

CD10 expression in non-small cell lung cancer

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Abstract. CD10 is a cell surface endopeptidase that inactivates various potentially growth stimulatory peptides. In lung cancer cell lines this downregulation has been associated with increased proliferation. Downregulation of CD10 in lung cancer tissue is described, suggesting a potential role in carcinogenesis and a possible use of CD10 as a prognostic marker. We aimed to determine the rate of CD10 expression in our non-small cell lung cancer (NSCLC) collection and to clarify its correlation with clinicopathological parameters and patient survival. 114 NSCLC were analysed immunohistochemically using a monoclonal CD10 antibody (clone NCL-CD10-270) on an NSCLC tissue micro array. The staining was semiquantitatively scored. CD10 expression was observed in 19% of cases, without any significant association with tumour type, -size, -grading, nodal status, clinical stage, and patient survival time. We conclude that a diagnostic use of CD10 immunostaining in NSCLC is unlikely.

Keywords: Non-small cell lung cancer, CD10, tissue micro array, immunohistochemistry

1. Introduction

Lung cancer is a major cause of death from neoplastic malignancy in the western world. In the USA alone 169,500 new cases were expected for the year 2001. The mortality rate of lung cancer is amongst the highest of all cancer types, with 157,400 expected deaths for 2001 alone [8]. In the last 20 years mortality rate could be lowered only by 6% due to improved therapies. So far the most promising approach appears to be primary and secondary prevention, as shown in recent studies [10].

The molecular biology of NSCLC is under intense international investigation in order to characterise new molecular marker genes which might be helpful in diagnosis or therapy [18]. A gene that has gained interest in neoplasia is CD10 (CALLA, NEP, enkephalinase). This is a cell surface bound 90–110 kDa endopeptidase which inactivates various potentially growth stimulatory peptides as bombesin, substance P, endothelin-1 and others [20,21]. Physio-

logically, CD10 is widely expressed in various tissues, e.g., granulocytes, lymphoid progenitor cells, enterocytes, placental trophoblast, glandular epithelium of prostate and gallbladder, myoepithelial cells, Schwann cells and renal tubulus epithelium [2,15]. In neoplasia CD10 expression is described in renal cell carcinoma, hepatocellular carcinoma, pancreatic solid-pseudopapillary tumour, gestational trophoblastic diseases (GTD) and endometrial stroma sarcoma [1–3, 11,16,24]. CD10 is a common diagnostic marker for the subclassification of acute leukaemias and non-Hodgkin lymphomas [13]. Furthermore, its expression has been associated with the chromosomal translocation t(14;18)(q32;q21) in diffuse large B-cell lymphoma [6]. In prostate cancer CD10 expression is often downregulated compared to normal prostate glands. This loss of expression has been linked to an androgen independent and thus more aggressive cancer phenotype [5,17,19].

There are few studies on the expression of CD10 in lung cancer. A downregulation of CD10 was found in a small set of small-cell lung cancer (SCLC) and NSCLC alike, though no clinicopathological associations were investigated [4,7,21].

The purpose of this study was to evaluate CD10 expression in a larger set of NSCLC and to examine its

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association with clinicopathological parameters, disease stage according to UICC and, most important, patient survival to clarify its possible use as a diagnostic or prognostic marker in this disease.

2. Patients and methods

2.1. Tumour samples

Our collective consisted of 114 patients between age 34 and 80 (median 63 years) who underwent thoracotomy for resection of NSCLC in the Department of Surgery of Charité University Hospital from 1995–1997. Clinical follow-up data was available of 78 patients. No adjuvant radiotherapy or chemotherapy was applied before surgery. Table 1 summarises the clinicopathological characteristics according to TNM criteria of the UICC [22]. The histopathological diagnosis was established in every case according to the WHO guidelines [23].

2.2. Tissue array generation

A tissue array was constructed as previously described [14]. Briefly, suitable areas for tissue retrieval were marked on standard haematoxylineosin (HE) sections, punched out of the paraffin block and inserted into a recipient block. The tissue arrayer was purchased from Beecher Instruments (Woodland, USA). The punch diameter was 0.6 mm. The lung tumour ar-

ray consisted of 127 NSCLC tissue samples. To allow comparison of the tissue array immunostaining with conventional slides, ordinary paraffin sections were cut from 18 cases that were incorporated in the tissue array.

2.3. Immunohistochemistry

The tissue array block was freshly cut (4 μ m), sections were mounted on superfrost slides (Menzel-Gläser, Germany) and dewaxed with xylene and gradually hydrated. Antigen retrieval was achieved by pressure cooking in 0.01 M citrate buffer for 5 minutes. The primary CD10 antibody (clone NCL-CD10-270, Novocastra, Newcastle, UK) was diluted 1:50 using a background reducing dilution buffer from DAKO (Germany). No other blocking agents were employed. The primary antibody was incubated at room temperature for 1 h. Detection took place by the conventional labelled-streptavidin-biotin (LSAB-kit, DAKO) method with alkaline phosphatase as the reporting enzyme according to the manufacturer's instructions. Fast-Red (Sigma-chemicals, Deisenhofen, Germany) served as chromogen, afterwards the slides were briefly counterstained with haematoxylin and aqueously mounted.

The CD10 staining was independently examined by two clinical pathologists (GK, YY). The staining intensity was semiquantitatively scored as absent, weak and strong (0, +, ++-) for each case. The results of both observers were identical but for a single case, which demonstrates the robustness of this grading system. Two tissue arrays were processed: the staining results of both arrays were almost identical. In only two cases different staining intensities were noted and the higher score was chosen for statistical analysis.

2.4. Statistical analysis

A two sided Fishers exact test was used to determine the strength of association between the investigated parameters. To compare the expression of CD10 expression with clinicopathological parameters, 2×2 contingency tables (e.g., CD10 score 0 vs. 1–2 and G1&G2 vs. G3) were set up and the resultant p-value was calculated. Cumulative survival curves were calculated according to the Kaplan–Meier Method, differences in the survival were assessed with the log-rank-test. P-values ≤ 0.05 were considered significant. All calculations were performed on a PC using the statistical software package SPSS, version 10.

Table 1
Study cohort

	CD10 negative	CD10 positive
Total No. (n = 114)	92	22
Adeno	43	12
SCC	47	10
LCLC	2	0
G1/G2 ¹	49	12
G3	43	10
pT1	21	4
pT2+ ²	71	18
pN0	50	16
pN1+ ³	42	6
Stage I–IIa	50	15
Stage IIb–IV	42	7

¹ G1 (n = 3), G2 (n = 58).

² pT2 (n = 77), pT3 (n = 10), pT4 (n = 2).

³ pN1 (n = 21), pN2 (n = 25), pN3 (n = 2).

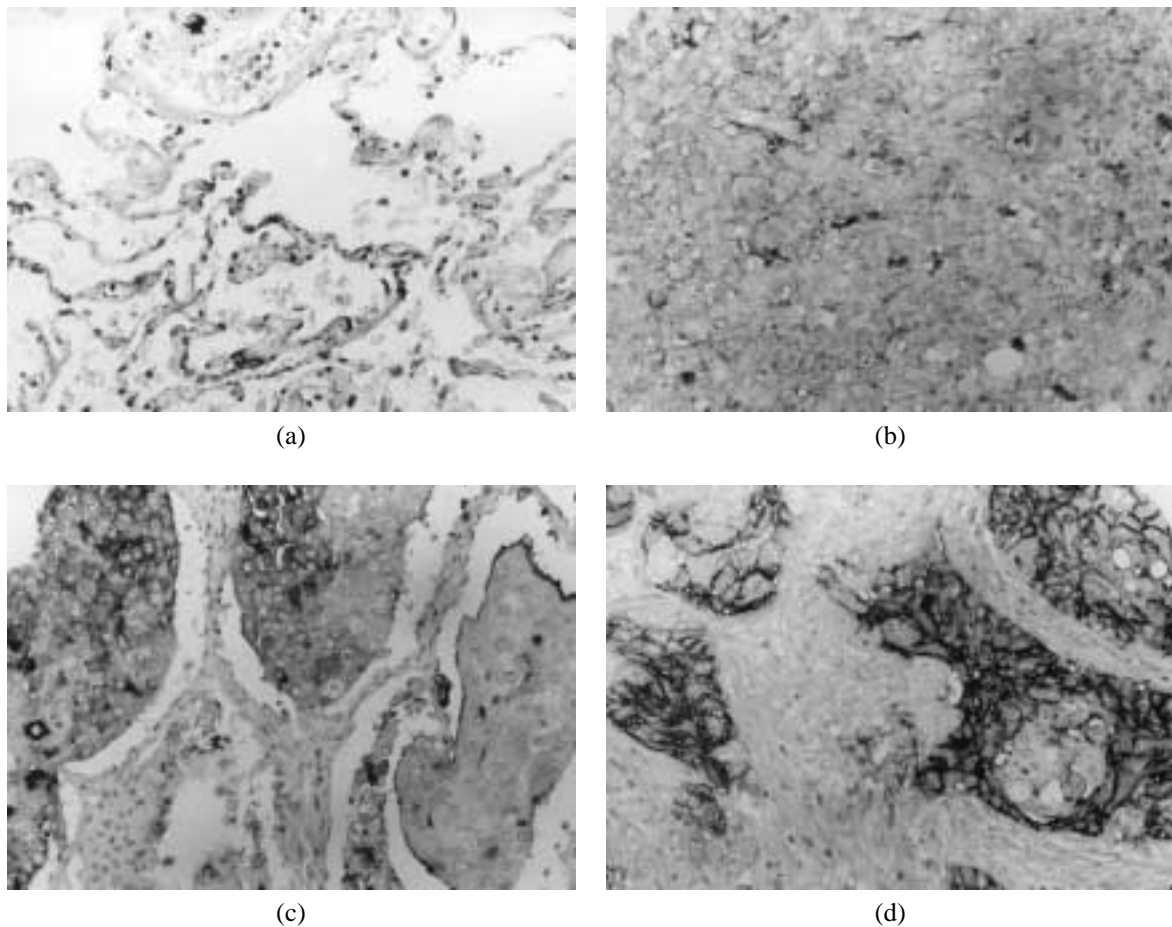


Fig. 1. CD10 Immunohistochemistry in NSCLC. (a) Normal lung parenchyma with CD10 expression in alveolar capillaries, (b) adenocarcinoma with weak CD10 expression (1+), (c) adenocarcinoma and (d) squamous cell carcinoma with strong CD10 expression (2+).

3. Results

3.1. Immunohistochemistry

Normal lung parenchyma showed no staining of mature pneumocytes or bronchial epithelium, only alveolar capillaries were strongly positive, whereas endothelia of greater vessels were negative (Fig. 1a). In tumours CD10 immunoreactivity was observed in 22 (19%) cases with a cytoplasmic and membranous staining pattern in adenocarcinomas and squamous cell carcinomas.

The gross sections showed a homogeneous staining pattern of the tumours which is a necessary condition to obtain reliable data from small tissue array punches. Moreover, on direct comparison of intensity grades given to conventional sections and arrayed tissue of the same cases ($n = 18$), we found 18 identical

scores. This clearly demonstrates the representativity of the CD10 staining of the tissue punch.

On the tissue array 92 cases (81%) exhibited no CD10 staining of the tumour. In 13 cases (11%) a weak staining was observed (Fig. 1b). Nine cases (8%) showed a strong staining signal (Fig. 1c,d). Taking group two and three together, 19% of tumours showed an expression of CD10 in this study (Table 1). This was opposed to no CD10 expression in the statistical analysis.

3.2. Statistical analysis

No correlation of CD10 expression was found with tumour type ($p = 0.64$), grading ($p = 1$), pT ($p = 0.78$), pN ($p = 0.15$) or disease stage according to UICC ($p = 0.34$). On Kaplan-Meier analysis of survival times no significant differences of CD10 positive or negative tumours became apparent (Fig. 2).

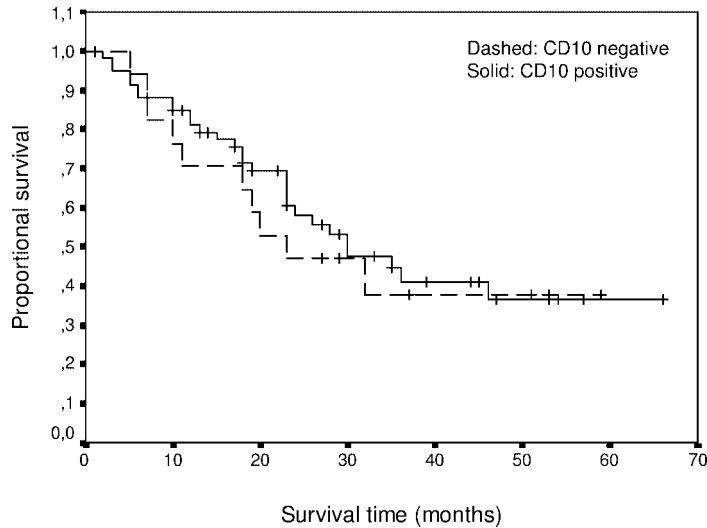


Fig. 2. Survival analysis: The Kaplan–Meier curve demonstrates no significant difference between CD10 positive and negative tumours.

The mean survival time of CD10 negative tumours was 37 months compared to 32 months for CD10 positive tumours ($p = 0.66$).

On stratification of the cases according to tumour type no significances could be demonstrated for either adenocarcinomas (grading $p = 0.75$; pT $p = 1$; pN $p = 0.21$; stage $p = 0.75$) or squamous cell carcinomas (grading $p = 0.74$; pT $p = 0.67$; pN $p = 0.47$; stage $p = 0.29$). The mean survival time of CD10 positive vs. negative tumours did not differ significantly in both subgroups (adenocarcinomas $p = 0.83$; squamous cell carcinomas $p = 0.69$). Even in the subgroup of small tumours without nodal metastases (pT1/2&pN0; $n = 63$) CD10 expression correlated neither to clinicopathological parameters (grading $p = 0.55$; tumour type $p = 0.87$) nor survival times ($p = 0.55$).

4. Discussion

In this study 114 NSCLC cases were immunohistochemically examined for the expression of CD10 protein using a well characterised monoclonal antibody. To our knowledge this is the largest set of tumours investigated in this respect so far.

We found that normal lung parenchyma has an expression of CD10 in alveolar capillaries exclusively, with no CD10 expression in pneumocytes, a finding that contradicts previous papers which found CD10 in pneumocytes [4,12]. Bronchial epithelium had no CD10 reactivity. In NSCLC 19% of cases had any

CD10 expression and only 8% had a strong expression. This is in concordance with published data: Chu et al. described an immunohistologically determined CD10 positivity of 18% ($n = 11$) of lung adenocarcinomas [2]. Cohen et al. found an expression rate (immunohistochemistry) of 12.5% ($n = 24$) in NSCLC and interpreted this in comparison to normal lung parenchyma as a downregulation [4]. The idea of CD10 as a down-regulated tumour suppressor gene is more tempting as *in vitro* evidence further supports it: in cell lines the inhibition of CD10 expression was linked to increased proliferation rates [7,21]. This endorses the hypothesis, that loss of CD10 expression could be linked to a more aggressive tumour behaviour. Another tumour entity for which a CD10 downregulation has been found is prostate cancer. Here, CD10 downregulation is especially marked in metastatic, androgen independent tumours, which usually have an unfavourable prognosis [5,17,19].

We found no association of CD10 expression in NSCLC with patient survival or any other clinicopathological parameter in our tumour sample. These findings do not encourage to use CD10 as a diagnostic or prognostic marker in surgical pathology. Still, we cannot rule out a possible prognostic role of CD10 expression in very specialized subsets of lung cancer which were not adequately represented in our study, e.g., very small (<1 cm) centrally localized squamous cell carcinomas without nodal metastasis. Further studies will have to address this issue.

Methodologically, we found the usage of tissue arrays very convenient to help processing a large set

of cases. The validity of this approach was tested for CD10 by comparison of the staining results with matching conventional sections, which we think is a necessary control when using tissue micro arrays. In addition, the data generated from tissue micro array analysis can be the basis for further correlations with the findings of other methods [9,14].

5. Conclusion

In summary, we found that CD10 is expressed in 19% of NSCLC without any predilection of tumour type, tumour size, nodal status and disease stage. No association of CD10 expression and prognosis could be demonstrated. We conclude that a diagnostic use of CD10 immunostaining in NSCLC is unlikely.

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