Expression of E-Cadherin and α-Catenin in T1 N0 Laryngeal Cancer

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Abstract. Aim: To determine whether modulation of expression of cell adhesion molecules occurs in neoplastic transformation of laryngeal epithelium and to investigate their possible role in clinical outcome. Materials and Methods: Fifty-five T1 N0 laryngeal biopsies were tested by immunohistochemistry for the E-cadherin/α-catenin adhesion complex. Results: High immunohistochemical expression of E-cadherin and α-catenin was found in 18% and 53% cases, respectively. Expression of both adhesion molecules decreased according to histological grading; a significant relationship was particularly found between high E-cadherin expression and G1 cases (p=0.013). High E-cadherin expression was statistically associated with in situ carcinoma (p=0.006). Non-statistical significance was evidenced between these adhesion molecules and tobacco use or site of occurrence. Regarding clinical outcome, recurrence was associated with low expression of both adhesion molecules. Conclusion: E-cadherin and α-catenin down-regulation might be associated with neoplastic transformation in laryngeal tissues and might be regarded as a risk factor for clinical recurrence.

Among head and neck squamous cell carcinomas (HNSCC), the larynx is one of the sites mostly implicated, being traditionally associated with tobacco and alcohol use, especially when in combination (1). As squamous carcinoma develops throughout a step progression in various stages of dysplasia, many molecular changes have been described (2); particularly, a de-regulation of adhesion molecules, such as cadherins, has been proven to be an important biomarker of the neoplastic process in laryngeal epithelium (3). E-cadherin (E-cad), which is an epithelial cell adhesion molecule, is a transmembrane protein with a cytoplasmic domain that connects the actin cytoskeleton through a complex with its associated cytoplasmic proteins, α-, β- and γ-catenins (4, 5). Cell adhesive properties mediated by E-cad are regulated by its association with catenins: adhesiveness at cell adhesion sites is enhanced when E-cad links to α-catenin (6). Loss of E-cad expression has been correlated with the in vitro invasive phenotype of cancer cell lines (7); furthermore, previous in vivo studies reported the reduced or aberrant expression of E-cad and/or α-catenin in different types of human cancer (8): in neoplastic thyroid tissue (9), in oesophageal cancer (10), in breast cancer (11, 12), in gastric (13) and pancreatic carcinoma (14), in bladder (15) and prostatic cancer (16), in several types of gynaecological cancers (17-19), in melanoma (20) and meningioma (21). Moreover, defects in the E-cad/α-catenin adhesion complex have been described in several types of laryngeal cancers and were associated with different prognostic outcomes (22). E-cad expression was found to be positively correlated with tumour differentiation and inversely with the metastatic process and clinical outcome in head and neck cancer (23). In addition, low expression of E-cad combined with high expression of epidermal growth factor receptor (EGFR), which is overexpressed in the majority of squamous cell carcinomas in upper aerodigestive tract cancers (24), seems to identify a subgroup of patients which may benefit from accelerated radiotherapy (25).

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With the present study, using immunohistochemistry assays in a series of 55 laryngeal biopsies with and without dysplasia, we aimed: a) to identify E-cad and α-cat tissue distribution according to the different biological and clinical findings in order to determine whether modulation of expression of these molecules occurs; b) to investigate their possible role as potential prognostic biomarkers in routine clinical practice.

**Materials and Methods**

Fifty-five T1 N0 or carcinoma in situ (CIS) laryngeal biopsies were selected from the archives of the Fatebenefratelli Hospital and Pascale Institute, from May 1992 to September 2004. All tissues were fixed in 10% buffered-formalin; haematoxylin and eosin-stained sections were examined under light microscopy and graded for dysplasia by two independent observers (A.V and A.F.), according to the World Health Organization (WHO) classification (26) as: CIS (four cases), squamous carcinoma (47 cases) and verrucoid carcinoma (four cases); three non-dysplastic lesions were included in the study. The mean age of the patients at diagnosis was 62 years (range, 42 to 77 years); sex, smoking history, histology and tumour location are summarized in Table I. The patients had not received any preoperative therapy and surgery was the main mode of given treatment; clinical follow-up, ranging from 24 to 48 months, was conducted with a median follow-up of 36 months.

E-cad and α-cat expression were assessed by using commercially specific mouse monoclonal antibodies (Zymed, S. Francisco, CA, USA): HECD-1, specific for human E-cadherin and α-cat-7A4 raised against a synthetic peptide corresponding to the C-terminus of mouse α-catenin (dilution 1:50). Immunohistochemistry was performed according to a standard streptavidin-biotin peroxidase complex method on 4-μm sections; microwave antigen retrieval in 0.01 M citrate buffer (pH 6.0) was used before immunohistochemical staining. Endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide and sections were then incubated in serum blocking solution to reduce non-specific labelling. Primary antisera was added and sections were then incubated for 1 h at room temperature (R.T.). Slides were then incubated for 30 min with biotinylated appropriate secondary antibody. The reaction was revealed by adding diaminobenzidine tetrahydrochloride (DAB) chromogen mixture. Positive controls were known E-cad and α-cat expressing epithelial tissues; negative controls were carried out by using unrelated isotype matching antibody. After haematoxylin counterstaining, slides were permanently mounted and analyzed for the presence and distribution of the immunostaining. Staining was scored based on semiquantitative assessment of the distribution pattern of staining (plasma membrane) and the number of immunoreactive epithelial cells. Following a previous study (12), high or low expression was defined as the degree of immunostaining in more (high expression) or less (low expression) than 50% positively-stained tumour cells, respectively; negative staining was confined when the sample showed no immunoreactivity.

**Results**

E-cad and α-cat immunohistochemical distributions are summarized in Table II.

With intense reactivity at the cell-to-cell borders of the epithelial cells; weak immunostaining was also present in the cytoplasm. No nuclear staining was observed.

In laryngeal cancer (Figure 1 A and B), E-cad and α-cat immunohistochemically were high (>50%) in 10/55 (18%) and 29/55 (53%) cases, respectively; whereas low/negative immunostaining was noted in 45/55 (82%) and 26/55 (47%) cases, respectively. It should be pointed out that high expression of adhesion molecules decreased according to the histological grading. In particular, high expression of E-cad was 33% for G1 cases and 7% for G2 cases, whereas no G3 case exhibited high expression; high α-cat expression was found in 62% of G1 cases, 48% of G2 cases and only one case (25%) of G3 showed such a pattern of expression. A significant relationship was found between high E-cad expression and G1 cases (p=0.013).

**Statistical analysis.** A cross-tabulation analysis of E-cad and α-cat immunohistochemical distribution with clinical and histopathological findings was performed by the Chi-square test for trend or by Fisher’s exact test. Cox proportional hazards regression was performed to analyse the effect of variables on recurrence times. A p-value <0.05 was considered statistically significant.
All in situ carcinomas highly expressed E-cad (p=0.006), whereas only one out of for (25% cases) was highly positive for α-cat; the high expression pattern in squamous and verrucoid carcinoma was 11% and 25% for E-cad and 55% and 50% for α-cat, respectively.

Regarding tobacco use, α-cat was highly expressed in 69% of tumours from non-smokers, whereas 79% of those from smokers exhibited a low/negative expression for E-cad.

Adhesion molecule expression was then correlated with anatomical sites: glottic carcinomas evidenced low/negative expression of E-cad and α-cat in 85% and 46% cases, respectively; 57% of supraglottic neoplasia exhibited low/negative expression for both adhesion molecules.

The prognostic impact of the different biological markers was evaluated: no statistical association was found between the presence of these adhesion molecules and clinical outcome (recurrence was evidenced in 78% of E-cad- and 44% of α-cat low/negative cases); however, multivariate Cox population hazard analysis for the time-to-recurrence showed a positive effect on recurrence of low expression of both adhesion molecules (with a hazard ratio of 1.21 for E-cad and 1.67 for α-cat) (Table III).
Discussion

Alterations in cell adhesion are among the hallmark characteristics of a malignant tumour, including irregularities in expression and distribution of adhesion molecules (4). Decreased expression of E-cad, a protein essential for the establishment of cell-cell contacts, has been detected in a significant number of tumours of different epithelial origin (7). Since the association of E-cad with catenins is essential for proper anchorage to the cytoskeleton and is necessary for the E-cad binding function (5-6), we investigated the co-expression of E-cad and α-cat in a retrospective series of 55 cases of T1 N0 laryngeal cancer, in order to determine whether modulation of expression of these molecules occurs.

We found a significant association only between high E-cadherin expression and G1 laryngeal cancer, as well-differentiated carcinomas exhibited a diffuse and strong pattern of expression (p=0.013). No statistical association was found between the high expression of E-cad and moderately/poorly-differentiated carcinoma, although an evident, but not significant, decline in E-cad expression with increasing de-differentiation of the tumour was observed. This morphological pattern differs from the one observed in previous studies regarding squamous cell cancer (17, 28), but, as Eriksen et al. (25) have postulated, this seems to be related to the evaluation of E-cad staining, since no consensus exists on the method to be used for this.

Our results showed that high immunohistochemical expression of E-cadherin was statistically associated with in situ carcinoma (p=0.006), where a strong and diffuse immunostaining pattern was observed; this was probably related to a preserved adhesive function of the E-cad molecule in such a pre-invasive form.

No relationship was found in this study between immunolabelling of laryngeal cancer cells for α-cat, as observed by previous studies (22, 27). No statistical significance was evidenced between the adhesion molecules and tobacco use or site of occurrence (glottic or supraglottic).

With the present study, we aimed to evaluate the possible impact of adhesion molecules as potential prognostic biomarkers in routine clinical practice, as the treatment of the clinically-negative (N0) neck in laryngeal cancer continues to be an area of controversy (29-30): we found no statistical relationship between strong expression of these adhesion molecules and recurrence, although, in multivariate Cox population hazard analysis for time-to-recurrence, conducted with a median follow-up of 36 months, a low expression of both adhesion molecules might be regarded as a risk factor for clinical recurrence.

Future prospective evaluations are required to establish the prognostic role of E-cad and α-cat in laryngeal cancer, particularly regarding the co-expression of such adhesion markers, since this might confirm their role in predicting the biological behaviour of laryngeal tumours.

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References


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