

Review

E-cadherin in oral SCC: An analysis of the confusing literature and new insights related to its immunohistochemical expression

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Summary. E-cadherin plays a crucial structural role in cell-cell contacts in epithelial tissues, and a functional role in signaling pathways that regulate cell proliferation, differentiation, and survival. Reduced immunoexpression of E-cadherin adhesions is largely considered as being equivalent to defective functionality and malignancy, and has been used as a prognostic parameter. A critical analysis of studies on E-cadherin immunoexpression in oral carcinomas revealed a wide range of both technical and interpretational aspects. This paper highlights biological characteristics of E-cadherin with respect to its expression in normal and neoplastic epithelial cells and to its interrelations with the tumor microenvironment that can have an impact on immunohistochemical results and their application in the clinical setting.

Key words: E-cadherin, Immunohistochemistry, Oral carcinoma, Extracellular and intracellular domain, Tumor microenvironment

Introduction

The formation of tight and compact cell-cell adhesions does not permit free cell movement in epithelial tissues. These adhesions serve as a positional indicator for the establishment of cell polarity. In addition to their structural roles, cell-cell contacts play an important part in signal transduction pathways through which cells can exchange a variety of information with their neighbors. This ultimately defines the status of differentiation of the epithelial tissue and

the integrity of its function (Pece and Gutkind, 2002; Ferber et al., 2008; Berx and van Roy, 2009). E-cadherin is a transmembrane cell surface molecule that has a key role in epithelial cell-to-cell adhesion contacts. The extracellular domain of E-cadherin (the -NH₂ terminal) contains the calcium-binding areas and is responsible for forming the homophilic ligations (Ferber et al., 2008; Schmalhofer et al., 2009). However, current evidence shows that E-cadherin is also involved in cell-to-extracellular matrix interactions (Dassen et al., 2010; Grover et al., 2010). The transmembrane domain is the least investigated of all the existing domains. Reported data suggest that interactions between these domains are important, but not critical, for the lateral association of E-cadherin molecules that, in turn, are required for cell-cell adhesion (Huber et al., 1999; Ozawa, 2002). The intracellular portion of the molecule (the -COOH terminal) connects the extracellular environment to the contractile cytoskeleton via intracellular interactions with a set of related proteins, collectively known as catenins. This intracellular segment of E-cadherin has a CH₂ domain (located at the membrane's proximal region) and a CH₃ domain (located at the distal region). The CH₂ domain interacts with p120-catenin (p120) and Hakai, while the CH₃ domain interacts with β -catenin (Ferber et al., 2008). The latter binds actin filaments and participates in the regulation of gene expression (Pece and Gutkind, 2002). Catenins, and, in particular, the β -catenin, are sequestered in a membranous E-cadherin-catenin adhesion complex, thus precluding the operation of β -catenin in the canonical Wnt pathway, which is ultimately expected to activate genes linked to oncogenetic activity, cell proliferation, production of metalloproteinases, and repression of the expression of E-cadherin. In addition, E-cadherin is a key inhibitor of the epidermal growth factor (EGF) receptor signaling, and a substantial contributor to the epithelial apicobasal

polarization (Berx and van Roy, 2009; Schmalhofer et al., 2009).

When the cell-cell contacts are disrupted, as, for example, by modifications in E-cadherin, the epithelial tissue becomes disoriented and cells stop functioning synchronously. Impairment in the expression of E-cadherin is a crucial step toward malignant transformation, which, together with additional changes (e.g., activation of proto-oncogenes or inactivation of tumor-suppressor genes), contributes to the accomplishment of a full-blown malignant phenotype (Berx and van Roy, 2009). Various types of carcinomas, including oral squamous cell carcinoma (OSCC), have been largely studied with regard to the immunohistochemical expression of E-cadherin, and it has been generally accepted that reduced expression is likely to be associated with poor prognosis (Berx and van Roy, 2009).

Accumulating data have recently cast doubt on the widely accepted direct relation between the immun-expression of E-cadherin and clinical outcomes. The aim of this study is to provide a critical analysis of the immun-expression of E-cadherin in OSCC based on evidence from studies published during the last decade, and to discuss new insights into the interpretation of E-cadherin immun-expression in order to refine it and correlate it more closely to clinical outcomes.

The principles of E-cadherin immunohistochemistry in normal and malignant tissues

Immunohistochemistry is the most common laboratory tool used in histopathology to demonstrate the presence or absence of E-cadherin. Antibodies can be targeted towards epitopes in either the extracellular or intracellular domains. Several scenarios of immunohistochemical staining patterns are feasible, depending on the status of each of the E-cadherin domains (Fig. 1). In normal (non-transformed) epithelial cells, an antibody aimed toward an extracellular or intracellular epitope is expected to be visualized as a membranous staining when that corresponding part of the molecule is intact. If there are structural changes on the extracellular epitope (as a result of genetic/epigenetic/transcriptional/proteolytic modulations), that epitope may no longer be recognized by an extracellular antibody, and the lack of membranous staining is interpreted as loss of E-cadherin expression. However, this may be somewhat erroneous, as only the cell-cell adhesion properties of the E-cadherin molecule are affected at this stage, while the intracellular part of the E-cadherin remains functional and no downstream signaling pathways are activated. Biologically, loss of the extracellular domain enables the first step of the metastatic cascade, but not the full-blown malignant phenotype (Onder et al., 2008). If the intracellular domain of E-cadherin is altered, the use of an antibody aimed at an extracellular epitope will not identify this condition, and a "normal"-appearing membranous

staining will be generated. Alterations in the intracellular domain of E-cadherin are believed to ultimately bring about the successful completion of the invasion-metastasis cascade. Under these conditions, an extracellular antibody might yield a positive reaction on a background that already operates malignant pathways. On the other hand, the use of an antibody aimed to identify an intracellular epitope may demonstrate a membranous pattern of staining as long as that portion of the molecule is recognizable at this location, but this type of antibody can also show a nuclear pattern as well. Specifically, this can occur if a portion of the intracellular domain of E-cadherin is enzymatically cleaved and transferred to the nucleus (Ferber et al., 2008; Céspedes et al., 2010).

Altogether, impairment in E-cadherin domains that occurs in association with malignancy may be differentially translated into immunohistochemical reactions, depending on the type of antibody employed. These observations may serve as an explanation as to why some recent studies could not find direct associations between the immun-expression of E-cadherin and the clinicopathologic and prognostic variables in various carcinomas, including OSCC (Mahomed et al., 2007; de Moraes et al., 2008; Rakha et al., 2010).

E-cadherin in oral squamous cell carcinoma: how genetic alterations are related to immunohistochemistry

Similar to carcinomas in other sites of the body, loss of expression of E-cadherin in OSCC constitutes a critical molecular event (Lyons and Jones, 2007), making the understanding of the mechanisms that control its expression all the more essential. Investigations have been performed at several levels, among which hypermethylation of the E-cadherin gene, *CDH1*, is considered to be principal. Promoter hypermethylation of the *CDH1* gene in OSCC has been examined in several studies (Saito et al., 1998; Nakayama et al., 2001; Chang et al., 2002; Yeh et al., 2002; Viswanathan et al., 2003; Kudo et al., 2004), and was reported as being from 17% (Saito et al., 1998) to 85% (Yeh et al., 2002). This considerable range could be attributed to different assays of varying sensitivity that were used in these studies, and to the fact that methylation patterns cannot be determined from very small amounts of DNA. This could also account for the occurrence of false-negatives due to degradation or loss of DNA and false-positives due to incomplete digestion (Viswanathan et al., 2003). The results were inconsistent when the methylation status in OSCC was related to the expression of the E-cadherin protein by means of immunohistochemistry (Nakayama et al., 2001; Chang et al., 2002; Yeh et al., 2002; de Moraes et al., 2008). The tumors usually comprised a mixture of hypermethylated and unmethylated cells and, although a trend was seen in which promoter methylation of the *CDH1* gene was

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associated with reduced E-cadherin expression, promoter hypermethylation was occasionally found together with preserved expression of the E-cadherin protein (Nakayama et al., 2001; Chang et al., 2002). Furthermore, unmethylation was found concomitantly

with reduced expression of E-cadherin (de Moraes et al., 2008). Yeh et al. (2002) concluded that the methylation status of E-cadherin in OSCC may be unrelated to the aberrant expression of E-cadherin protein, and furthermore it is assumed that promoter methylation of

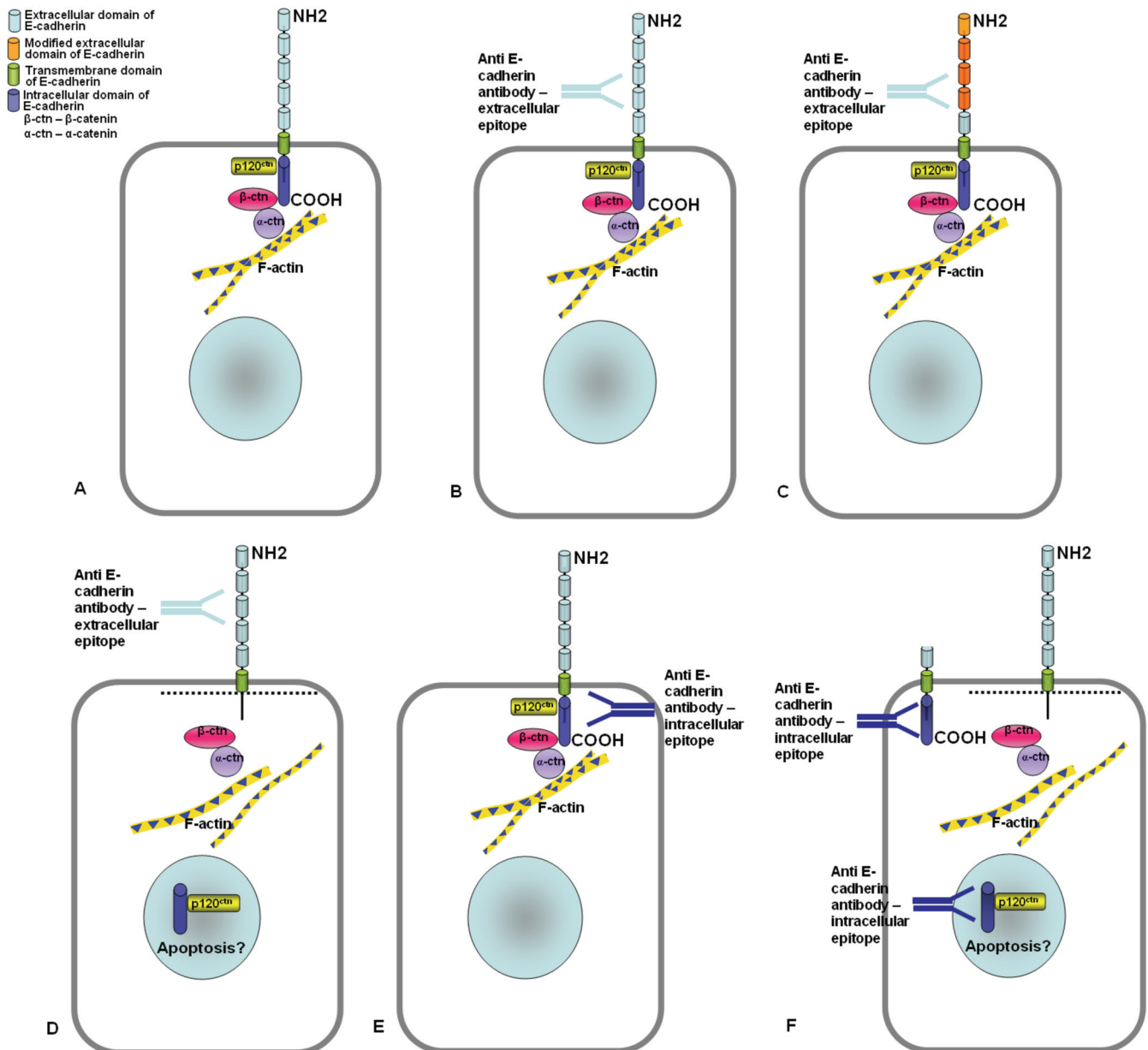


Fig. 1. Relations between the status of E-cadherin molecules and the interpretation of the immunohistochemical findings. **A.** In the normal epithelial cell, the transmembrane E-cadherin is seen to be bridging between the extracellular microenvironment and the intracellular cytoskeleton. **B.** An antibody directed toward an epitope on the extracellular domain will generally identify E-cadherin at a membranous location. **C.** An antibody directed toward a modified extracellular epitope tends to yield an apparent negative immunoreponse. The intracellular domain may be intact and fully functional. **D.** An antibody directed toward an epitope on the extracellular domain will tend to identify E-cadherin but it would not be able to detect changes in the intracellular domain. **E.** An antibody directed toward an epitope on the intracellular domain will generally yield a positive immunoreaction at the cell's membrane. **F.** An antibody directed toward an epitope on the intracellular domain of E-cadherin can detect this segment at a nuclear location after it has been cleaved from the rest of the molecule.

the E-cadherin gene is heterogeneous, dynamic, unstable, and can be modulated by the tumor microenvironment in cancer (Bers and van Roy, 2009). Therefore, the immunoeexpression of E-cadherin warrants investigation, not only among the tumor cells, but also in the broader context of the tumor and its microenvironment.

Analysis of studies from the literature on the immunoeexpression of E-cadherin in OSCC

Literature search

All relevant studies published in the English language literature during the last decade were retrieved using the PubMed database (the key words included "E-cadherin" and "oral carcinoma") and the reference lists of relevant articles as the source for the literature survey. The studies were analyzed with respect to the anti-E-cadherin antibody that was used, the methodology of assessing the immunohistochemical staining results, and the main findings and their clinical associations (Table 1). Only studies involving human patients were included. Results of the immunostaining referred only to findings from primary OSCCs and not from recurrent tumors or lymph node metastases. A total of 24 studies were found to be suitable for inclusion. The number of patients analyzed in these studies ranged between 15 (Lopes et al., 2009) and 135 (Kurtz et al., 2006). Most of the tumors were from the oral cavity, and only a few came from the oropharynx (Kurtz et al., 2006).

Classification of studies

Study classification was done according to the epitope against which the antibodies were targeted, resulting in four main categories: studies (n=7) in which the antibodies targeted epitopes on the extracellular domain of E-cadherin, studies (n=5) in which antibodies targeted the intracellular domain, studies in which the used antibody was produced by a recombination assay and the epitope was undefined (n=3), and studies (n=9) in which only the manufacturer of the E-cadherin antibody was stated but information on the specific type of antibody used was not provided. In the latter group, several of the antibodies could not be traced over the Internet in spite of considerable efforts on our part. The main point that this classification intended to emphasize was that the choice of the antibody and its targeted epitope could be critical in the interpretation of the resulting staining pattern when attempting to investigate the immunohistochemical expression of any molecule that is characterized by a transmembrane configuration. As noted earlier, there are different fates for different portions of the E-cadherin molecule under differing conditions, depending on whether it is located in the extracellular or intracellular domains (Onder et al, 2008).

Methods used for the assessment of the immunoeexpression of E-cadherin

The variability of the methods used to assess the immunohistochemical staining results of E-cadherin was even greater than the assortment of the antibodies that were used. Some studies used a subjective qualitative methodology that occasionally employed vague terms, such as "normally stained cells", "heterogeneous population of cells", "less than normal but recognizable" (Nakayama et al., 2001; Yeh et al., 2002; Okamoto et al., 2002; Franz et al., 2009). Other studies either differentiated between the findings at the front of invasion from those in the center parts of the tumor, or focused only on the area at the front of invasion (Mahomed et al., 2007; de Moraes et al., 2008; Franz et al., 2009; Wang et al., 2009; Liu et al., 2010). The controls that were routinely employed to assess E-cadherin staining in the tumors consisted of samples of normal oral mucosa from healthy patients free of carcinoma (Mahomed et al., 2007; Bankfalvi et al., 2002a; Diniz-Freitas et al., 2006; Wang et al., 2009) or of what appeared to be normal-looking oral mucosa in the vicinity of the tumors (Chang et al., 2002; Bankfalvi et al., 2002b; Nakanishi et al., 2004). The control tissues often exhibited continuous membranous E-cadherin staining in all the epithelial cells of the spinous layer and to a lesser extent in the basal cells, while the epithelial cells almost lacked E-cadherin staining in the superficial layers. Tumors were scanned for evidence of membranous staining of E-cadherin compared to the controls, but this was performed in an almost "free-style" manner. Although most studies shared a technique whereby the results of the staining were expressed as an assessment of the percent of membranous stained tumor cells from the entire tumor mass, they differed remarkably in the threshold percent that was considered as representing a positive result. This vast diversity included studies in which the threshold ranged between 11% and 100% of stained cells (Diniz-Freitas et al., 2006; Wang et al., 2009) whereas it was 36.5% (de Moraes et al., 2008), 50% (Chow et al., 2001; Chang et al., 2002; Lim et al., 2004, 2005; Kurtz et al., 2006; Hung et al., 2006; Mahomed et al., 2007; Zhong et al., 2007; Foschini et al., 2008), 75% (Bankfalvi et al., 2002a,b; Tanaka et al., 2003), 80% (Nakanishi et al., 2004; Liu et al., 2010), and even 90% of all stained cells (Ueda et al., 2006) in others. In light of these variations, it was not surprising to find that E-cadherin-positive cases in the analyzed studies ranged between 8.5% (Franz et al., 2009) to 75% (Wang et al., 2009).

Correlations between the immunoeexpression of E-cadherin and clinical outcomes

It was generally reported that patients with a reduced expression of E-cadherin had more frequent lymph node involvement and a shorter survival compared to patients

Table 1. Studies on the immuno-expression of E-cadherin classified according to the location of the immunogenic epitope.

	Authors	Manufacturer	Assessment method for the expression E-cadherin (% refers to stained tumor cells)	Preserved/highest expression of E-cadherin (% of cases)	Associations between loss of expression of E-cadherin and clinical outcomes
Monoclonal antibody directed against an extracellular epitope (HECD-1 or G-10*)	Okamoto et al., 2002	Takara, Shiga, Japan	Normal: uniformly stained. Aberrant: negative or heterogeneous (mixed populations of positive and negative cells)	44%	None with delayed node metastasis
	Bankfalvi et al., 2002a	TaKaRa Biomedicals, Shiga, Japan	Preserved \geq 75%	The exact number of cases attributed to each score was not stated	Shorter survival
	Bankfalvi et al., 2002b	TaKaRa Biomedicals, Shiga, Japan	Preserved \geq 75%	28%	Poor prognosis
	Nakanishi et al., 2004	Transduction, Lexington, KY, USA	Preserved $>$ 80%	31%	Lymph node metastasis, advanced stage, greater depth of invasion and shorter survival time
	Mahomed et al., 2007	Zymed, San Francisco, CA, USA	Preserved $>$ 50% (assessed separately for superficial tumor and invasive front)	53% Superficial tumor 7% Invasive front	None with nodal status
	Pyo et al., 2007	Non-commercial/ (HECD-1)	Preserved $>$ 75%	21%	Lymph node metastases, none with survival.
	Lim et al., 2005	Santa Cruz, Santa Cruz, CA, USA*	Preserved $>$ 50%	48%	Worse prognosis
Monoclonal antibody directed against an intracellular epitope (clone 36)	Chang et al., 2002	Transduction, Lexington, KY, USA	Preserved $>$ 50%	17%	Independent prognostic factors for tumor-free survival
	Tanaka et al., 2003	Transduction, KY, Kentucky, USA	Preserved \geq 75%	42%	Shorter survival and lymph node metastasis
	Lim et al., 2004	Transduction, Lexington, KY, USA	Preserved $>$ 50%	32%	Late cervical metastasis
	Diniz-Freitas et al., 2006	Transduction, Lexington, KY, USA	Preserved \geq 10%	70%	Regional recurrence, shorter disease-free period
	De Moraes et al., 2008	Transduction, Lexington, KY, USA	Preserved $>$ 36.5%	No neck metastases: 50% Neck metastases: 29%	None with survival
Monoclonal antibody directed against an undefined epitope (clone NCH-38)	Foschini et al., 2008	Dako, Glostrup, Denmark	Preserved $>$ 50%	Range 10%-90%	Lymph node metastases
	Lopes et al., 2009	DakoCytomation, Carpinteria, CA	Pattern of staining: 1, absent; 2, focal heterogeneous; 3, reduced homogeneous; 4, strong homogeneous. Extent of staining: 1, $<$ 25%; 2, 26%-50%; 3, 51%-75%; 4, $>$ 75%	The exact number of cases attributed to each score was not stated	Not analyzed
	Franz et al., 2009	Dako, Germany	Preserved: majority of cells	8.5%	Not analyzed
Antibody not detailed	Nakayama et al., 2001	Transduction, Lexington, KY, USA	(+): less than normal but recognizable, (-): completely lost in all cells. (\pm): heterogeneous [some tumor cells (+), (\pm), and (-) or homogeneous (all (\pm)]	22%	Not analyzed
	Chow et al., 2001	Transduction, Lexington, KY, USA	Preserved $>$ 50%	15%	Regional recurrence, shorter survival
	Yeh et al., 2002	Santa Cruz, Santa Cruz, CA, USA	(+): less than normal but recognizable, (-): completely lost in all cells, (\pm): heterogeneous [some tumor cells (+), (\pm), and (-) or homogeneous (all (\pm)]	The exact number of cases attributed to each score was not stated	Not analyzed
	Kurtz et al., 2006	BD Biosciences, Pharmingen San Diego, CA, USA	Preserved $>$ 50%	62%	Shorter survival
	Hung et al., 2006	Santa Cruz, Santa Cruz, CA, USA	Preserved $>$ 50%	18%	Lymph node metastases and advanced clinical stage, none with survival
	Ueda et al., 2006	Transduction, Lexington, KY, USA	Preserved $>$ 90%	40%	Lymph node metastasis
	Zhong et al., 2007	Zymed, USA	Preserved $>$ 50%	48.5%	Shorter survival
	Wang et al., 2009	Maxim, Fuzhou, China	Preserved $>$ 11%	75% center of tumor 57.7% invasion front	Shorter survival (expression at tumor front)
	Liu et al., 2010	ZM-0092, Zymed, San Diego, CA, USA	Score: intensity X proportion. Intensity: 0, negative; 1, weak; 2, moderate; 3, strong. Proportion: 0, negative; 1, $<$ 10%; 2, 11-50%; 3, 51-80%; 4, $>$ 80%. Negative: score: 0; Preserved: score: $>$ 4. (Note: center of tumor and invasive front were assessed separately)	37.5% Center of tumor 26.5% Invasive front	Shorter survival

with a preserved expression of E-cadherin. Furthermore, based on these "significant" clinical associations, it was concluded that E-cadherin could be used as an independent marker for survival. However, given the variability in the thresholds set for E-cadherin positivity, this conclusion can be problematic. For example, a patient with a tumor showing 60% E-cadherin-stained cells would be expected to have a shortened survival time according to studies that set a high threshold ($\geq 75\%$). In contrast, according to studies in which the threshold was lower ($\geq 50\%$), that patient would be expected to survive longer.

In summary, the present analysis of the recent literature on the expression of E-cadherin in OSCC reveals that critical assessment of the data exposes drawbacks in study design and lack of standardization of the laboratory techniques and assessment methodology that may lead to confusion and inconsistent information, and, in turn, to inaccurate clinical conclusions. Given that the expression of the E-cadherin protein is not necessarily directly related to the status of its gene activation, newer ways to interpret the immunoreaction of E-cadherin, a molecule with complex biological activity, should be pursued.

New insights into the interpretation of E-cadherin immunoeexpression

The importance of understanding the differential functional roles and biological implications between the extracellular and intracellular domains of E-cadherin have already been emphasized earlier. It is further highlighted by Chetty et al. (2008) who showed that the same cases of carcinoma of the pancreas that apparently yielded a negative E-cadherin immunoreaction when an "extracellular" antibody was used unexpectedly became E-cadherin-positive when using an "intracellular" antibody. Furthermore, it has been shown that the intracytoplasmic end of the E-cadherin molecule is enzymatically cleaved, whereby a fragment of it is released into the cytosol (Ferber et al., 2008). Within the cytosol, the E-cadherin fragment is coupled to one of the membranous cadherin-binding catenins, p120. p120 not only enables the nuclear translocation of the newly produced complex of cleaved E-cadherin-p120, but also facilitates the specific binding of this complex to DNA. Identification of the specific DNA-binding sites and clarification of the *in vivo* functions of nuclear E-cadherin are still under investigation, the preliminary assumption being that it is involved in the regulation of apoptotic pathways (Ferber et al., 2008). This is consistent with observations from several studies which reported immunohistochemical identification of nuclear E-cadherin (Gervais et al., 2007; Salahshor et al., 2008). In OSCC, initial evidence on the identification of nuclear E-cadherin has been provided (Pugalagiri and Cheng, 2011). These findings raise the possibility that routine use of immunohistochemistry with antibodies

against E-cadherin concomitantly with antibodies against catenins, in particular anti-p120 catenin, may provide greater certainty in the identification of genuine E-cadherin-positive cells (Rakha et al., 2010). Identification of membranous E-cadherin does not exclude the possibility that the cleaved fragment of E-cadherin does not function at a nuclear level. The possibility that the membranous and nuclear localizations of E-cadherin might confer this molecule opposing functions in terms of neoplastic transformation (i.e., membranous E-cadherin preserves the integrity of the epithelial morphology and function while the nuclear E-cadherin functions as an anti-apoptotic factor) needs to be examined by using adequate antibodies corresponding to each of the domains of E-cadherin in the same case. A general consensus on the most suitable antibody for the immunohistochemical detection of E-cadherin is needed so that all laboratories produce uniform and comparable findings. Subsequent correlations of the immunoeexpression of E-cadherin in each of these cellular compartments with the clinical outcomes could more reliably determine the prognostic potential of E-cadherin.

The expression of E-cadherin was heterogeneous, not only in different tumors, but also in different areas of the same tumor, thus reflecting spatial and temporal variations in the expression of E-cadherin (Schmalhofer et al., 2009). As such, the expression of E-cadherin can be modulated to be "on" or "off" as a factor of the needs and status of the tumor cells, which, under appropriate conditions, reduce the expression of E-cadherin in order to facilitate invasion, whereas the tumor cells re-express E-cadherin and renew the intercellular adherence when the surrounding conditions are less favorable. Therefore, what is actually seen by E-cadherin immunohistochemistry can be interpreted as one "still" picture within a dynamic setting.

It is now believed that the expression of E-cadherin within malignant cells is not dictated solely by the carcinomatous cells themselves, but that the *tumor microenvironment* (TME) contributes to the expression as well. There is a series of reports that show that the expression of E-cadherin can be modulated by the presence of stromal collagen I (Araújo et al., 2009) and laminin-1 (Benton et al., 2009) or under hypoxic conditions (Imai et al., 2003). Furthermore, in a very elegant model, Gaggioli et al. (2007) have shown that physical matrix remodeling by fibroblasts in the vicinity of the tumor enables the collective invasion of carcinoma cells that retain their epithelial phenotype. As a rule, cells that retain epithelial markers (e.g., E-cadherin) are unable to remodel the surrounding matrix, but instead follow within tracks the extracellular matrix behind the stromal fibroblasts, thus weakening the notion that tumors with a high expression of E-cadherin are less invasive. Acquisition of motile behavior by SCC cells is not sufficient for invasion, in that both force-mediated and protease-mediated matrix remodeling are

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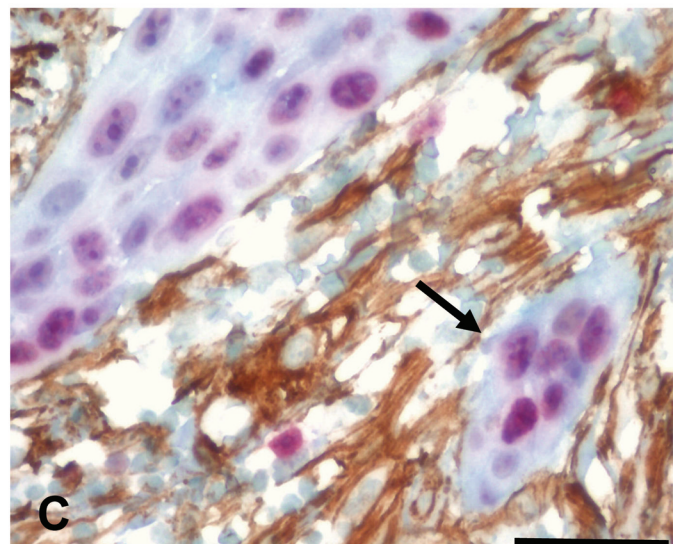
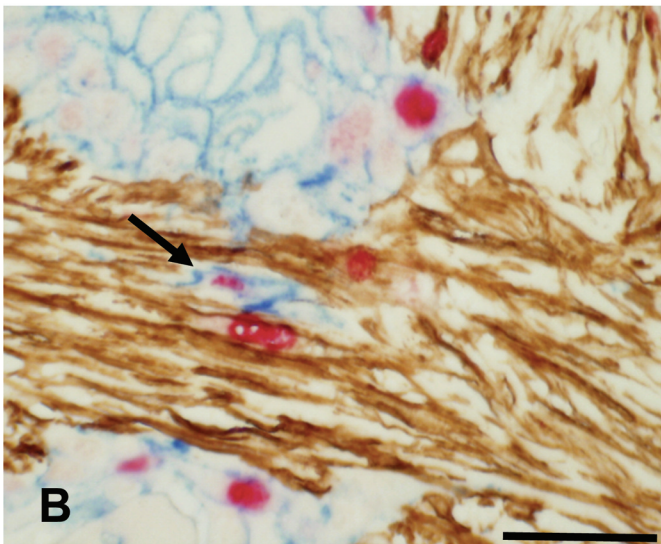
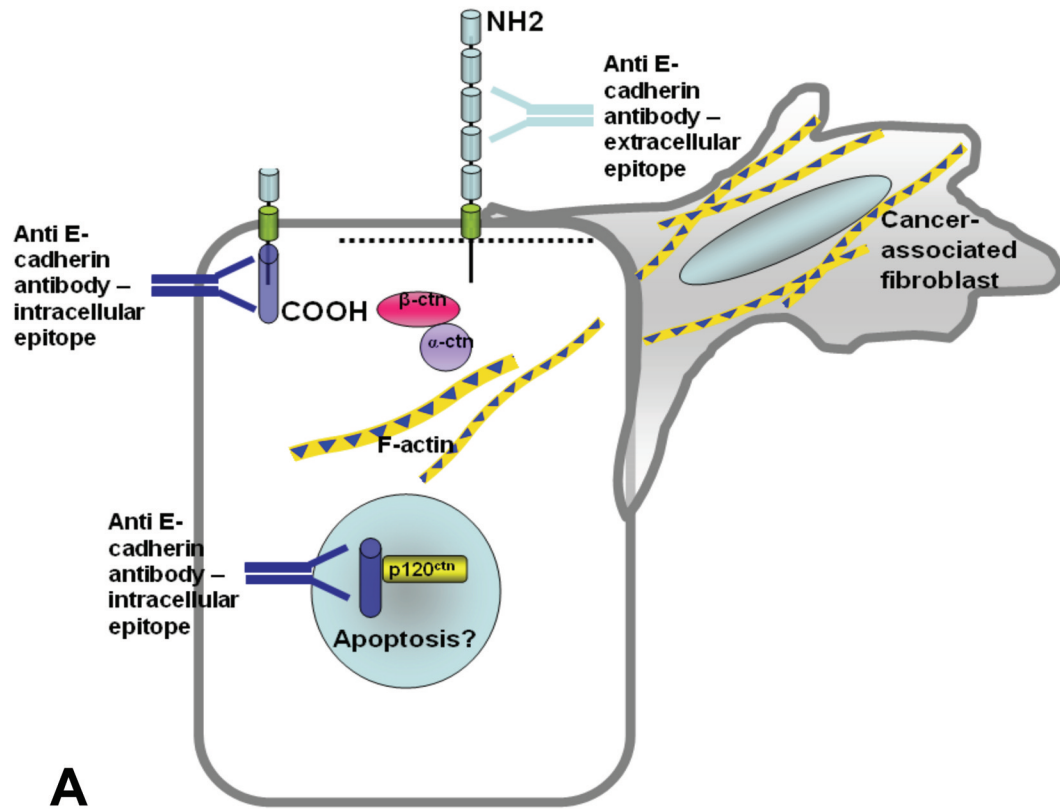


Fig. 2. A. The malignant epithelial cell can be led by cancer-associated fibroblasts, irrespective of the status of the E-cadherin expression. **B** and **C.** Photomicrographs of two cases of tongue carcinoma stained with a triple immunostaining assay that includes anti-E-cadherin (clone NCH38) for identification of the carcinoma cells (blue), anti- α -smooth muscle actin for identification of cancer-associated fibroblasts (CAFs; brown), and anti-Ki-67 for identification of proliferating cells (reddish-purple). The carcinoma cells generally preserved expression of E-cadherin. **B.** The arrow points to a single tumor cell detached from the rest of the tumor island entirely surrounded by CAFs that may be led away by the latter and spread into the adjacent tissues. The carcinoma cells are seen to have lost E-cadherin expression. **C.** The arrow points to a small, detached cluster of carcinoma cells surrounded by CAFs. The latter are in close proximity to the tumor cells, which may enable their collective movement led by the CAFs. Scale bar: 50 μ m.

required. These activities are provided by stromal fibroblasts that lead collectively invading chains of cells, irrespective of the status of E-cadherin expression (Vered et al., 2010). As illustrated in Fig. 2, where we stained human tongue SCCs using a triple immunohistochemical procedure, which contained antibodies against E-cadherin (NHC-38), cancer-associated fibroblasts (alpha-smooth muscle actin) and proliferation marker, Ki-67, we found carcinomatous cells that showed a very close relationship with the surrounding cancer-associated fibroblasts, as if these two types of cells had merged together. This holds true for both E-cadherin-positive and E-cadherin-negative cancer cells, pointing to the possibility that the tumor cells were led to an invasive status by the cancer-associated fibroblasts, irrespective of the expression of E-cadherin.

In summary, the use of immunoreactions of E-cadherin as a prognostic parameter in several types of carcinomas and the possibility that this application might depend to a considerable extent on technical-laboratory and subjective aspects, demands re-appraisal of the approach of the immunohistochemistry of this molecule. In this context, it is fundamental to understand that E-cadherin is a transmembrane molecule that probably has separate and differential functions of the extracellular and intracellular domains, and that the use of a single antibody may not reflect the entire range of biological functions of this molecule. Therefore, a more reliable assessment of the immunoreaction of E-cadherin should include concomitant examination of both the membranous and nuclear reactions (e.g., the use of multiple antibodies). Furthermore, such an assessment should be coupled with that of a related catenin (i.e., p120). Finally, there is a need for an in-depth investigation of the relationships between the expression of E-cadherin and TME.

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