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Expression of Small Heat Shock Protein alpha-B Crystalline in Non-Small Cell Lung Cancer

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To evaluate the expression profile of the small heat shock protein alpha-B crystallin in NSCLC and to analyse its correlation with Ki-67 and p53 expression.
Immunohistochemistry with alpha-B crystallin, Ki-67 and p53 was applied on 25 tissue samples of patients with non-small cell lung cancer.

70% of the samples are positively stained for alpha-B crystallin. Both nuclear and cytoplasmic staining is observed. Fifty-two per cent of the tissue samples are positive for p53 and 40% were positive for Ki-67.

The small heat shock protein alpha-B crystallin is a novel biomarker that is largely expressed in non-small cell lung cancer. It is not predominantly expressed in any histologic type. There was a tendency for a more intensive nuclear staining in adenocarcinomas. Alpha -B crystalline negative cells were positive for Ki-67 and p53.

Key words: non-small cell lung cancer, p53, Ki-67, alpha-B crystalline.

Introduction

Stress proteins or small heat shock proteins are synthesized under deleterious environmental conditions, that alter the protein conformation. In such conditions they are a performing a chaperoning structure, thus counteracting the formation of aberrantly folded polypeptide aggregates, and providing their renaturation during stress recovery[1, 3]. αB-crystalline belongs to the family of small heat shock proteins. In humans there are more than 10 different such proteins, but only part of them act as real heat shock proteins (Hsp 27, Hsp 22, αB-crystalline), whose synthesis is stimulated under stress. αB-crystallin exists as a homo- or heteroolygomer (with other small heat shock proteins) with a molecular mass 700-800 kDa. Under certain conditions it can be phosphorylated on three serine residues Ser 59,45,19, which provokes the disassociation of the large oligomeric complexes and the formation of smaller ones [4, 5]. The equilibrium of the two structural forms of alpha-B crystalline is closely associated with its functions as a molecular chaperone. Its oligomeric form has predominantly chaperone or antioxidant

activity, which significantly decreases under phosphorylation. It is assumed that phosphorylation increases its antiapoptotic functions [2]

It is a major component of the fibrillar aggregates, that are accumulated in cataract, desmin-associated myopathy, Alzheimer disease, Parkinson disease, Alexander neuropathy, senile systemic amyloidosis, macular degeneration, familial amyloidogenic polyneuropathy, haemodyalisis associated amyloidosis. Alongside with this it is widely expressed in many oncological diseases where it is assumed as a pathological factor, prognostic and predictive marker [6, 8]. For the first time data about high expression is reported in brain tumors (glioma multiforme, astrocytoma, oligodendroglioma). Later high expression was reported in renal cell carcinoma, thyroid carcinoma, basal-cell and metaplastic breast cancer. αB-crystalline acts as an oncogene in these carcinomas and provokes the neoplastic transformation-changed cellular architectonic, increased proliferation, repressed apoptosis, regulates invasion and metastasis [7].

Non-small cell lung cancer is among the commonest oncological diseases, and is characterized as extremely heterogenous in both histological and genetic aspects. Because of lack of data about the role of αB-crystalline in lung oncogenesis we decided to study its expression.

Materials and Methods

Whole tissue sections of 25 patients operated on non-small cell lung cancer are studied. Patients were operated on non-small cell lung cancer during 2005-2006 and none of them has received prior chemo- or radiotherapy.

Tissue slides were deparaffinized in xylene and hydrated through graded ethanol. Endogenous peroxidase activity was blocked by a 5-min incubation in 3% hydrogen peroxide. The slides were next incubated with 10% normal horse serum for 30 min at room temperature to reduce nonspecific background staining. A primary monoclonal anti-mouse antibody – p53 (DO-7). Ki-67 (MIB-1) Dako Cytomation, Carpinteria (CA) and monoclonal anti-rabbit αB-crystalline antibody (at a 1:200 dilution with PBS) were applied for 24 hours at 2-4 °C. Secondary antibody was detected by using an antimouse horseradish peroxidase-labelled polymer secondary antibody from the LSAB (Labeled Streptavidin Biotin System) (Dako). The slides were rinsed in PBS between procedures and visualized by a 3-min incubation with diaminobenzidine (DAB). Finally, the slides were counterstained with hematoxylin. In negative control experiments, normal horse serum was used and the primary antibodies were omitted.

Evaluation of immunohistochemistry

Immunostaining was classified as follows: 0 – lack of staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining. The predominated grade of staining was determined by the percentage of cells of the same grade.

Results

The study group consisted of 13 squamous cell carcinomas, 5 adenocarcinomas, 3 bronchoalveolar carcinomas and 4 adenosquamous carcinomas.

In four of the squamous cancers there was a lack of staining. The others showed moderate to intensive cytoplasmic staining with granular characteristic. All tissue

samples had moderate to intensive nuclear staining that varied between 50% to 100% of the counted cells. Neither the intensity of nuclear, nor the intensity of the cytoplasmic staining correlated to the grade of differentiation.

Three adenocarcinomas and two bronchoalveolar carcinomas were with intensive cytoplasmic staining. In the rest of the cases there was no staining. The nuclear staining was intensive and varied largely from 60% to 100% of the cells. In comparison to the squamous cell carcinomas the intensity and percentage of the positivity of the nuclear staining was distinctively higher.

The adenosquamous group of cancers that were only four had intensive nuclear and cytoplasmic staining and the number of positive cells was 80-100%.

In 40% (9/25) of the samples Ki-67 positivity was detected. The number of the positive cases predominated in tissue samples, where no or weak positivity was observed. The samples that turned to be positive for alpha-B crystallin were negatve for Ki-67 except one case.

P53 was positive in 52% (13/25) of the cases. Forty per cent had intensive and twelve per cent had moderate staining. No difference could be observed between the two major histologic types considering this marker.

Discussion

Overall 70% (16/25) of the patients were positively stained for alpha-B crystalline. Both nuclear and cytoplasmic staining was observed. In adenocarcinomas there was a tendency for a higher percentage of cells with intensively stained nuclei. Fifty-two per cent of the tissue samples were positive for p53 and 40% were positive for Ki-67. None of these two markers turned to be typically expressed in the two histologic types. There was a tendency between Ki-67 positivity in alpha-B crystalline negative cases.

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