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## Histochemical polymorphism of keratin pearls in squamous cell carcinoma of the lung

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### ABSTRACT

The aim of the research is to study histochemical features of keratin pearls in keratinizing squamous cell carcinoma of lung irrespective of its localization. Methods: histochemical methods of staining have been also used during the investigation: neutral fats stained with Sudan III and with finish hematoxylin staining. Combined histochemical staining has been also used: neutral mucoproteins and acid glycosaminoglycans with PAS-reaction-alcian blue and PAS-reaction with tionine blue. The results of histological and histochemical analyses detected three types of keratin pearls of different structures and tinctorial features: eosinophilic or tionine-positive, PAS-positive (containing glycogen) and light or sudanophilic (containing lipids). Taking into consideration the obtained data, it can be assumed that tumor clusters with heterogenous keratin pearls by tinctorial features are differed according to the degree of cells' differentiation, keratinization and related to various stages of tumor progression. Histochemical analyses of keratin pearls are not normally taken into consideration during morphological studies and it is regarded as differentiated keratinizing cancer. However, the results of morphological investigations show that it is necessary to consider histochemical features of cancerous clusters for adequate morphological analysis of tumor progression.

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## 1. Introduction

Lung cancer, the development of which is associated with clearly defined exogenous factors, remains relevant medical-social and environmental problem of current oncology. According to WHO (World Health Organization) classification, histological type of keratinizing squamous cell carcinoma of lung is assigned

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to organ nonspecific one, as it occurs in various organs (skin, esophagus, neck of uterus, larynx, bronchi, etc.) (Akopov 2011; Travis et al. 2004).

Histological structure of such type of cancer is characterized by the formation of a keratin pearl. The latter one is formed due to concentric arrangement of eosinophilic keratinous squamulae (Filenko 2012).

Squamous cell carcinoma is mostly found in central or peripheral localization of tumor in lungs. The following identical zones have been detected regardless of the localization of the given histological type: zone of cancrioid corpuscles, proliferation zone and invasion zone (Boyko et al. 2014). According to the publications, keratinizing carcinoma occurs due to metaplasia of multirowed ciliated epithelium into pavement one. The prominent oncologists consider smoking as one of the major etiological factors of the development of metaplasia (Zaridze 2004; Radzikowska et al. 2002; Proctor 2001; Wang et al. 2009).

The lack of the unified concept of morphogenesis determines the need for detailed morphological studies of lung cancer.

## **2. Purpose**

The aim of the research is to study histochemical features of keratin pearls in keratinizing squamous cell carcinoma of lung irrespective of its localization.

## **3. Object and Methods**

In compliance with the objectives of the study a complex of histological and histochemical methods has been applied during the investigation. The analysis has been carried out on post operational material, taken from 25 patients with keratinizing squamous cell carcinoma of lung. The obtained material was first fixed in 10% buffered solution of neutral formalin with further paraffinization. The obtained sections were stained with hematoxylin and eosin in compliance with standard techniques.

Histochemical methods of staining have been also used during the investigation: neutral fats stained with Sudan III and finish stained with hematoxylin. Combined histochemical staining has been used, too: neutral mucoproteins and acid glycosaminoglycans with PAS (Periodic Acid-Schiff)-reaction-alcian blue and PAS-reaction with tionine blue.

Since lipids are dissolved in alcohol and xylene in handling of specimens in compliance with conventional technique, then, during traditional histological hematoxylin and eosin staining, the cytoplasm of cellular lipid elements becomes glassy. Consequently, during the investigation detections of intracellular lipid inclusions were initially based on identification of cellular elements with visually glassy cytoplasm and further justified by specific histochemical Sudan III staining on neutral fats and with finish hematoxylin staining on prefrozen sections.

Stained specimens have been analyzed on the "Olympus BX-41" digital light microscope with  $\times 10$ ,  $\times 20$ ,  $\times 40$ ,  $\times 100$  lenses, and their exposure have been performed by the "Olympus C 4040" digital camera.

#### **4. Results and Discussion**

In microscopic analysis keratinizing squamous cell carcinoma of any localization consists of tumor clusters of various sizes formed by atypical cells of different sizes with polymorphous heterochromatic nuclei.

Keratin pearls are found in the depth of cancerous clusters, which are formed due to concentric arrangement of acanthocytes. Gradual growth of keratinization occurs in the direction from the periphery to the center that is justified by the presence of eosinophilic keratinous squamulae, forming the sinuous structures in the focus of pearls.

It should be noted that necrotic zones of various sizes are detected in the focus of some keratin pearls. Their sizes probably depend on the degree of differentiation and vascularization, mainly due to neoangiogenesis. The more the necrotic zone is, the less tissue vascularization is.

Stroma of tumor, in depth of which the strongly pronounced areas of tumor cells were found, was strongly pronounced and presented in the form of coarse-fibered connective tissue and vessels of various sizes in some cases.

The results of histological and histochemical analyses detected three types of keratin pearls of different structures and tinctorial features: eosinophilic or tionine-positive, PAS-positive (containing glycogen) and light or sudanophilic (containing lipids).

Eosinophilic keratin pearls are formed of concentric keratinous fusiform angular cells. Cytoplasm of such cells is homogeneously intense eosinophilic. In some cells aggregation of fine intense eosinophilic granules are identified. Nuclei are prolate, hyperchromatic with coarsely granular chromatin. Such keratin pearls are surrounded by polymorphous atypical cells and a zone of moderately pronounced inflammatory infiltrate.

In finish staining with tionine-blue such keratin pearls are dark-purple, i.e., tionine-positive. They consist of individual heterochromatic keratinized cells. Peripheral part of a cell is more hyperchromatic than the central one (Fig.1).

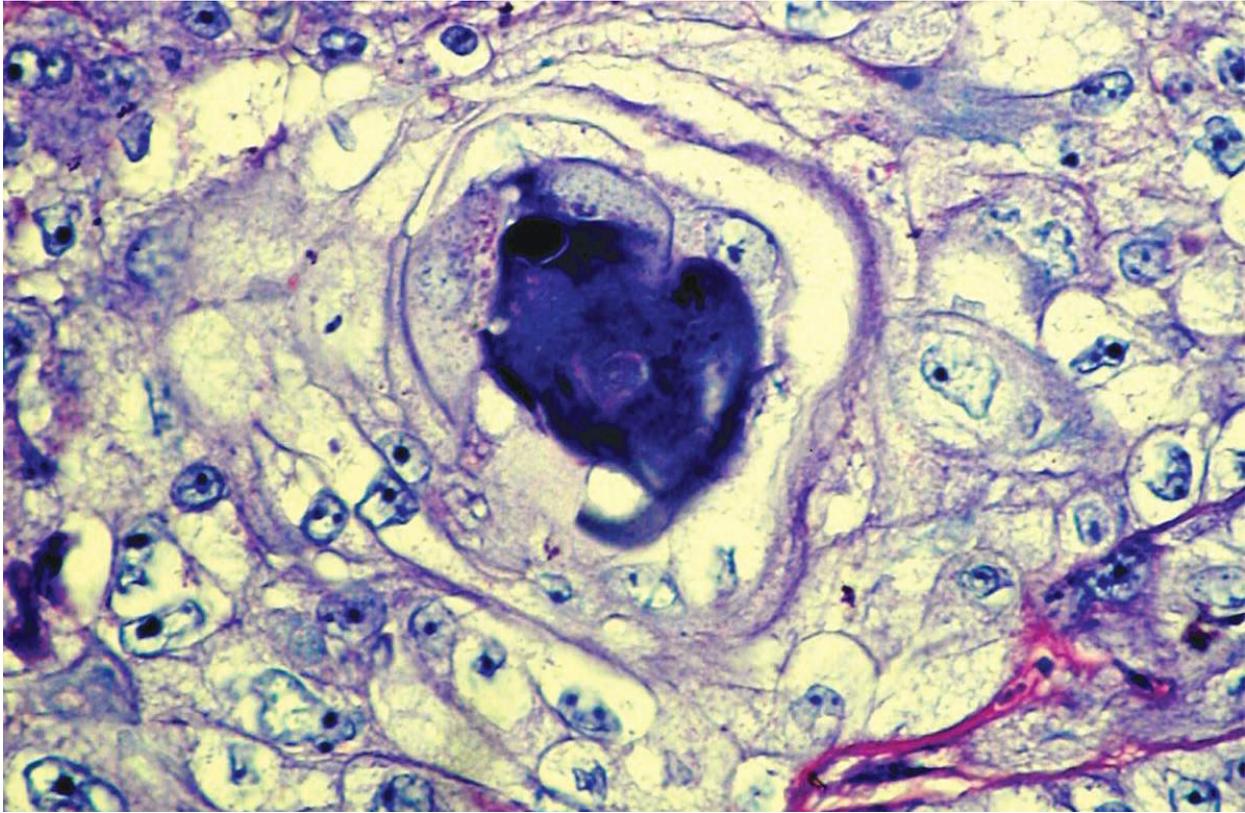


Fig. 1. Tionine-positive keratine pearl in keratinizing squamous cell carcinoma of lung. Tionine-blue stain. Original magnification  $\times 1000$ .

It can be assumed that such tinctorial features of keratin pearls indicate the processes of intensified formation of keratohyalin, i.e., hyperkeratosis. Keratin pearls, containing glycogen, are formed with keratinized cells of larger size, in comparison with eosinophilic pearls. Their cytoplasm is light eosinophylic in conventional histological staining. Nuclei of such cells are prolate-ovoid, hyperchromatic with coarsely granular chromatin. Karyopyknosis is found in some cells.

Presence of glycogen in such keratin pearls is justified by the results of histochemical analyses made by the PAS-reaction. Cells of PAS-positive keratin pearls contain glycogen staining their cytoplasm purple.

In combined PAS-tionine blue staining it is detected that such keratin pearls contain tionine-positive central part surrounded with concentric PAS-positive homogeneous structures on the periphery. Keratin pearls, mostly consisting of only PAS-positive concentric structures, are also often found (Fig. 2).

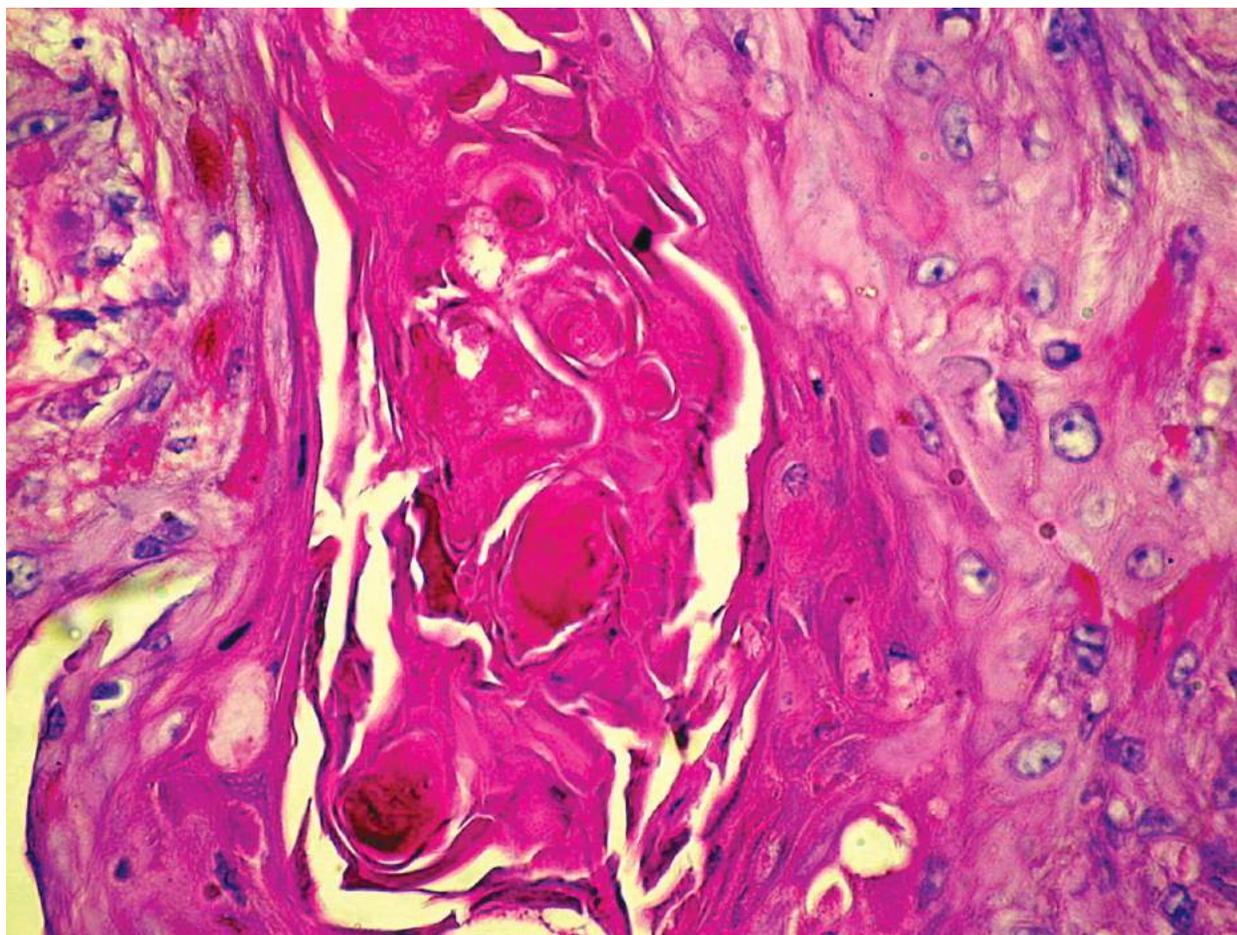


Fig. 2. PAS-positive keratin pearls in keratinizing squamous cell carcinoma of lung. PAS-reaction stain. Original magnification  $\times 400$ .

It can be assumed that such staining of PAS-positive keratin pearls is due to development of parakeratosis, i.e. the process of keratinization abnormality.

In conventional histological hematoxylin and eosin staining the third type of keratin pearls is revealed, which are characterized with weak eosinophilic staining of cells' cytoplasm or contained foam cytoplasm. Nuclei of such cells, located on the periphery, are large and ovoid, hyperchromatic with mainly medium granular chromatin. Cells with karyopyknosis and karyolysis are identified in the central part of keratin pearls.

The study concluded that cells of such keratin pearls contain lipids, which are dissolved in alcohol and xylene while handling of specimens in compliance with conventional techniques.

This assumption is confirmed by the results of histochemical analyses, using Sudan III staining. Sudanophilic keratin pearls are strombuliform ones and consist of sudanophilic structures. Clusters of cancer cells of keratin pearls containing lipids are orangophilic indicating the content of fats in them. Thereafter lipids are found both in cells' cytoplasm and intercellular space. Cells, in which lipids are arranged intracellular, are characterized by saving the nucleus; karyopyknosis, karyorrhexis and karyolysis are found in extracellular

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lipid aggregation. While lipids are going beyond the cytoplasm boundaries, a highly dispersed or atomized sudanophilic substance is identified in the intercellular spaces (Fig. 3).

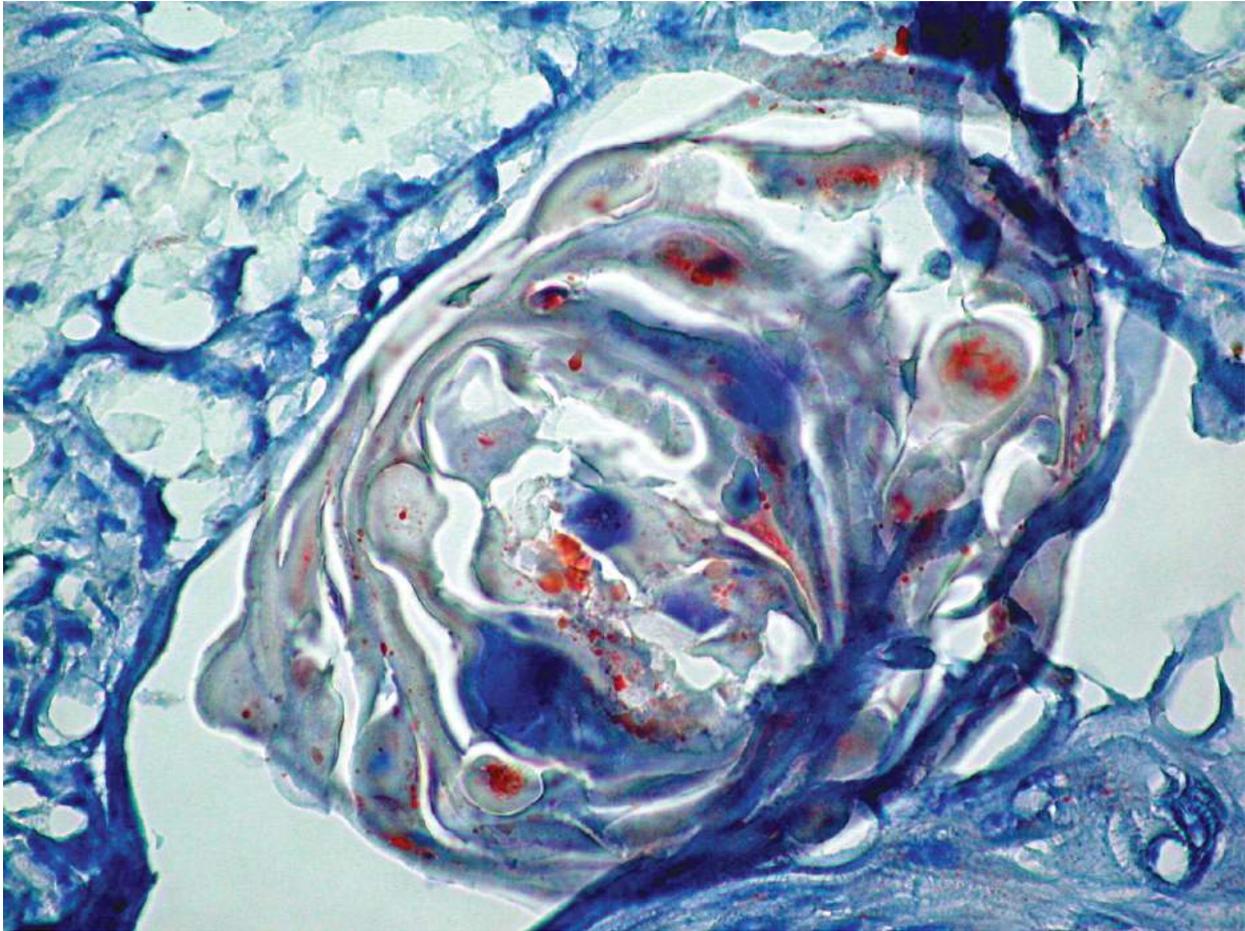


Fig. 3. Sudanophilic keratin pearl in keratinizing squamous cell carcinoma of lung. Sudan III staining with finish hematoxylin staining. Original magnification  $\times 400$ .

Such pearls are formed due to development of dyskeratosis in them.

## 5. Conclusions

Taking into account the obtained data, it can be assumed that tumor clusters with heterogenous keratin pearls by tinctorial features are differed according to the degree of cells' differentiation, keratinization and related to various stages of tumor progression.

The zone of tumor clusters with keratin pearls is characterized by the formation of heterogenous keratin pearls by the histochemical features: eosinophilic or tionine-positive, PAS-positive or glycogen-comprising and sudanophilic or lipid-comprising ones.

Histochemical analyses of keratin pearls are not usually taken into consideration during morphological studies and it is regarded as differentiated keratinizing cancer. However, the results of morphological investigations show that it is necessary to consider histochemical features of cancerous clusters for adequate morphological analysis of tumor progression.

## 6. Perspectives of further research

As for further research, it is planned to carry out cytogenetic and immunohistochemical study of tumor clusters of squamous cell lung cancer with keratin keratinizing squamous cell carcinoma of lung.

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