Ann Hematol (1994) 68:81–83

DGHO PRESENTATION

M. Böhm · B. Totzeck · I. Wieland

Differences of E-cadherin expression levels and patterns in human lung cancer

Received 2 November 1993 / Accepted 8 November 1993

Summary Fifty-two lung carcinomas obtained at surgical resection were examined by immunofluorescence for their expression levels and patterns of the calcium-dependent intercellular adhesion molecule E-cadherin. In well-differentiated squamous cell and adenocarcinomas expression of E-cadherin was confined to the lateral cell border, similar to the expression level and pattern of normal lung tissue. The E-cadherin level was reduced and the expression pattern was spotty or diffuse in moderately and poorly differentiated squamous cell carcinomas and in small cell carcinomas of the lung. Also, most metastases resected had a reduced level and an altered pattern of E-cadherin expression. In contrast, no such correlation was found in adenocarcinomas of the lung. This indicates that different cellular mechanisms are responsible in the progression of squamous cell carcinomas versus adenocarcinomas of the lung.

Key words E-cadherin · Lung cancer
Dedifferentiation · Metastasis

Introduction

Progression of a tumor to malignancy includes profound alterations in cell-to-cell and cell-to-substratum interactions. These alterations are evident in epithelial tumors when malignant cells begin to invade the surrounding tissue and metastasize to distant organs. Apparently, a prerequisite for this process is that the carcinoma cells detach from their primary site and become motile, i.e., that these cells have reduced adhesion to neighboring tumor cells. E-cadherin is a mediator of epithelial cell-to-cell adhesion and is considered to be an important member of the family of calcium-dependent cell-adhesion molecules: E-cadherin plays an essential role in organogenesis during embryonic development and in maintaining the histoarchitecture in adult tissue [1].

In human squamous cell carcinoma of the head and neck, lack of E-cadherin expression is correlated with dedifferentiation and metastasis [2]. So far, there are only few studies of E-cadherin expression in lung carcinomas, and an association of down-regulation of E-cadherin expression and dedifferentiation and invasiveness has been described only in some lung carcinoma cell lines [3, 4]. In this study we analyzed expression levels and expression patterns of E-cadherin by immunofluorescence staining of surgically resected normal lung tissue and lung carcinomas.

Materials and methods

Biopsies of lung carcinomas and of normal lung tissue were resected from 52 patients undergoing routine surgery. The biopsies were immediately frozen and stored in liquid nitrogen; the time between ligation of the supplying blood vessels and freezing was less than 15 min. Serial 6-μm frozen sections were stained with hematoxylin/eosin, or incubated with anti-E-cadherin monclonal antibody 6F9 (Boehringer Mannheim) or anti-pancytokeratin monoclonal antibody KL1 (Dianova) as described elsewhere [2]. Dichlorotriazinyl-aminofluoresceine-conjugated polyclonal goat anti-mouse antibody (Dianova) was used as a second fluorescent antibody. Only vital tumor tissue representative of routine formalin-fixed paraffin-embedded diagnostically relevant tumor tissue was evaluated under the microscope with respect to expression level and expression pattern. Histological classification and grading of the tumors was made according to the criteria of the World Health Organization [5].

I. Wieland (✉) · M. Böhm[1]
Institut für Zellbiologie (Tumorforschung),
Universitätsklinikum,
Virchowstrasse 173, D-45122 Essen, Germany

B. Totzeck
Chirurgische Abteilung, Evangelisches Krankenhaus,
Hordeler Strasse 5–7, D-44651 Wanne-Eickel, Germany

This work was presented at the Annual Congress of the German and the Austrian Society of Hematology and Oncology, Essen, 10–13 October 1993

[1] *Present address:* Urologische Klinik, Universitätsklinikum, Hufelandstrasse 55, D-45122 Essen, Germany

Table 1 E-cadherin expression levels and patterns in human lung carcinomas

	Expression level[a]			Expression pattern[b]		
	++	+	−	Linear	Spotty	Diffuse
Squamous cell carcinomas[c]						
G1	**3**	0	0	**2**	1	0
G2	6	**14**	4	3	**13**	4
G3	2	3	**4**	0	**3**	2
Adenocarcinomas[c]						
G1	**3**	0	0	**3**	0	0
G2	**6**	1	1	**5**	1	1
G3	**4**	1	0	**3**	1	1
Small cell carcinomas[c]						
G4	0	3	1[d]	0	2	1

[a] E-cadherin expression level was classified as normal (++) when fluorescence intensity was similar to that of normal epithelium and more than 90% of the tumor cells were stained, as reduced (+) when fluorescence intensity was less than half of that of normal epithelium and/or 90–95% of the tumor cells were stained, and as absent (−) when the staining was not distinguishable from background or less than 5% of the tumor cells were stained
[b] E-cadherin expression pattern was classified as linear when staining was uninterrupted and confined to the cell borders, as spotty when it was interrupted but confined to the cell borders, and as diffuse when it was not confined to the cell borders
[c] Histological grading according to WHO criteria [5]. The largest group for each grade is printed in bold numbers. In four biopsies both squamous cell and adenocarcinomas were distinguished and were evaluated separately
[d] Metastasis obtained by mediastinoscopy

Table 2 E-cadherin expression levels and patterns in primary lung carcinomas and their metastases. Only pairs, consisting of primary tumor (*P*) and its metastasis (*M*), are listed

	Expression level[a]			Expression pattern[b]		
	++	+	−	Linear	Spotty	Diffuse
Squamous cell carcinomas[c]						
G2	P	M			P	M
G2	P		M		P	
G2		P	M		P	
G2		P	M		P	
G2		P	M		P	
G3		P, M			P, M	
Adenocarcinoma[c]						
G2			P, M			
Small cell carcinoma[c]						
G4		P	M		P	

[a–c] See footnotes to Table 1

Results

The 52 lung carcinomas consisted of 36 squamous cell carcinomas, 16 adenocarcinomas, and four small cell lung carcinomas (summarized in Table 1). Of the squamous cell carcinomas three were well differentiated, 24 were moderately differentiated, and nine were poorly differentiated. The well differentiated carcinomas expressed E-cadherin at levels found in the normal lung epithelium, and in two of the three E-cadherin was confined to the lateral cell borders. In 14 of 24 moderately differentiated forms, however, the amount of E-cadherin was reduced and expression appeared in a spotty or diffuse pattern. An additional four expressed no E-cadherin. In seven of nine poorly differentiated squamous cell carcinomas E-cadherin was either reduced and expressed in a nonlinear pattern or absent. Down-regulation of E-cadherin expression was also observed in all lymph node metastases of squamous cell carcinomas (Table 2). These results suggest that down-regulation and/or alterations in the expression pattern of E-cadherin correlates with a less differentiated and more malignant phenotype of squamous cell lung carcinomas. In the few small cell lung carcinomas examined here, E-cadherin was also down-regulated and expressed in a nonlinear pattern (Table 1).

In contrast, most (10/13) moderately and poorly differentiated adenocarcinomas of the lung expressed E-cadherin at about normal levels and in a linear pattern

(Table 1). The difference between squamous cell and adenocarcinomas was evident even within the same carcinoma: In lung carcinomas of the adenosquamous type, the adenoid parts exhibited a normal, linear E-cadherin expression, while the squamous parts showed a reduced, spotty expression. A weighted analysis of variance showed a correlation between grading and the E-cadherin expression level in squamous cell carcinomas ($p=0.02$), but not adenocarcinomas ($p=0.77$). It also showed a correlation between grading and the E-cadherin expression pattern in squamous cell carcinomas ($p=0.03$), but not adenocarcinomas ($p=0.30$).

Discussion

In a previous study, loss of E-cadherin expression was demonstrated to be associated with progression of squamous cell carcinomas of the head and neck, i.e., carcinomas that originate in the mucosa of the aerodigestive tract [2]. No such close association has been reported for lung carcinomas [3], although these tumor cells are derived from related epithelia of endodermal origin. A normal E-cadherin expression was observed in the majority of well differentiated squamous cell carcinomas and adenocarcinomas of the lung. In contrast, in nearly half of the poorly differentiated squamous cell carcinomas E-cadherin expression was absent. Accordingly, lack of E-cadherin appears to be associated with poor differentiation in squamous cell lung carcinomas, which is similar to the finding in squamous cell carcinomas of head and neck. In the majority of moderately differentiated squamous cell carcinomas E-cadherin was still expressed, albeit at reduced levels. However, the pattern of expression was spotty or diffuse.

None of the metastases exhibited normal E-cadherin expression. All five metastases of moderately differentiated squamous cell carcinomas showed a reduced E-cadherin expression as compared with the respective primary tumor. This suggests that down-regulation of E-cadherin expression is associated with metastasis in this subgroup of squamous cell carcinomas. Alternatively, as all five primary tumors showed a nonlinear expression pattern, an altered E-cadherin expression pattern in the primary tumor could be related to metastasis.

In contrast, no association between dedifferentiation and alterations in E-cadherin expression was found in lung adenocarcinomas. These results suggest that different mechanisms are responsible in the progression of squamous cell carcinomas and adenocarcinomas of the lung [6].

Acknowledgements We thank Gerd Neuhaus for his help with the specimen collection, Dr. W. Birchmeier and Dr. U. Hellerich for suggestions and carefully reading the manuscript and Dr. W. Birchmeier also for monoclonal antibody 6F9. This work was supported by the *Deutsche Forschungsgemeinschaft* and the *Wilhelm-Sander-Stiftung.*

References

1. Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455
2. Schipper JH, Frixen UH, Behrens J, Unger A, Jahnke K, Birchmeier W (1991) E-cadherin expression in squamous cell carcinomas of head and neck: Inverse correlation with tumor dedifferentiation and lymph node metastasis. Cancer Res 51:6328–6337
3. Shimoyama Y, Hirohashi S, Hirano S, Noguchi M, Shimosato Y, Takeichi M, Abe O (1989) Cadherin cell-adhesion molecules in human epithelial tissues and carcinomas. Cancer Res 49:2128–2133
4. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D, Birchmeier W (1991) E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 113:173–185
5. Kreyberg L (1981) Histological typing of lung tumours, 2nd edn. WHO, Geneva, p 1–36
6. Böhm M, Totzeck B, Birchmeier W, Wieland I (1994) Differences of E-cadherin expression levels and patterns in primary and metastatic human lung cancer. Clin Exp Metastasis 12:55–62