Expression of the candidate tumor suppressor gene nm23 in the bronchial system of patients with squamous cell lung cancer

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Abstract

Objective: A number of oncogenes and tumor suppressor genes participating in tumorigenesis have been identified, one of them being nm23. The expression of the candidate tumor suppressor gene nm23 depends on the cell type of tumors. Both, reduced expression as well as overexpression of nm23 is associated with a high potential of malignancy. In a variety of tumor cell lines secretion of the nm23 protein can be detected. In an earlier investigation we showed, that the nm23 expression in squamous cell lung carcinomas is considerably elevated. In order to establish the potential diagnostic value of this finding we investigated the nm23 expression in healthy and diseased lungs in patients with squamous cell lung cancers.

Methods: We examined bronchial lavage samples of 20 patients with bronchogenic squamous cell carcinoma. The lavage was separately performed in the bronchus of the tumor bearing lobe and in the corresponding bronchus of the unaffected contralateral lung.

Results: Using Western blot analysis we found 2–7 fold elevated amount of nm23 protein in the bronchial lavage of the tumor bearing lung in comparison to the healthy side. This finding was neither related to tumor stage nor to tumor location. Thus we have a strong hint that the nm23 protein is secreted by the bronchogenic squamous cell carcinoma. Conclusions: With respect to these results the proof of nm23 protein in bronchial lavage fluid might be of relevance to establish the diagnosis when pulmonary nodules of unknown etiology are found. © 1997 Elsevier Science Ireland Ltd.

Keywords: Nm23 gene expression; Squamous cell lung cancer; Bronchial lavage

1. Introduction

A variety of oncogenes and tumor suppressor genes have been identified, which can, if they malfunction, produce tumorigenic transformation and metastatic dissemination [4–6,14,22,34]. One of these genes putatively involved in regulating metastases is nm23 [31]. The nm23 gene was originally identified in differential colony hybridization experiments involving murine K-1735 melanoma sublines with low and high metastatic potential. The expression of the nm23 gene has been shown to be inversely related to the metastatic potential of these melanoma cells [31]. In consequence ‘nm’ is an acronym for ‘non-metastatic’. Two distinct nm23 genes, nm23-H1 and nm23-H2, have been isolated [29,30], and mapped to the same region of chromosome 17 [3]. Both genes encode the 88% identical nucleoside diphosphate kinase (NDPK) A and B polypeptides, respectively [11]. However, the relation between enzymatic and antimetastatic effects is largely unknown. Depending on the cell type the malignant potential of several tumors is correlated both with reduced nm23 expression, and with overexpression of nm23 [7,28,32]. Recently nm23 protein with NDPK activity was found in culture supernatants and in the membrane fraction of mouse myeloid leukemia cells [26]. In earlier investigations we

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could demonstrate a strong expression of nm23 in squamous cell lung carcinoma and found a positive correlation between the nm23 gene expression and the tumor stage [8,19].

This, and the finding of nm23 protein with NDPK activity in culture supernatants prompted us to investigate whether NDPK can be detected in the bronchial secretion of patients with squamous cell carcinoma of the lung.

2. Material and methods

2.1. Bronchial lavage

We collected preoperatively bronchial lavage samples of 20 patients with squamous cell carcinomas of the lung. The patients were intubated with a Robertshaw tube and remained in supine position for the procedure. During the bronchoscopical control of the tube position a bronchial lavage with 20 ml of saline solution was performed twice in the bronchus belonging to the tumor bearing lobe as well as in the corresponding lobe of the unaffected contralateral lung (Olympus LF-2 bronchoscope, Olympus Optical). The fluid was injected with low pressure and sucked back immediately. Thus between 5–10 ml of fluid could be extracted during each procedure.

2.2. Western blotting

The bronchial lavage samples were centrifuged at 10 000 × g for 15 min to remove cells and solid material. No further processing of the sediment was performed with respect to existence or amount of malignant cells. Aliquots (20 µl) of each supernatant were mixed with SDS sample buffer, boiled for 1 min and applied to 14% polyacrylamide SDS gels. After electrophoresis, the samples were transferred to PVDF membranes (Millipore) by semi-dry blotting. The membranes were incubated with anti-nm23 polyclonal antibodies which recognize both nm23-H1 and nm23-H2 proteins. The final detection was done using horseradish peroxidase coupled secondary antibody in combination with an enhanced chemiluminescence (ECL) detection kit (Amersham). Nm23 proteins were quantified by densitometric analysis. The value of the nm23 protein level in the bronchial secretion of the unaffected lung was defined as 1.0 and the value of the nm23 protein level in the bronchial secretion of the tumor bearing lobe was referred to it.

3. Results

The tumors were classified according to the TNM staging system of the UICC published in 1987 [17]. Eight patients were in stage I, three patients in stage II, and ten patients in stage IIIa, respectively. The nm23 protein concentrations were 2–7 fold higher in the bronchial lavage of the tumor bearing lung. We found this constellation in every tumor stage without significant differences between the stages (Table 1). In a second analysis of the same data the patient population was divided into three groups.

**Group A**: the preoperative diagnosis was made from biopsy specimen obtained bronchoscopically from a visible, i.e. central tumor.

**Group B**: the tumor was not visible bronchoscopically. The diagnosis of non-small cell lung cancer was made up cytologically from endoscopically gained material however.

**Group C**: patients with peripheral tumors in whom the diagnosis squamous cell carcinoma was made after histological examination of the resected specimen.

Group A comprised 8 patients, group B 7 patients, and group C 5 patients, respectively. In all groups we found the same constellation of higher nm23 concentrations in the lavage fluid from the tumor bearing lobe. However, there existed no significant differences of the nm23 gene expression between the three groups, (Table 2).

<table>
<thead>
<tr>
<th>Tumor stage</th>
<th>n</th>
<th>Elevation*</th>
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<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>2–6 fold higher expression</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>2–5 fold higher expression</td>
</tr>
<tr>
<td>IIIa</td>
<td>9</td>
<td>2–7 fold higher expression</td>
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*Elevation of the nm23 protein levels in tumorous lungs compared to the healthy lung of the same patient.

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**Table 1**

Nm23 protein expression in the bronchial lavage

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4. Discussion

Two human nm23 genes have been identified, designated nm23-H1 and nm23-H2 [29, 30], which encode the NDPK A and NDPK B polypeptides, respectively [11]. The function of NDPK is to supply all nucleoside and deoxynucleoside triphosphates except ATP to the cell. This main enzymatic function of nm23 protein appears not to be involved in the process of tumorigenic transformation and metastatic dissemination [12]. The first link between the nm23-H2 gene and tumor progression was provided by the identification of the nm23-H2 protein as a transcription factor [27]: a protein that initiates the activity of cellular myc-oncogene, which is known to have cancer causing potential. In addition, nm23 proteins have been shown to interact with GTP-binding proteins [20]. Regulation of microtubule polymerization in the mitotic spindle is also an nm23 protein function [25]. A more recent study provided evidence that nm23 proteins are capable of transferring a phosphate group to other proteins. This function as protein phosphotransferase possibly plays a role in signal transducing process of the cell and therefore could explain the regulatory function of nm23 [9].

In human breast tumors [16, 18, 31], in malignant melanomas [10], and in hepatocellular carcinomas [24] lower levels of nm23 gene products are correlated with a poor prognosis. In contrast there is an increased nm23 gene expression in neuroblastomas [13, 21], in human pancreatic cancer [23], as well as in colon carcinomas [2, 15] positively correlated with a poor prognosis.

In healthy lung tissue only a weak expression of the nm23 gene takes place whereas in squamous cell carcinoma tissue a 2–18 fold increase of nm23-mRNA is found [19]. As nm23 protein with NDPK activity is not only detected in the tumorous tissue itself but also in supernatants of tumor cell cultures [1, 26, 33] similar patterns of behaviour could be expected in the bronchial secretion of patients with squamous cell carcinoma of the lung. This was confirmed in the present investigation.

A major difficulty consists in the lack of ‘norm levels’ of nm23 protein in healthy lung tissue which are gone beyond in tumors. Thus until now only the intra-individual comparison of the activity of nm23 in lavage fluid from the healthy as well as the diseased lung proved to be meaningful. Considering these prerequisites a 2–7 fold higher activity of nm23 protein in bronchial lavage fluid of tumor bearing lungs compared with the contralateral lung is found. This constellation did not depend upon the tumor stage nor on the location of the malignancy (i.e. central or peripheral). The individual levels of nm23 protein showed a wide range of variation but no correlation between the activity and the size and location of the malignancy could be stated. Therefore a further processing of the sediment was omitted. After resection of the diseased lung tissue further intraindividual comparisons are impossible. Thus follow-up studies were not performed.

The diagnostic relevance of testing the nm23 protein levels in bronchial lavage fluid should not be overestimated, particularly, because nm23 levels were not yet measured in patients with inflammatory processes or benign tumors. Nevertheless the investigation appears meaningful in the work of yet undiagnosed unilateral pulmonary nodules, as well as after resection of squamous cell lung cancer, if recurrent intrapulmonal nodules appear to be metastasis, or if local recurrence in the bronchus stump after lobectomy is suspected but cannot be proved.

The present paper makes the following conclusions:

1. A secretion of nm23/NDPK by squamous cell lung cancer into the bronchial system takes place.
2. The amount of elevation of nm23 proteins seems to be independent of size and location of tumors.
3. In consequence of the wide range of variation of gene activity only intraindividual evaluations are meaningful and of diagnostic relevance until now.

References


Appendix A. Conference discussion.

Dr J. Hasse (Freiburg, Germany): Thank you very much, Dr Huwer, for this fine investigation. Let me ask you two questions. How much alveolar lavage are you asking to get back? Unfortunately, the test applies only for squamous cell carcinoma, or at least not for adenocarcinoma. So this is a limitation of your conclusions and outlook, that it could serve as a screening for carcinoma. Let me just continue with a short question. What is the cost of the Western blot in this investigation?

Dr H. Huwer: Firstly, we have performed bronchial lavages, not bronchoalveolar lavages and we got back about 20–50% of the lavage fluid. Secondly, we did not investigate bronchial lavage samples of patients with adenocarcinomas. With respect to your second question, our former investigations dealt with the nm23 gene expression in the primary tumor and lymph node metastases of squamous cell carcinomas. We believe that we are able to characterize and interpret the levels of nm23 gene expression in the base of mRNA and protein, whereas these investigations are still lacking as far as adenocarcinomas are concerned. Thirdly, the costs of Western blot kits of nm23 protein/NDPK are about 500DM for analysis of 20 samples.

Dr K. Moghissi (Hull, UK): I think it is fair to say that if you want to conclude that the nm23 expression has relevance in the diagnosis, you have to show that after you have actually resected the tumor that you would have a negative result. Have you done that?

Dr Huwer: We could show that the nm23 protein levels are lower in the healthy, unaffected contralateral lung of the same patient, but you have to show that after you have actually resected the tumor that you would have a negative result. Have you done that?

Dr W. Klepert (Vienna, Austria): My question is quite similar to Dr Moghissi’s question. Have you done any investigation of the nm23-suppressor gene in benign conditions with chronic inflammation? And, on the other hand, have there been any tumors with gross inflammation in your series, and was there any difference between small tumors without inflammation and those with a large amount of poststenotic inflammation?

Dr Huwer: In our paper we found that even in the bronchus of lobes with peripheral coin lesions the nm23 protein concentration was elevated, but we have not found a correlation of nm23 with inflammatory processes.