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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.

Keywords: Cervical cancer; β-catenin; E-cadherin; Squamous carcinoma; Adenosquamous carcinoma; Adenocarcinoma; Premalignant lesions; Normal tissue

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.

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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 catenin-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 neoplasic progression within different histologic types of cervical cancer.

Materials and methods

Tissue samples

Normal tissue samples and tumors from the uterine cervix were collected from archives of the Department of Gynecology at the Hospital General Manuel Gea Gonzalez and the Department of Pathology at the Instituto Nacional de Cancerología, 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 hydratation 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% H_2O_2. 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