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Immunohistochemical analysis of cathepsin B and cathepsin S in tumors, parenchyma and regional lymph nodes of the lung

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Introduction

The detection of antigens on tissue sections by using specific antibodies (immunohistochemistry (IHC)) is an important approach in identifying cell types, which express the specific antigens. Although the immunohistochemical analysis (IHA) is a relatively well established and simple method, the major problem remains to detect low concentrations of specific antigens. In this respect, the efforts are mainly focused on improved detection systems after binding of the specific primary antibody.

Cathepsin (cath) S is present at rather low levels in lung tissue extracts[1], therefore low expression of cath S at the cellular level is expected. In fact, cath S was hardly detected by using standard procedures described in the literature. To solve this problem we established a highly sensitive staining protocol based on the CSA detection system from DAKO (Hamburg, Germany) in combination with antigen retrieval approach.

Material and methods

In order to identify cell populations producing cath S, we performed IHC on tissue sections deriving from formalin-fixed, paraffin-embedded tumors (n = 36), non-tumors parenchyma (n = 6) and regional lymph nodes of the lung (n = 10). In addition, the expression of cath S in lung cell populations was compared with those of cath B.[2] Furthermore, the expression of both cysteine-cathepsins was correlated with clinical-pathological parameters of lung cancer patients.

Results

Cath S is expressed in alveolar type II cells, macrophages, bronchial epithelial cells and lymphocytes. The enzyme showed a lysosomal distribution over the cytoplasm. In addition, we observed differences in the staining intensity of cath S in macrophages, alveolar type II cells and lymphocytes, as well. In some bronchial epithelial cells a more restricted localization in the basal proliferating zone of the epithelium was observed. Immune reactive cath S was not found in alveolar type I cells. However, cath S could also be detected in lung tumors, independently of their origin. We found a positive reaction in adenocarcinomas, squamous cell carcinomas and small cell carcinomas. Remarkably, a weak positive reaction at sides of interaction between tumor cells of the small cell carcinoma and the extracellular matrix of the alveoli could be observed. It should be noted that also in alveolar duct cells a focal positive reaction was visible.

Cath B could easily be detected using a standard protocol in combination with the ABC-system (Vector Laboratories, Serva Heidelberg), but without the need of an additional enhancing step. This clearly indicates that

cath B is expressed at much higher levels in various cell populations compared with cath S. Cath B could also be detected in alveolar type II cells, macrophages, bronchial epithelial cells and various tumors. In contrast to cath S, we were not able to localize cath B in lymphocytes. The expression of cath S did not correlate with clinical-pathological parameters of lung cancer patients, while for cath B ΣpN1/PnP/pN3 primary tumors were more frequently labelled than PnP tumors.

Conclusion

We established a highly sensitive IHC protocol for the detection of cath S in tissue sections deriving from tumors, parenchyma and regional lymph nodes of the lung. Our results show that of the two cysteine cathepsins (B and S), only cath S seems to be produced by lymphocytes, This indicates that cath S may be involved in regulatory mechanisms of the immune response.

References

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