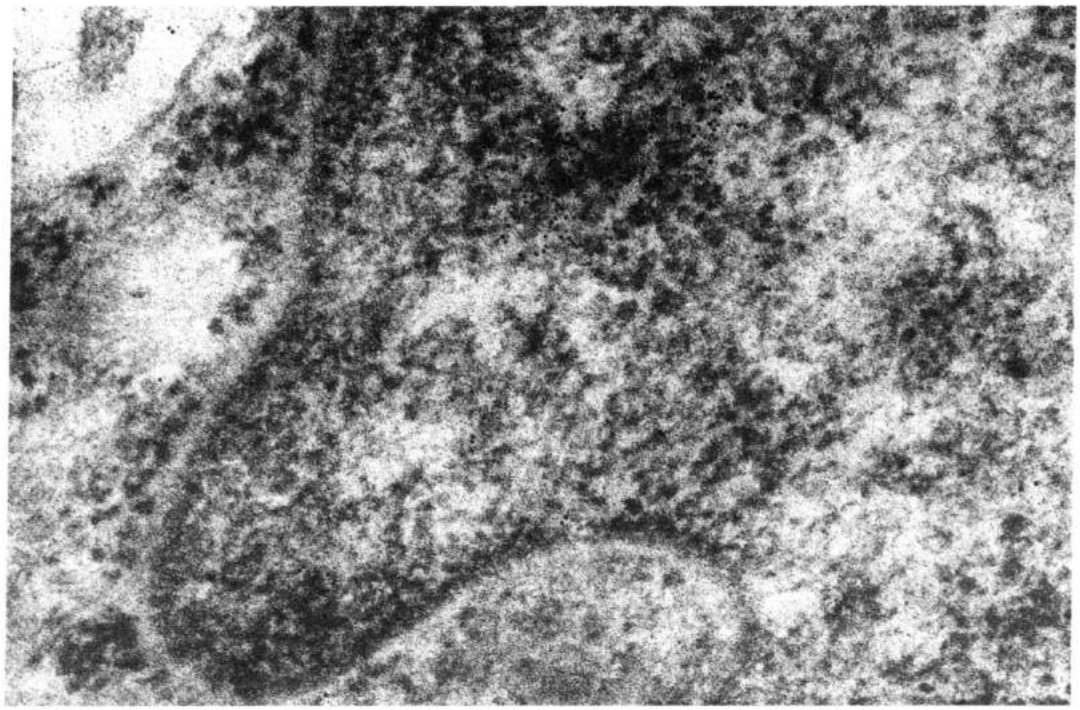

FIG. 2. Localization of PCNA protein by using gold method in tissue section obtain from laryngeal squamous cell carcinoma. Gold particles are seen in nucleus. X 80 000

Discussion

These studies demonstrated comparison of overexpression of the p53 protein with proliferating cell nuclear antigen (PCNA) at the ultrastructural level. There was correlation between positive staining for PCNA which allow the identification of proliferation with p53 protein which is implicated in growth control of the cell. PCNA positive immunostaining demonstrates the tumour's proliferative fraction and might be used as an indicator of increased malignant potential in different cancer (2, 12, 24, 26, 27). The p53 gene is known as anti-oncogen and its function is controlling the cell cycle. It is responsible for the death of the cells with damaged DNA as well. The mutations of the p53 gene are responsible for synthesis of the damaged protein which unable the control of the cell cycle (20). There is a good evidence that abnormalities of the p53 gene are impotent in many types of cancer (3,11,14,). These studies showed significant correlation between expression of p53 protein and proliferating cell nuclear antigen, thus, our results and others support

the hypothesis that accumulation of p53 protein is associated with a high tumour proliferation rate (15, 17, 19). There were some studies which, results are in agreement with ours in these studies the correlation between overexpression of p53 protein and PCNA was observed (15, 17, 19). Suto et al. (23) found that p53 positivity was no related to the percentage of PCNA labeled cells. In spite of this report which we found in the literature there are some of them where the expression of the p53 protein and PCNA were correlated and they both seem to have prognostic significance in many types of cancer.

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Streszczenie

W przeprowadzonych badaniach oceniono korelację dwóch białek PCNA i p53 na poziomie ultrastrukturalnym u pacjentów z rakiem krtani. PCNA i białko p53 na poziomie ultrastrukturalnym zlokalizowano przy użyciu 5 nm złota koloidalnego. Do oceny PCNA i białka p53 zastosowano metodę po zatopieniu w eponie. Barwienie immunocytochemiczne dla PCNA obserwowano w 9 z 15 przypadków, stanowiło to 60% wszystkich przebadanych przypadków. 5 z 15 próbek od pacjentów z płaskonabłonkowym rakiem krtani wykazało pozytywne znakowanie dla białka p53, stanowi to 33.3% wszystkich przebadanych próbek. PCNA uwi-

doczniony przy użyciu cząsteczek złota koloidalnego został wykryty w tych samych 5 próbkach, w których zaobserwowano również pozytywną reakcję dla białka p53.