Role of metallothionein expression in non-small cell lung carcinomas

Dzięgiel P[1], Jeleń M[2], Muszczyńska B[3], Maciejczyk A[4], Szulc A[4], Podhorska-Okołów M[1], Cegielski M[1], Zabel M[1, 5]

[1]Chair and Department of Histology and Embryology, University School of Medicine in Wrocław; [2]Chair and Department of Pathomorphology, University School of Medicine in Wrocław; [3]Lower Silesia Centre of Tuberculosis and Lung Diseases in Wrocław; [4]Lower Silesia Centre of Oncology in Wrocław; [5]Chair and Department of Histology and Embryology, University School of Medicine in Poznań

Abstract

Metallothionein (MT) is a low molecular weight protein, which participates in differentiation and proliferation of normal and tumour cells. In some malignant tumours (mammary, renal, ovarian cancers), its increased expression is thought to represent an unfavourable prognostic factor. Non-small-cellular lung cancers (mainly squamocellular cancer and adenocarcinoma) are characterised by ill-defined prognosis, which poses problems in the selection of effective post-surgical therapy. The present study aimed at demonstration of the prognostic significance of MT expression in cells of non-small cell lung cancers, attempting to correlate the intensity of MT expression with G grade and with the intensity of proliferation-associated antigen, Ki-67 expression. The studies were performed on archival paraffin blocks with samples of 25 cases of non-small cell lung cancers (5 squamous cell cancers, 20 adenocarcinomas). In paraffin sections of the studied tumours, immunocytochemical reactions were performed, using mouse monoclonal anti-MT and anti-Ki-67 antibodies. The expressions of MT and Ki-67 were demonstrated in all the studied tumours. An analysis of correlation between the expression of MT, Ki-67 antigen and G grade demonstrated a strong positive relation between the latter two parameters ($r=0.70$; $p<0.05$). Less pronounced positive correlations were disclosed between MT expression and G grade ($r=0.44$; $p<0.05$) and between MT expression and the expression of Ki-67 antigen ($r=0.41$; $p<0.05$). In addition, in 15 cases of examined tumours, survival analysis was performed, which disclosed a shorter survival in patients with high MT expression. The obtained results confirmed the relationship between MT expression and Ki-67 antigen expression, indicating an involvement of the proteins in processes of tumour cell proliferation. In turn, the shorter survival of patients with high expression of MT pointed to prognostic significance of the protein in non-small cell lung cancers.

Key words: metallothionein, nonsmall cell lung carcinoma.

ADDRESS FOR CORRESPONDENCE:
Piotr Dzięgiel
Chair and Department of Histology and Embryology
University School of Medicine in Wrocław,
Chałubińskiego 6A; 51-650 Wrocław, Poland
tel. +4871/784-00-81; fax. +4871/784-00-82;
e-mail: piotr@hist.am.wroc.pl

Introduction

Metallothioneins (MT) are low molecular weight (7 kDa) proteins. They consist of a polypeptide chain of 61 to 68 amino acids, of which around 30% are cystein residues. Due to their structure and function, four basic types of the protein are distinguished: MT-I, MT-II, MT-III and MT-IV, coded by genes, located in chromosome 16 [1]. MT are commonly present in normal tissues, as well as in cells of various tumours [2, 3, 4]. The earliest recognized function of MT involves their capacity to bind both toxic ions (Cd, Pb, Hg) and ions indispensable to the body (Zn, Cu). Sharing the potential to bind Zn ions, MT act as controllers of zinc-dependent enzymes, which participate in DNA replication, transcription, translation and in cell metabolism [1]. Augmented expression of MT in cells of some tumours is linked with worse prognosis, resistance to cytostatic drugs and to radiotherapy [2, 3]. The group of non-small cell lung carcinomas (NSCLC) includes squamous cell cancer, adenocarcinoma and large cell carcinoma. In such patients, the prognosis used to be difficult to define, among others, due to frequent non-uniform histological character of NSCLC. This creates problems in the selection of effective post-surgery treatment (chemo- and/or radiotherapy) [5]. The present study aimed at demonstrating the prognostic significance of MT expression in NSCLC cells and in the attempts to correlate the intensity of its expression with malignancy grade, G and with the intensity of expression of the proliferation-associated antigen, Ki-67 antigen.

Figure 1. Expression of metallothionein (MT) in cell nuclei and cytoplasm of cells in squamous cell lung cancer. x200; background staining with haematoxylin.

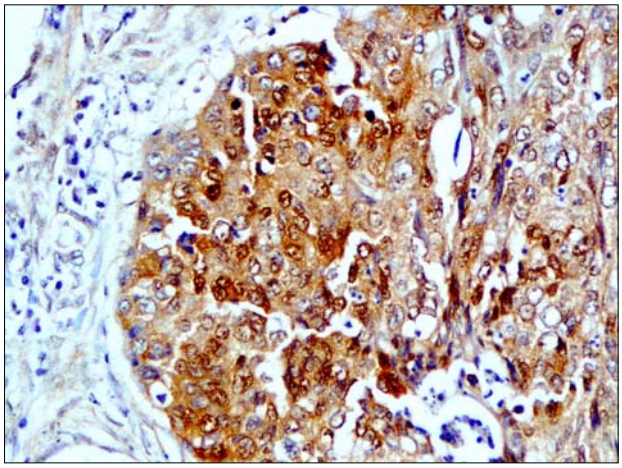
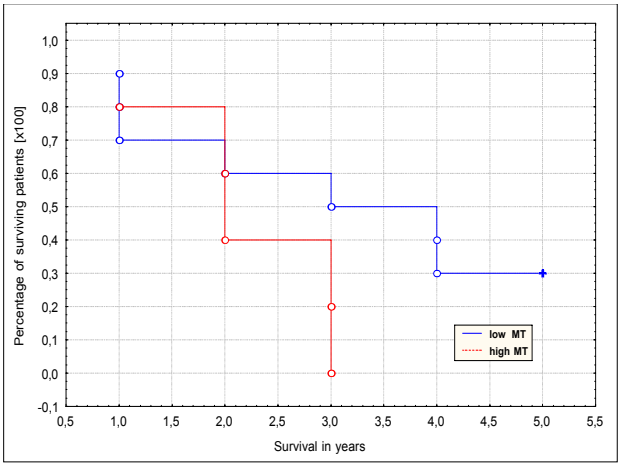


Figure 2. Duration of patients' survival in non-small cell lung cancers NSCLC) of a low (1-4 pts, n = 7) or a high (6-12 pts, n = 8) expression of metallothionein (MT).



Material and Methods

The studies were performed on archival paraffin blocks, containing 25 histopathologically verified cases of NSCLC (T2N0 - 5 squamous cell carcinomas, 20 adenocarcinomas), subjected to surgery during the years 1998-99 at the Lower Silesia Centre of Tuberculosis and Lung Diseases in Wrocław. In paraffin sections of studied tumours, immunocytochemical reactions were performed, using mouse monoclonal antibodies, directed to MT-I and MT-II (clone E9) and to Ki-67 (clone MIB-1). The investigated antigens were visualised, using LSAB2 kit and diaminobenzidine (DAB). All the antibodies and reagents were purchased in DAKOCytomation (Denmark). MT expression was evaluated, using the semiquantitative IRS scale, according to Remmele [6] (0-12 points), which took into account the intensity of the colour reaction and the number of positive cells. The expression of Ki-67 antigen was appraised, considering the proportion of cells with nuclear colour reaction (0 pts - lack of reaction; 1 pt - 1-10%; 2 pts - 11-25%; 3 pts - 26-50%; 4 pts - over 50% positive cells). Grade of malignancy, G, was evaluated as recommended by WHO [7]. Clinical data (TNM classification, duration of survival) were recovered from the archives of the Lower Silesia Centre for Tuberculosis and Lung Diseases in Wrocław. The obtained results were subjected to statistical analysis, using the STATISTICA PL software (StatSoft, Poland) and employing Spearman's correlation tests and survival analysis of Kaplan-Meier.

Results

In all the examined tumours, variable expressions of MT and of Ki-67 antigen were observed. The most pronounced intensity of nuclear-cytoplasmic expression of MT (Fig. 1) and of Ki-67 antigen was noted in squamous cell cancers. The analysis of correlations between the expressions of MT, Ki-67 antigen and G grade of malignancy demonstrated a strong positive relationship between the two latter variables ($r = 0.7$; $p < 0.05$). Less pronounced positive correlations were disclosed between MT expression and G grade ($r = 0.44$; $p < 0.05$) and between MT expression and the expression of Ki-67 antigen ($r = 0.41$; $p < 0.05$). In addition, in 15 cases of NSCLC (7 squamous cell cancers, 8 adenocarcinomas), survival analysis was performed which demonstrated shorter survival times in patients with high expression of MT (Fig. 2).

Discussion

NSCLC represent a non-uniform group of tumours, regarding the histological structure, clinical course or the prognosis. This creates problems in the selection of appropriate therapeutic approach [8]. For several years, independent markers have been searched for, which could help in choosing an effective way of treatment after removal of the primary tumour. The expression of MT in normal cells and in some tumours is linked to cell proliferation and differentiation [9]. This has prompted us to examine the intensity of expression of the protein in NSCLC in attempts to use it in prognostic evaluation of the patients. However, the few papers, published on the subject till now, have presented contradictory results. Tcheocharis and collaborators [10] dealt with a group of 89 NSCLC, evaluating by immunocytochemistry, i.a., the expression of MT-I and MT-II. In part, they obtained results similar to those obtained by us, noting the highest intensity of MT expression in squamous cell cancers and a less pronounced expression in adenocarcinomas. However, in contrast to us, they found no correlation between MT expression and the other clinical and pathological variables. Volm et al. [11], as well as Mattern et al. [12], examined NSCLC in respect to their resistance to cytostatic drugs. Both in *in vitro* and *in vivo* experiments, they noted more extensive resistance to doxorubicin of MT (+) cells. Moreover, the intensity of MT expression was positively related to malignancy grade G and to the expression of another protein responsible for multi-drug resistance to cytostatic drugs, glutathion-s-transferase-Π. Similarly to tumours of other organs, the results obtained by us in NSCLC demonstrated a positive correlation between the expression of MT and Ki-67 antigen [2, 13]. This indicated participation of the protein in the processes of NSCLC cell proliferation. In turn, the shorter survival of patients with high MT expression may argue for prognostic significance of its expression in NSCLC.

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