Abnormal distribution of E-cadherin and β-catenin in different histologic types of cancer of the uterine cervix

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Abstract

Objective. The goal of this study was to analyze the cellular distribution and possible alterations of β-catenin and E-cadherin proteins in different histologic types of uterine cervical cancer and precursor lesions, compared to normal controls.

Methods. We performed an immunochemical staining analysis of the cellular distribution of β-catenin and E-cadherin proteins in biopsy samples from 20 normal exocervical squamous epithelium, 43 premalignant lesions, and a large series of 126 invasive tumors of different histologic types that included 68 squamous carcinomas, 31 adenosquamous carcinomas, and 27 adenocarcinomas. Statistical significance was evaluated by the chi-square or Fisher’s Exact test.

Results. We observed β-catenin abnormally distributed in the cytoplasm of 62% of premalignant lesions and more than 70% of invasive cancers, statistically significant when compared with normal tissue (P < 0.05). Similarly, we found that E-cadherin exhibit a significant abnormal distribution in the cytoplasm of 58% of premalignant lesions (P < 0.05) and in more than 71% of squamous carcinoma and adenosquamous carcinoma when compared with normal tissue (P < 0.05). We found no differences in the distribution of E-cadherin between adenocarcinomas compared with control samples. Interestingly, we found that both, β-catenin and E-cadherin, were absent in the membrane of nearly 40% premalignant lesions. Nuclear staining of β-catenin was rarely seen in any cases, contrary to what has been reported for this and other neoplasias.

Conclusion. Our findings indicate that cellular alterations of both β-catenin and E-cadherin are frequent in tumors of the uterine cervix of different histologic types, and support a role for these proteins in cervical cancer development.

Introduction

In Mexico, cervical cancer is still the leading cause of death among women with cancer, with almost 4000 annual deaths and 23,000 new cases every year [1]. The main risk factors for its development are persistent infections with oncogenic or “high” risk types of Human Papillomavirus (HPV) such as types 16 and 18 [2]. “High” risk HPV types have been found in more than 99% of cervical carcinomas, suggesting a necessary role in tumor development [3]. While persistent viral infection is necessary, genetic alterations at the cellular level that are involved in the progression of precursor lesions to malignancy are still poorly understood.
Recent reports have demonstrated that β-catenin is involved in two major cellular activities: signal transduction and intercellular adhesion [4]. β-catenin is a downstream transcriptional activator of the Wnt signaling pathway [5]. In the absence of Wnt signals, phosphorylation of β-catenin by glycogen synthase kinase 3-β (GSK-3β) marks it for ubiquitin-mediated degradation through the proteasome pathway [6]. Activation of the Wnt pathway inhibits GSK, after which unphosphorylated β-catenin is translocated to the nucleus where it interacts with members of the T-cell factor/lymphoid-enhancer binding factor (TCF/LEF). This interaction subsequently forms a transcription complex that activates specific target genes [7], including major regulators of cell proliferation, such as cyclin D1 [8] and c-myc [9]. Different types of alterations affecting β-catenin signaling pathway have been found in several neoplasias [10–14].

In addition to its role in signal transduction, β-catenin has a major role in intracellular adhesion. It is localized at the calcium-dependent adherent junctions (AJs), where it binds the cytoplasmic domain of E-cadherin. Formation of this complex links the actin cytoskeleton with AJs via α-catenin and constitutes a key element in cell–cell adhesion [15]. In fact, mutant cadherin molecules whose cadherin-binding sites have been deleted are impaired in their cell-binding function [16]. Several groups have proposed that E-cadherin functions as a tumor invasion suppressor gene, such that its loss allows or enhances invasion of adjacent normal tissues [17]. In addition, reduced E-cadherin expression has been observed in gastric cancers (42%) [18], infiltrated lobular breast carcinoma (85%) [19], colorectal carcinoma (57%) [20], lung endocrine tumors [21], and human cervical carcinoma-derived cell lines [22].

The role of β-catenin and E-cadherin has not been extensively studied in cervical cancer. Catenin gene expression was observed altered and associated with absent or reduced E-cadherin levels in cervical cancer-derived cell lines [22]. Normal mRNA levels of E-cadherin, and α- and β-catenin were observed in primary tumors from the cervix, compared with low levels in more advanced tumors [23]. On the contrary, increased β-catenin mRNA levels were found in 5 analyzed by micro-array technology [24]. Previous results from our laboratory showed that nearly 50% of tumors from the uterine cervix exhibited increased levels of expression or/and altered patterns of localization of β-catenin [25].

Here, we have analyzed the distribution of β-catenin and E-cadherin in samples from normal epithelium, premalignant lesions and a large series of invasive cancer of different histologic types that included 68 squamous carcinomas, 31 adenosquamous carcinomas, and 27 adenocarcinomas. The aim of this study was to evaluate differences in the distribution of these molecules during various stages of neoplastic progression within different histologic types of cervical cancer.

Materials and methods

Tissue samples

Normal tissue samples and tumors from the Department of Gynecology at the Hospital General Manuel Gea Gonzalez and the Department of Pathology at the Instituto Nacional de Cancerologia, SSA, in Mexico City. Tissues were routinely fixed in 4% formalin and embedded in paraffin. A total of 189 specimens was analyzed, whose diagnosis were normal squamous epithelia (20 cases), low- and high-grade squamous intraepithelial lesions (SILs) (43 cases), invasive squamous carcinoma (68 cases), adenosquamous carcinoma (31 cases), and adenocarcinoma (27 cases). The median age of the patients was 48 years (range 21–85 years).

As normal controls, we used sections from formalin-fixed, paraffin-embedded tissues from 20 normal cervices from patients who underwent amputation of the cervix for prolapse (HTA and cervicitis) (age range, 20–74 years; median age, 33 years). The specimens were reevaluated blindly by experienced pathologists (R.D. and A.M.). All series included positive and negative controls. Replacement of the monoclonal antibody with mouse IgG1 protein of the same concentration was used as negative control. All controls gave satisfactory results. SW480 cell line was used as positive control. Actin, ECM, and vimentin were used as internal controls.

Immunohistochemical staining

Serial sections (3 μm) were obtained from paraffin-embedded tissues and mounted on glass slides pre-treated with poly-l-Lysine. Sections were stained with hematoxylin and eosin for histologic classification or used for immunohistochemical detection of E-cadherin or β-catenin after deparaffinization in xylene and hydration in alcohol. To improve antigen reactivity, sections were pre-treated as described below. In short, sections were boiled for 10 min in citrate buffer (pH 6), cooled down in the same buffer, and subsequently incubated 5 min in 0.3% H₂O₂. Monoclonal antibodies (mAbs) against β-catenin (clone 14) and E-cadherin (clone 36) were obtained from Transduction Laboratories (Lexington, KY) and were used at 1:1000 dilutions. Tissue sections were incubated with primary antibodies for 30 min at room temperature in a humid chamber, rinsed in PBS, and treated with anti-mouse Envision System (Dako, Glostrup, Denmark). Diaminobenzidine was used as chromogen and sections were counterstained with hematoxylin. All slides were evaluated by two independent pathologists (R.D. and A.M.).

Determination of the immunoreactivity index

The proportion of stained cells and the cellular distribution of the epitope were used to score semiquantitatively the
immunohistochemical staining of β-catenin and E-cadherin. Percentage of positive tumor cells were scored as follows: 0 = no positive cells; 1 = 1–25% positives; 2 = 26–50% positives; 3 = 51–75% positives; and 4 = 76–100% positives. The intensity was estimated in comparison with the control and scored as follows: 0 = absence of staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining.

Statistical analysis

Statistical analyses were performed using the STATA 7.0 program. Differences between proportions were evaluated by the chi-square test and by Fisher’s Exact test accordingly. P values equal or less than 0.05 was considered statistically significant.

Results

Abnormal distribution of β-catenin protein in normal cervical tissue, premalignant lesions, and invasive cancer

In the present study, we first evaluated expression levels and cellular distribution of β-catenin in a series of 188 samples by immunohistochemistry. The series included 20 normal squamous epithelial samples, 42 low- and high-grade squamous intraepithelial lesions (SILs), and 126 cases of invasive carcinoma (68 squamous carcinoma, 31 adenosquamous carcinoma, and 27 adenocarcinoma).

In normal epithelia, immunoreactivity was strongly observed in all cases at the plasma membrane of both basal and parabasal cells. No immunoreactivity was observed in superficial layers of normal epithelium (Figs. 1A and 2A).

On the other hand, while the majority of SILs (64%) exhibited β-catenin at the plasma membrane (27 out of 42), a positive immunoreactivity was also frequently observed at their cytoplasm (62%, 26 out of 42) (Figs. 1B–C). Interestingly, absent immunostaining at the membrane (36%) was observed in a large number of SILs. When we compared β-catenin staining between normal and SILs (both low and high grade), we found a significant difference between them (P = 0.001) (Table 1). It is worth nothing that, in these samples, β-catenin immunostaining was rarely observed in the nucleus. When β-catenin staining was compared between SIL and cancer, we found significant differences between them both at the membrane (P = 0.0453) and the cytoplasm (P = 0.0084).

In invasive carcinomas, while strong staining of β-catenin was found at the cytoplasm and predominantly at the invasion front, nuclear expression was also rarely observed (Fig. 1F). In squamous tumors, while β-catenin was frequently (75%) observed at the membrane (51 out of 68 cases), staining at the cytoplasm was also as frequently observed (76%; 52 out of 68 cases). Again, absent staining in the membrane was found in 25% (17 out of 68) of the samples (Figs. 1D–F). In adenosquamous carcinoma and adenocarcinoma, β-catenin was observed at the cytoplasm in most cases (91%; 28 out of 31; Figs. 2B–C, and 85%; 23 out of 27; Figs. 2D–E, respectively). When we compare cytoplasmic distribution of β-catenin either between normal tissue and adenosquamous carcinoma or between normal tissue and adenocarcinomas, we found significant differences (P < 0.05). Immunostaining at the membrane was found in more than 70% of both types of cancer (Figs. 2B–E). However, we did not find significant differences of β-catenin staining at the membrane between normal tissue

![Fig. 1](image.png)
and adenosquamous carcinomas ($P = 0.2746$). Nuclear staining was observed in only a few cases: one adenosquamous carcinoma (out of 31) and two adenocarcinomas (out of 27) (data not shown). The majority of squamous samples corresponded to stage II tumors, while adenosquamous and adenocarcinomas were between stages II and III. No significant differences were observed when the clinical stage of the tumors was compared to the localization of β-catenin.

Abnormal distribution of E-cadherin in normal cervical tissue, premalignant lesions, and invasive cancer

In this study, we have also analyzed E-cadherin expression and distribution patterns in these tumors (Table 2). Homogenous but weak cytoplasmic staining was observed in normal squamous epithelium, with intense reactivity at cell-to-cell borders in all samples analyzed (12 out of 12; Fig. 3A).

In SILs, E-cadherin was mainly localized in the basal and parabasal layers. In general, immunostaining was less intense and seemed to disappear in cell–cell junctions in 40% of the cases (17 out of 43). In the majority of SILs cases, E-cadherin was found both at the membrane (26 out of 43 samples; 60%) and the cytoplasm (25 out of 43; 58%). When compared to normal tissue, staining in SILs was found significantly different between them, both at the membrane ($P = 0.0108$) and the cytoplasm ($P = 0.0002$) (Fig. 3B). On the contrary, we did not find significant differences for E-cadherin staining between SIL and invasive cancer either at the membrane ($P = 0.7230$) or the cytoplasm ($P = 0.4149$).

In squamous tumors, E-cadherin expression was observed in 66% of the cases (45 out of 68) at the cellular membrane ($P = 0.015$) and in 73% of the cases (50 out of 68) at the cytoplasm ($P = 0.0068$; Figs. 3C–D). In only 4 out of 68 cases analyzed, E-cadherin staining was observed in the nucleus (data not shown). In adenosquamous tumors, E-cadherin was mainly localized in the cytoplasm in 71% of these cases (22 out of 31, $P \leq 0.05$; Figs. 3C–D). In adenocarcinomas, E-cadherin expression was observed in 36% of these samples (11 out of 31). In adenocarcinomas, E-cadherin expression was observed at the membrane in 55% of the cases (15 out of 27) ($P = 0.0068$), as well as in the cytoplasm in 37% of the cases (10 out of 27; $P = 0.017$) (Fig. 3F). Again, in 45% of these cases, E-cadherin was found both at the membrane (26 out of 43 samples; 60%) and the cytoplasm (25 out of 43; 58%).

### Table 1

Distribution of β-catenin in normal squamous epithelium, premalignant lesions, and invasive cancer

<table>
<thead>
<tr>
<th>Cellular localization</th>
<th>Immunostaining</th>
<th>Control $n = 20$</th>
<th>SIL $n = 42$</th>
<th>$p^b$</th>
<th>Invasive cancer $n = 68$</th>
<th>Squamous $P^b$</th>
<th>Adenosquamous $P^b$</th>
<th>Adenocarcinoma $P^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane</td>
<td>Moderate/Strong</td>
<td>20 (100%)</td>
<td>27 (64%)</td>
<td>0.001</td>
<td>51 (75%)</td>
<td>0.009</td>
<td>28 (91%)</td>
<td>0.275</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Absent/Weak</td>
<td>20 (100%)</td>
<td>26 (62%)</td>
<td>0.000</td>
<td>52 (76%)</td>
<td>0.000</td>
<td>28 (91%)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td></td>
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</tbody>
</table>

Superscript:

a SIL: squamous intraepithelial lesion.

b Fisher’s Exact test. $P$ value of each group is the result of its comparison with the control group.
cases membrane immunostaining of E-cadherin was not observed (12 out of 27 cases). No E-cadherin was observed in the nucleus of any adenosquamous carcinoma or adenocarcinoma. When adenosquamous carcinoma and adenocarcinoma were compared for E-cadherin staining at the cytoplasm, we found significant differences \( (P = 0.0096) \).

**Discussion**

Alterations in cell adhesion are among the common features that describe a tumor, including irregularities in adhesion molecules \[26\]. Decreased function of E-cadherin has been detected in cervical squamous carcinoma and carcinoma in situ \[27,28\]. We previously detected an aberrant localization of β-catenin in a small group of squamous tumors of the uterine cervix \[25\]. Our aim in this study was to determine if β-catenin and E-cadherin are altered during neoplastic progression of uterine cervical cancer by analyzing their localization in normal epithelial tissue, premalignant lesions, and invasive cancer of different histologic origin (squamous carcinoma, adenosquamous carcinoma, and adenocarcinoma).

Our finding that, in normal cervical epithelium, β-catenin is localized only at the plasma membrane in cells of the basal and parabasal layers agrees with its known function in cell adhesion \[29\]. Namely, that it remains at the membrane due to its binding to the cytoplasmic domain of E-cadherin. In contrast, frequent cytoplasmic localization of β-catenin was observed in SILs. In fact, it was clearly detected at the membrane and was present in the cytoplasm in 62% of all SILs analyzed.

In the present study that included 126 invasive carcinomas of different histologic types, we found a striking cytoplasmic localization of β-catenin in the majority of the cases (more than 76%). These data suggest that the presence of β-catenin in the cytoplasm may be related to the malignant phenotype of epithelial cells from the uterine cervix. Altered localization can be due to stabilization and subsequent accumulation of β-catenin in the cytoplasm, possibly due to activation of the Wnt signaling pathway, mutations in the APC gene, dysfunction of GSK3β, or mutations of the β-catenin gene itself \[30\]. However, no mutations in the β-catenin gene were found in 40 of these samples analyzed (data not shown and \[25\]). Another explanation may be that alterations in E-cadherin, possibly...
affecting its binding to $\beta$-catenin, can induce its membrane detachment and consequently increase its cytoplasmic levels. Importantly, although differences in protein localization were found among various histologic types of cervical cancer, there was no significantly different proportion (28/31 of adenosquamous carcinoma, 23/27 of adenocarcinoma, and 52/68 of squamous carcinoma).

Loss of cell–cell interactions have been reported to be important in tumor progression. It is worth mentioning that although we found an aberrant localization of $\beta$-catenin in the cytoplasm of invasive cervical carcinoma cells, membrane localization of this protein was not totally absent. Moreover, a strong positive signal at membrane was found for $\beta$-catenin in more than 70% of the cases. Even some studies report an increase [29], other report a reduction [31]. Therefore, we can speculate that cell adhesion may be altered but not completely lost in cervical cancer, or that the loss of the $\beta$-catenin/E-cadherin complex is not necessary.

Recently, Van de Putte et al. found a reduction in membrane-associated expression of $\beta$-catenin (10%) and an increase in its cytoplasmic localization (75%), while nuclear staining was not relevant in early squamous cervical carcinoma [32]. These data are in agreement with our results.

It is worth emphasizing that no $\beta$-catenin expression was found in the nucleus of any of the samples analyzed. This could be linked to the fact that probably in our samples, wild type APC could be exerting its ability to act as a nuclear exporter of $\beta$-catenin from the nucleus [33]. Absent nuclear $\beta$-catenin staining in our samples could also stress the fact that some well-known transcriptional targets of this molecule (like c-myc and cyclin D1) are now known to be independent of its regulation, indicating that nuclear accumulation of $\beta$-catenin is not mandatory in some cases [34]. These results differ from those reported previously for colon cancer and hepatocellular carcinoma, where nuclear localization was frequently observed [35,36]. In this study, as well as that of Imura et al. [37], nuclear translocation was not seen as frequently as in other carcinomas. Their data and those presented here suggest that nuclear translocation of $\beta$-catenin may be an infrequent event in cervical cancer progression, and that nuclear translocation might not be associated with malignant potential or development of not only cervical adenocarcinoma, but also squamous and adenosquamous carcinomas.

Decrease or loss of E-cadherin expression is a common finding in many human epithelial cancers, including cervical cancer [38,39], but a decreased expression in this molecule had also been described in metastasis, but not primary tumors [23,27]. Hypermethylation of CDH1 has been proposed as an explanation for decreased E-cadherin expression [40,41], and was even suggested as a potential marker for identifying cervical cancer patients at high risk for relapse [42]. As expected, we found that, in normal cells, E-cadherin localizes only at the cellular membrane, both in basal and parabasal cell layers. However, in SILs and invasive tumors, E-cadherin was observed both at the plasma membrane and in the cytoplasm. A reduction of protein levels at the membrane was clear in all cases, with an important decrease that ranges from 36% to 45%. This situation may result in reduced cell adhesion normally observed in cervical cancer and intraepithelial lesions [38,43].

The progressive reduction of E-cadherin expression in basal cells of the intermediate and superficial layers of the cervix epithelium (30% of all cases) indicates a possible participation of this molecule in the scaling process of the stratified epithelium. It also suggests that E-cadherin may play an important role in the maintenance of adult tissue structures [27]. Recently, Van de Putte et al. found a diminished expression of membrane-associated E-cadherin in early squamous cervical carcinoma and proposed this changes as early events in cervical carcinogenesis [32]. E-cadherin was observed, both at the membrane and in the cytoplasm, in 13 SIL, although in five cases it was absent. It is interesting to note that in 12 out of 17 cases of SIL in which E-cadherin remained absent at the membrane, there was an abnormal localization of this protein in the cytoplasm, suggesting that the absence of E-cadherin at the membrane with its consequent localization in the cytoplasm could be an important event in SIL progression. This fact is strongly associated with the loss of $\beta$-catenin that we previously observed at the plasma membrane. In summary, our findings indicate that alterations of these proteins are frequent in cancer of the uterine cervix, suggesting that they may play an important role in their development.

Acknowledgments

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