Oral Oncol, Eur J Cancer, Vol. 29B, No. 4, pp. 303–305, 1993
Printed In Great Britain

0964–1955/93 $6.00 + 0.00

E-cadherin Expression in Normal, Hyperplastic and Malignant Oral Epithelium

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E-cadherin is a calcium-dependent cell adhesion molecule which is important in cell–cell interactions in epithelium and plays a major role in maintaining the structure and integrity of epithelial sheets. The purpose of this study was to examine E-cadherin expression in normal and malignant oral epithelium. Ten specimens of normal oral epithelium, five specimens of hyperplastic epithelium and 15 squamous cell carcinomas were stained using a standard immunoperoxidase technique and a monoclonal antibody to E-cadherin. Normal and hyperplastic epithelium showed strong pericellular staining in the basal, suprabasal and prickle cell layers. The keratinising superficial layers were negative. E-cadherin expression did not correlate to the degree or pattern of keratinisation and was not altered in the hyperplastic epithelium. All cases of squamous cell carcinoma showed heterogenous staining with areas of loss or fragmentation of staining. No tumour was completely negative. The amount or pattern of loss showed no apparent correlation to the degree of tumour differentiation. These findings suggest that loss of E-cadherin is not essential for the acquisition of a malignant phenotype but may be important in the invasive process. This supports the view that E-cadherin may be the product of a tumour suppressor gene important in tumour progression.
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INTRODUCTION

THE CADHERINS are a family of calcium-dependent cell adhesion molecules found on all cells which form solid sheets [1]. They are transmembrane glycoproteins which form homophilic cell–cell interactions. The family is divided into subclasses that are found in different tissues, at least a dozen different cadherins are known so far [2]. Included in this family is E-cadherin (epithelial cadherin or uvomorulin) which is present in all living layers of stratified squamous epithelium and is therefore found in all cell layers except the superficial keratinising layer [3]. E-cadherin plays a major role in the maintenance of intercellular junctions in normal epithelial cells in most organs. It plays a regulatory role in the organisation of adherens junctions and desmosomes and its absence is associated with abnormal stratification [4]. In cell culture, loss of E-cadherin results in de-differentiation of epithelial cells which assume a fibroblastoid phenotype and become invasive [5, 6] suggesting that E-cadherin acts as an invasion suppressor [7]. Reduction in expression of E-cadherin has been demonstrated in carcinomas of the oesophagus, breast, stomach [3], liver [8] and in squamous cell carcinomas of the head and neck [9].

The purpose of this study was to examine the expression of E-cadherin in normal and neoplastic oral epithelium in an attempt to relate expression of E-cadherin to the degree of differentiation of the tumours.

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Received 26 Jan. 1993; accepted 11 Feb. 1993.

MATERIALS AND METHODS

The material studied included 10 specimens of normal oral epithelium, five specimens of hyperproliferative epithelium and 15 squamous cell carcinomas. Normal buccal mucosa (five specimens) was obtained with permission from patients undergoing routine removal of third permanent molars, when the tissue flap required trimming. Normal palatal tissue (five specimens) was obtained adjacent to benign lesions which had been biopsied for diagnostic purposes. The specimens used as examples of hyperproliferative epithelium were biopsies taken for diagnosis of non-specific ulcers or pyogenic granulomas. Squamous cell carcinomas were collected from patients either at initial biopsy of the tumour or when undergoing definitive surgical removal. All specimens were transported to the pathology laboratory in transport medium, mounted in OCT compound embedding medium (Tissue Tek, Miles Inc., Elkhart Indiana, U.S.A.) and snap frozen in liquid nitrogen. Sections were cut at 6 μm, mounted on aminopropyltriethoxysilane coated slides, air dried and fixed for 10 min in 100% acetone.

Sections were stained using a routine avidin–biotin peroxidase method. Briefly, sections were air dried for 1 min, washed in phosphate buffered saline (PBS), and incubated with the primary antibody against E-cadherin (Eurodiagnostics) diluted 1:10 in PBS for 1 h at room temperature. After washing in PBS, biotin conjugated secondary antibody (Sigma Chemical Company, St Louis, U.S.A.) at a dilution of 1:200 was applied for 30 min at room temperature. After a further wash and incubation with avidin–biotin-peroxidase complex the reaction was visualised with 3.3,-diaminobenzidene tetra-

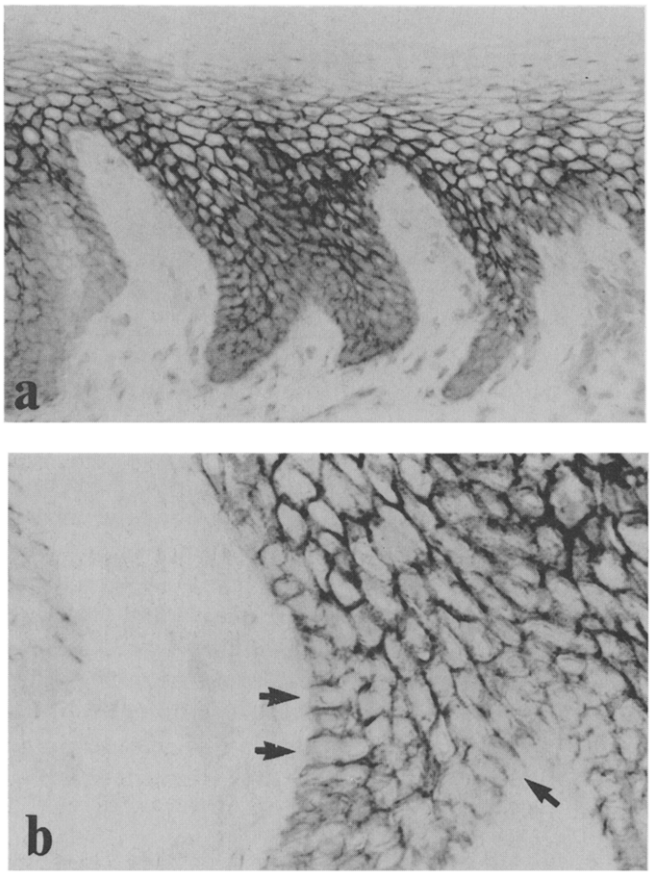

Fig. 1. E-cadherin expression in normal oral epithelium. (a) There is strong pericellular staining of basal, suprabasal and prickle cell layers, the superficial keratinising layer is negative (×40). (b) The basal aspect of basal keratinocytes adjacent to the basement membrane is negative (arrows) (×100).

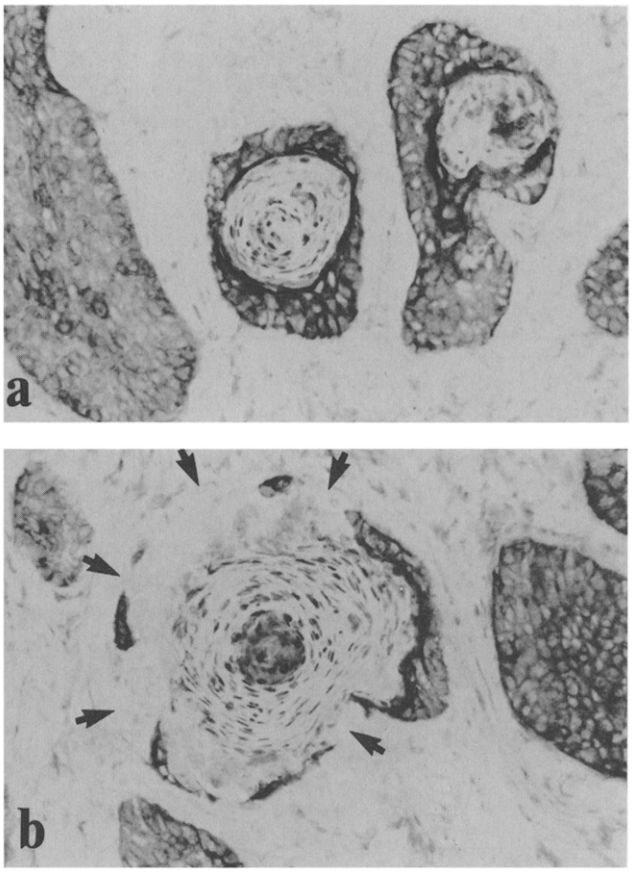

Fig. 2. E-cadherin expression in a moderately well differentiated carcinoma. (a) A normal distribution with strong basal and suprabasal staining, the central keratinising cells are negative (×40). (b) Another island from the same tumour, there is extensive loss of E-cadherin expression (arrows) (×40).

hydrochloride and 1% hydrogen peroxide. Sections were counterstained with Meyer's haematoxylin.

The negative control involved the omission of the primary antibody. The positive control was intrinsic as the normal distribution of the molecule is known.

Staining was considered positive if a brown pericellular reaction product was seen. A subjective assessment of the expression of E-cadherin was undertaken.

RESULTS

Normal and hyperproliferative epithelium displayed the same pattern of staining which was present in a pericellular distribution throughout the basal, suprabasal and prickle cell layers. Staining was weak or absent on the basal aspect of basal keratinocytes and was absent in the most superficial keratinising layers (Fig. 1). Although the pattern of staining was the same in all sections examined the staining varied in intensity, this did not appear to be related to the keratinisation of the tissue.

Ten of the squamous cell carcinomas were moderate or well differentiated and five were poorly differentiated. All the tumours showed reduced and heterogenous staining. No tumour was completely negative for E-cadherin and there was no apparent correlation between the degree of loss and tumour differentiation. Most tumours showed areas of loss of staining adjacent to areas with a normal E-cadherin distribution (Fig. 2). Many small islands of tumour or single infiltrating epithelial cells were negative (Fig. 3). This pattern did not appear to relate to the depth of tumour invasion. In other tumours and in areas of poor differentiation the pericellular E-cadherin expression was fragmented with loss of staining

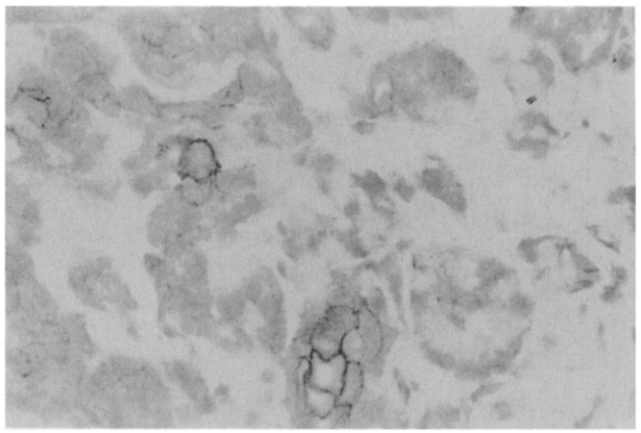

Fig. 3. The infiltrative front of a moderately differentiated carcinoma. Some islands show strong pericellular staining but many small islands and single infiltrating cells are negative (×63).

coinciding with loss of cellular adhesion and separation of cells within tumour islands (Fig. 4).

DISCUSSION

E-cadherin is a calcium-dependent cell–cell adhesion molecule found in epithelium [1, 3]. It is present in both basal and terminally differentiating keratinocytes [10], but is absent from the most superficial keratinising cell layers in stratified squamous epithelium [3].

In carcinomas of the head and neck expression of E-cadherin has been correlated to differentiation since poorly differentiated tumours were completely negative [9]. Histological grade of gastric carcinoma correlated well with loss of E-cadherin expression although no correlation could be demonstrated in tumours of breast and oesophagus [3]. E-cadherin was absent in an undifferentiated hepatocellular carcinoma but was expressed in tumours which were more differentiated [8]. E-cadherin mRNA levels were reduced in squamous cell carcinoma lines compared to cell lines of normal keratinocytes [10], and highly metastatic ovarian tumour cell lines expressed less E-cadherin than less metastatic cell lines [11]. Human cell lines derived from carcinomas with a fibroblastoid phenotype were more invasive and expressed less E-cadherin than cell lines derived from tumours with an epithelioid phenotype, which were not invasive [6]. These studies suggest that E-cadherin acts as an invasion suppressor molecule [7] and may be the product of a tumour suppressor gene. Indeed the gene for E-cadherin is located on chromosome 16q [12] which is close to the site of a recently characterised tumour suppressor gene which is lost in liver carcinomas [13] and carcinomas of the breast and prostate [14, 15].

The expression of E-cadherin in oral epithelium has not been examined previously. In this study the distribution of E-cadherin expression in normal and hyperplastic oral epithelium was similar in all specimens. The intensity of staining varied between specimens but was not dependent on whether the epithelium was orthokeratinised or parakeratinised.

All the squamous cell carcinomas examined displayed a heterogenous pattern with areas of loss of E-cadherin staining.

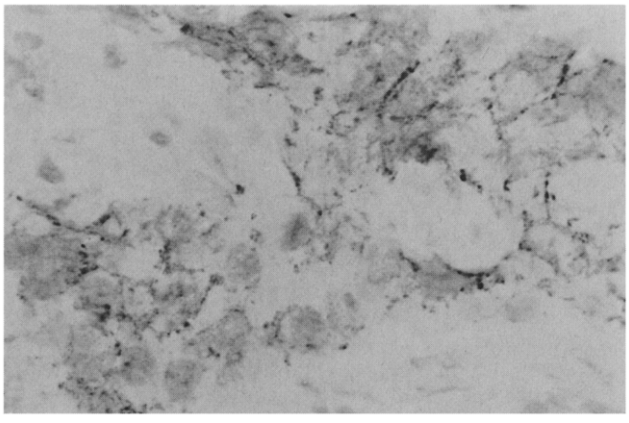

Fig. 4. A poorly differentiated carcinoma showing fragmentation of E-cadherin staining associated with loss of cellular adhesion and disruption of tumour islands (× 100).

There was no correlation between the pattern of staining and the histological grade of the tumour, although small infiltrative islands and single tumour cells were found to be negative. This suggests that loss of E-cadherin is not necessary for the acquisition of the malignant phenotype, but may be involved in the infiltrative process. This correlates to the *in vitro* studies where increased infiltrative or invasive potential was associated with low levels of E-cadherin expression while non-invasive but still malignant lines showed high levels [11]. The function of E-cadherin depends on the formation of an intracellular molecular complex with catenin [16] and it is possible that E-cadherin could be present but non-functional. Further studies will concentrate on the role of E-cadherin in modulating the behaviour of cells *in vitro*.

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