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Abnormal Immunoexpression of Cell Adhesion Molecules (CAMs) in Cervical Cancer

Toro de Méndez Morelva, PhD,[1] and Llombart Bosch Antonio, PhD[2]

Abstract

The purpose of this study was to examine the immunoexpression of cell adhesion molecules (CAMs) E-cadherin, CD44s, and CD44v3 in cervical cancer and compare it with that in benign exo-endocervical tissue. In all, 81 cervical cancer biopsy specimens and 22 benign controls were included. Primary monoclonal antibodies NHC-38, F10-44-2, and 3G5 for E-cadherin, CD44s, and CD44v3 were used, respectively. Statistical significance was evaluated by the χ^2 test. Antigen expression was significantly different in cervical cancer specimens compared with controls, showing marked decrease in membrane expression: E-cadherin, 6.5% and 77.3% ($P < .000$); CD44s, 3.9% and 81.8% ($P < .000$); and CD44v3, 0% and 81.8% ($P < .000$), respectively. The immunoexpression was significantly heterogeneous in carcinomas ($P < .034$) and adenocarcinomas ($P < .000$) for E-cadherin and CD44s. For CD44v3, no case of cancer showed immunostaining in membranes. These findings reaffirm that cell adhesion is markedly altered in cervical cancer. The authors suggest that these proteins could serve as markers for invasive cervical neoplasia.

Keywords

cervical cancer, E-cadherin, CD44s, CD44v3, immunohistochemistry

Introduction

Cell adhesion is measured by different types of molecules commonly called cell adhesion molecules (CAMs). They are involved in many biological processes of vital importance such as embryogenesis, tissue repair, differentiation, growth, apoptosis, communication, and cellular mobility. They carry out very crucial functions. They join specific ligands located in other cells or in the extracellular matrix to facilitate cellular interactions and their migration to different tissues; also, they generate regulation signals after interacting with their ligands[1-6]

Selectins, integrins, the immunoglobulin family, extracellular matrix proteins, CD44s and their isoforms, and cadherins are included in the CAM family. Cadherins make up the main components of the adherens junctions of the desmosomas. They are monomeric forms of transmembrane glycoproteins that intervene in the cellular interconnection between homotypic cells in the presence of Ca++ ions, which permit the tissue to which it belongs to keep its architecture and morphology by inducing epithelial differentiation and suppressing cellular motility.[4] When the intercellular union zones mediated by E-cadherin are affected, changes occur in the phenotype with an increase in motility and cellular invasion.[7,8]

The CD44 family (cluster differentiation 44) includes a group of glycoproteins widely distributed at the transmembrane level. They are made up of 20 exons: 10 exons vary between exon 5 (v1) and exon 15 (v10), thus, forming an heterogeneous CD44 family. The smallest form of CD44 is the standard one and does not contain any of the exon variables. This protein has been involved in different cellular functions.[9]

CAMs show strong expression in benign epithelial tissue with no exception. On the contrary, in neoplastic tissue, a disposition toward reducing or losing E-cadherin immunoreactivity as well as CD44, or isoforms, is observed. This observation suggests that the impaired CAM-mediated adhesion system is a characteristic of cells with malignant transformation, and the impaired expression of these glycoproteins is frequently observed in tumors with aggressive

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histopathological characteristics.[2,10,11] Immunoexpression decrease in aggressive tumors with a high invasive potential may also be observed.[4]

Cells with malignant potential reduce contact with neighboring cells. This characteristic is reflected in the loss of architecture that gives rise to the destruction of the histological structure belonging to neoplastic growth.[3] The loss or alteration in cellular adherence is also characteristic of squamous and glandular cervical cancer as well as their respective precursor lesions.[12-16] During the development of cervical lesions, alterations essential in CAM expression occur, which are either qualitative in character (cytoplasmic or membrane localization) or quantitative (changes in expression).[11,14,17]

The abnormal expression of E-cadherin in cervical cancer could be one of the factors responsible for the invasion and metastasis of this neoplasm.[18] The selective loss of E-cadherin could generate undifferentiation and invasivity of malignant cervical cells. This suggests that it acts as an invasion suppressor, and as such, it is considered a useful malignancy parameter and marker of minor cellular differentiation.[19,20] The localization of E-cadherin is an important parameter, given that it could be related to the degree of malignity of the cervical lesion in such a way that it could be expressed at the cytoplasmic level instead of its usual membranous localization.[12] In high-risk HPV (human papillomavirus) DNA-positive cervical carcinomas, decreased E-cadherin or lack of expression in E-cadherin is associated with viral integration.[21]

The purpose of this study was to examine the immunoexpression of the CAMs E-cadherin, CD44s, and CD44v3 in cervical cancer and compare it with that in benign exo-endocervical tissue.

Materials and Methods

Benign tissue samples and cancer specimens from the uterine cervix were collected from the archives of the Anatomical Pathology Service of the University Hospital of Valencia, Spain. Two groups of cervical cancer samples were included: the first one with cases diagnosed from 2000 to 2005 and the second one with 15 cases of cervical cancer diagnosed between 1969 and 1998. The original hematoxylins of all the cervical cancer cases were reevaluated to corroborate the original diagnosis and to classify the cases according to the original epithelial tissue.[22] Tumoral samples with one or more of the following characteristics were excluded: excessive necrosis and bleeding, insufficient tumor material, poorly preserved tumor material, and those cases in which there were no corresponding paraffin blocks. Also, 22 cases of benign exocervical and endocervical tissue were included as a control group. These cases were used as controls with which to compare the immunohistochemical reactivity of cervical cancers.

Immunohistochemistry

The tissue microarrays were constructed with distinct tumor areas of squamous cell cervical carcinoma (SCC) and cervical adenocarcinoma. Tissue microarrays of benign exocervical (n = 22) and benign endocervical (n = 22) tissue were also prepared taking the exocervical and endocervical sections from each block. Immunohistochemical staining was then carried out. In summary, deparaffinization in xylene and hydration in decreasing alcohol series was performed. Antigen retrieval was carried out with citrate buffer, pH 6.0, and cooling was done in the same buffer; subsequently, endogenous peroxidase activity with 3% hydrogen peroxide at room temperature for 30 min was blocked. Similarly, tissue collagen activity to avoid nonspecific unions with a 20% horse serum solution at room temperature for 20 min was blocked. The tissue microarrays were then incubated with primary E-cadherin antibodies, NHC-38 (Dako, A/S, Denmark); CD44s, F10-44-2 (Novocastra, Newcastle, UK); and CD44v3, clone 3G5 (R&D System, Abingdon, UK) at room temperature for 1 h. The tissue microarrays were then incubated with biotin-labeled secondary antibody contained in the LSAB + system-HRP, and Liquid DAB + substrate chromogen system (DAKO Cytomation, Denmark) was used to develop the reaction. All rinses were carried out with PBS (pH = 6), and the tissue microarrays were counterstained with hematoxylin. As a negative control, a sample of neoplastic cervical tissue processed in the same conditions without including the primary antibodies was included. Positive controls were made up of neoplastic tissue with known reactivity before the biomarkers under study, from a vesicular tumor, a breast tumor, and a lymphoma. These controls were included in each one of the tissue microarrays. Cell counting was carried out including the entire diameter of tumor disc as the total (100%). The positive reaction against the antibodies in the study was represented by the presence of brown precipitation at the membrane or cytoplasm or in both cellular compartments. The percentage of neoplastic or benign cells with reactivity was estimated considering a minimum of 100 cells per tissue. The distribution of the immunoreactive cells was carried out using a semiquantitative scale: negative, ≤5%; +, ≥5% to <25%; ++, between 25% and 50%; +++, ≥50%. Moreover, we estimated staining intensity and scored as follows: low, moderate, or high. All tissue microarrays were evaluated first by an observer and later by an experienced pathologist who did not know the clinical data regarding the pathologies. Cases with no agreement were reviewed

again by 2 observers using a monitoring system adapted to the microscope for simultaneous observation. The objective was to have a consensus for final immunoreactivity.

Detection and Genotypification of HPV DNA

Only tumoral samples were submitted for HPV evaluation. A paraffin block with no embedded tissue (empty block) was prepared previously. The following recommendations were strictly followed: gloves were used and changed regularly; the microtome was cleaned first with disinfectant, then with xylene, and finally with ethanol; and a new knife was used to cut each block and its corresponding empty paraffin block. We made 5-µm thick cuts were made and placed them in 1.5 mL Eppendorf tubes. We removed the block, cleaned as indicated previously, changed the knife, and cut the corresponding empty paraffin block. The same procedure was used for the other tumor blocks. For isolation of cell DNA, 5 cuts were submitted for deparaffinization with xylene, which were then washed with absolute ethanol. The pellet was resuspended in 500 µL of lysis solution (SDS 0.5%, Tris–HCl 10 mM, pH 8; NaCl 0.15 M; EDTA 5 mM). Then, 25 µL of proteinase K (0.5 mg/mL) was added to each tube and incubated overnight at 55°C. DNA was extracted using phenol–chloroform–isoamilic alcohol and then precipitated by adding 0.1 volume of sodium acetate and 2.5 volumes of ethanol. The pellet was resuspended with enough bidistilled water according to the pellet size (5-30 µL). A polymerase chain reaction (PCR) test was carried out before estimating the quality of DNA extracted through amplification of the interferon gene INF primers: INF150DR, CTGGGATGCTCTTCGACCTC; and INT150DF, TCTTTTCTTTCCCGATAGGT. Samples with a clear band of electrophoresis for INF150 were submitted for amplification of the 65 bp segment of the L1 region of HPV DNA using the set of SPF10 primers (INNO-LiPA, Innogenetics Inc, Belgium). The reaction was carried out at a final volume of 50 µL containing 2 mM of $MgCl_2$, Triton X-100 at 0.1%, 200 µM for each dNTP, 10 µL of the mixture of SPF10 biotinylated primers, 1.5 UI of AmpliTaq Gold DNA polymerase (Perkin-Elmer), and 10 µL of isolated DNA. The PCR conditions were as follows: initial denaturalization and activation of the polymerase for 9 min at 94°C was followed by 40 cycles of 30 s at 94°C, 45 s at 52°C, and 45 s at 72°C, and a final extension for 5 min at 72°C. Positive and negative HPV 6 (Innogenetics, Belgium) controls containing only master mix reaction were included. PCR products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide. If the analyzed sample contained HPV DNA, the band was expected to have approximately 65 pb. The samples resulting from positive HPV DNA for SPF10PCR were submitted for genotypification of HPV using the reverse hybridization band probe assay method (INNO-LiPA, Innogenetics Inc, Belgium). The SPF10PCR products were mixed with a denaturing and hybridized solution with specific probes to identify 24 different HPV types (namely, HPV-6, -11, -16, -18, -31, -33, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -66, -68, -70, and -74).

Statistical Analysis

Association between different variables was determined using the χ^2 test with the Yates test and the Fisher exact probability test. The SPSS statistical package was used for statistical analysis, and statistical significance was set at $P < .05$.

Results

For this study, the age for each cervical cancer case was obtained from the clinical information originally found at the hospital service (range 28-79 years; mean 50.67 years). For the control group the age range was 36-75 years, with a mean age of 51.82 years. Data is not shown. A total of 81 cases of cervical cancer were studied: 62 (76.5%) SCCs and 19 (23.5%) cervical adenocarcinomas. In SCCs, the moderately differentiated (MD) group tumors predominated (43.5%), followed by well-differentiated (WD) ones (21%). The group of carcinomas with less differentiation was made up of 22.6% immature (IM) and 12.9% of immature and undifferentiated (IU) ones. The greater portion of adenocarcinomas belonged to the endocervical type (42.1%), followed by the endometrioid type (26.3%); the villoglandular and papillary types reached 15.8% and 10.5%, respectively; the remaining 5.3% represented a mucinous type. We also included 44 benign cervical tissues (control group: 22 exocervical and 22 endocervical).

Of the 81 cervical tumors, 3 (3.7%) were excluded because DNA was inadequate; for the remaining 78 (96.3%) cases, SPF10PCR was carried out, and amplification of the HPV DNA sequences present in these samples was obtained. Then, they were considered HPV positive. In these, 67 (85.9%) cases showed a single HPV infection, and 11 (14.1%) showed multiple HPV-type infections. The most frequent specific viral type was HPV16 in 45 (71.4%) cases, followed by HPV18 in 12 (19.4%), HPV45 in 3 (4.5%), and HPV58 in 2 (3%); viral types HPV31, HPV66, HPV68, and HPVX manifested in equal numbers, with 1 of each (1.5%). The SCCs as well as the adenocarcinomas showed predominantly 1 type of viral sequence 83.1% and 94.7%, although they did not reach statistical

Table 1. Summary of Immunoreactivity and Localization of CAMs in Controls and Cervical Cancer Histological Types

			E-cadherin		CD44s		CD44v3	
			No (%) Positive	No (%) Negative	No (%) Positive	No (%) Negative	No (%) Positive	No (%) Negative
	Cases		59 (76.6)	18 (23.4)	70 (90.9)	7 (9.1)	79 (97.5)	2 (2.5)
	Controls		34 (77.3)	10 (22.7)	36 (81.8)	8 (18.2)	36 (81.8)	8 (18.2)
Histological types	Carcinoma		47 (77.0)	14 (23.0)	59 (98.3)	1 (1.70)	60 (96.8)	2 (3.2)
	Adenocarcinoma		12 (75.0)	4 (25.0)	11 (64.7)	6 (35.3)	19 (100)	–
	P		<.034		<.000		.230	
Immunoreactivity	Carcinoma	+	13 (21.3)		40 (66.7)		39 (62.9)	
		++	20 (32.8)					
		+++	14 (23.0)		19 (31.7)		21 (33.9)	
	Adenocarcinoma	+	9 (56.3)		10 (58.8)		10 (52.6)	
		++	3 (18.8)		<0.000			
		+++	–		1 (5.9)		9 (47.4)	
	P		<.034				<0.230	
Localization	Cases	M	5 (6.5)		3 (3.9)		–	
		C	19 (24.6)		25 (32.5)		47 (58.0)	
		M/C	35 (45.5)		42 (54.5)		32 (39.5)	
	Controls	M	34 (77.3)		36 (81.8)		36 (81.8)	
		C	–					
		M/C	–					
	P		<.000		<.000		<.000	
	Carcinoma	M	5 (8.2)		3 (5.0)		–	
		C	9 (14.8)		20 (33.3)		30 (48.4)	
		M/C	33 (54.1)		36 (60.0)		30 (48.4)	
	Adenocarcinoma	M	–		–			
		C	10 (62.5)		5 (29.4)		17 (89.5)	
		M/C	2 (12.5)		6 (35.3)		2 (10.5)	
	P		0.064		0.479		0.688	

Abbreviations: M, membrane; C, cytoplasm.

significance ($P = .208$). HPV16 was the most frequent viral type in SCC 35 (71.4%) as well as in cervical adenocarcinomas 10 (55.6%), with equal *P* values of .248; HPV18 was detected in 44.4% of the cervical adenocarcinomas and in 10.2% of SCCs, which were statistically significant ($P < .001$). These results are not shown. Immunoreactivity results for the CAMs—E-cadherin, CD44s, and CD44v3—and cellular immunolocalization in cervical cancers and the control group are summarized in Table 1.

E-cadherin. In all, 76.6% of cervical tumors were E-cadherin positive in membrane, cytoplasm, or in both cellular compartments, whereas 77.4% of the control group were E-cadherin positive in membrane, resulting in significant differences ($P < .000$) according to cellular localization, when compared with the control group. The greatest proportion of cases of both histological types (59) showed between + and +++ reactivity. Interestingly, 56.3% of the adenocarcinomas had less than 25% of stained cells, and no case had +++ reactivity. This suggests an important decrease in cellular cohesiveness for this tumor type in comparison with SCCs ($P < .034$). In relation to SCCs, 54.1% showed E-cadherin expression in the membrane as well as in the cytoplasm (M/C), followed by the cytoplasmic (C) localization (14.8%), but only 8.2% showed only membrane (M) expression. In the adenocarcinomas, 62.5% had localization C and 12.5% in M/C; there were no cases with M staining. E-cadherin immunolocalization was significantly different between the 2 histological types of tumors ($P < .000$).

Membrane localization of E-cadherin in malignant squamous cells was more intense than in cytoplasm; in this, it was more homogeneous and clear. Membrane staining of the adenocarcinomas was more marked on the lateral borders than on the basal portion; the edge of the apex was left without staining. The cytoplasmic staining was less intense and homogeneous; this observation corresponded to the 2 cases of adenocarcinoma with M/C staining. Figures 1A to 1F show membrane and cytoplasm staining in both histological types of cervical cancer.

Comparison of E-cadherin localization between tumors and controls was significantly different ($P < .000$). In tumors, it was variable but with cytoplasmic trends, whereas in controls, it was located basically in the membrane. Only some cells from the deep layers showed slight

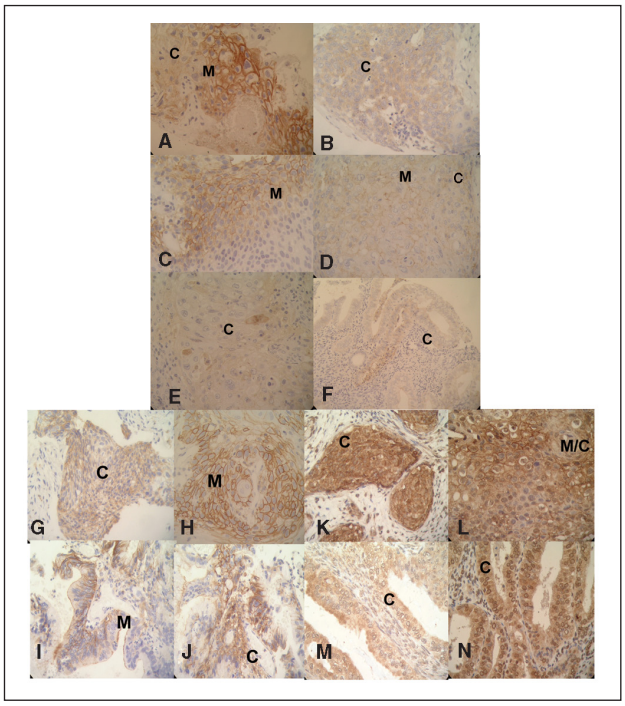

Figure 1. Immunoreactivity of CAMs in cervical cancer showing cell localization. E-cadherin: A-E, squamous cell carcinoma, 40×; F, adenocarcinoma villoglandular, 10×. CD44s: G, H, squamous cell carcinoma, 20×, 40×, respectively; I, J, endocervical adenocarcinoma, 20×. CD44v3: K, L, squamous cell carcinoma, 20×, 40×, respectively; M, N, endocervical adenocarcinoma, 20×, 40×, respectively.
Abbreviations: M, membrane; C, cytoplasm.

Table 2. Immunoreactivity and Cell Immunolocalization of Adhesion Molecules in Squamous Cell Cervical Carcinomas According to Degrees of Differentiation

Biomarker	WD (%)	MD (%)	IM (%)	IU (%)	*P*
E-cadherin					
<5%	23.1	7.7	21.4	75	.006
5-<25%	15.4	15.4	50	–	
>25-50%	30.8	50	14.3	12.5	
>50%	30.8	26.9	14.3	12.5	
E-cadherin localization					
Negative	23.1	7.7	21.4	75	.004
Membrane	–	11.5	14.3	–	
Cytoplasm	7.7	15.4	28.6	–	
M/C	69.2	65.4	35.7	25	
CD44s					
<5%	–	–	–	14.3	.330
5%-50%	53.8	73.1	78.6	42.9	
>50%	46.2	26.9	21.4	42.9	
CD44s localization					
Negative	–	–	–	14.3	.070
Membrane	15.4	–	–	14.3	
Cytoplasm	30.8	19.2	57.1	42.9	
M/C	53.8	80.8	42.9	28.6	
CD44v3					
<5%	–	–	–	25	.008
5%-50%	46.2	66.7	71.4	62.5	
>50%	53.8	33.3	28.6	12.5	
CD44v3 localization					
Negative	–	–	–	25	.063
Cytoplasm	53.8	48.1	42.9	50	
M/C	46.2	51.9	57.1	25	

Abbreviations: WD, well differentiated; MD, moderately differentiated; IM, immature; IU, immature and undifferentiated; M/C, membrane/cytoplasm.

cytoplasm staining (Table 1 and Figures 1A to 1F; Figures 2A and 2B). Table 2 shows E-cadherin immunoexpression in the SCC group according to degrees of differentiation; it was significantly variable ($P < .006$); the IUs were the least reactive (75%). The greater proportion of cases showed localization. M/C and IUs were predominantly negative ($P < .004$). The immunoreactivity of E-cadherin in different histological subtypes of adenocarcinoma was 100% endometrioid (cytoplasmic), mucinous (cytoplasmic), and villoglandular (M/C), followed by 62.5% endocervical (cytoplasmic) and 50% papillary in M/C; there were no significant differences. The E-cadherin localization was predominantly cytoplasmic and M/C. There were no significant differences. These data are shown in Table 3. E-cadherin was expressed intensely in exocervical tissue. It had a fine granular appearance at intermediate layers and a tenuous appearance at the deep layers, whereas at the superficial layers, appearance progressively disappeared. Basal and parabasal cell staining was predominantly cytoplasmic and occasionally membranous (Figure 2A). In benign endocervical cells, there was also E-cadherin expression; this was also observed in those membranes close to the basal portion, the nucleus, and, occasionally, the cytoplasm. Reserve cells did not react before the antibodies against E-cadherin,[23] and elements of the underlying stroma did not show staining (Figure 2B).

CD44s. In membrane, cytoplasm, or in both cellular compartments, 90.9% of cervical tumors revealed immunoreactivity for CD44s, whereas 81.8% of the control group responded positively but only in membrane, with significant differences ($P < .000$) in cellular localization when compared with the control group. The greater proportion of cases of both histological types (50) showed between + and ++ reactivity for CD44s; only 5.9% of the adenocarcinomas had +++ reactivity, with significant differences ($P < .000$). In 60% of SCCs, the CD44 expression was in M/C followed by C localization (33.3%) and only 5% in M. In the adenocarcinomas, 35.3% of reactivity was present in M/C and 29.4% in C. No case revealed

Table 3. Immunoreactivity and Cell Immunolocalization Adhesion Molecules in Histological Subtypes of Cervical Adenocarcinoma

Biomarker	Endocervical (%)	Mucinous (%)	Endometrioid (%)	Papillar (%)	Villoglandular (%)	*P*
E-cadherin						
<5%	37.5	–	–	50	–	.766
5%-25%	37.5	100	100	–	100	
>25%	25	–	–	50	–	
E-cadherin localization						
Negative	37.5	–	–	50	–	.064
Cytoplasm	62.5	100	100	–	–	
M/C	–	–	–	50	100	
CD44s						
<5%	25	100	25	50	50	.606
5%-50%	75	–	50	50	50	
>50%	–	–	25	–	–	
CD44 localization						
Negative	25	100	25	50	50	.479
Cytoplasm	50	–	–	50	50	
M/C	25	–	75	–	–	
CD44v3						
5%-50%	25	100	60	50	100	.012
>50%	75	–	40	50	–	
CD44v3 localization						
Negative	–	–	–	–	–	.688
Cytoplasm	87.5	100	80	100	100	
M/C	12.5	–	20	–	–	

Abbreviation: M/C, membrane/cytoplasm.

immunoreaction in M. CD44s immunolocalization was significantly different between both histological types ($P < .000$). Malignant cells after membrane staining showed a thick, brown, and sharp-edged appearance; a fine–granular homogeneous cytoplasm was observed. Staining intensity varied between moderate and marked (Figures 1G to 1J). The comparison of CD44s immunolocalization between tumors and controls showed cytoplasmic tendencies in cervical cancer (87%), whereas 81.1% of immunopositive controls were membranous with significant differences ($P < .000$). See Figures 1G to 1J and 2C to 2D. Even though differences were not significant, it is worth mentioning that the IU revealed less expression for the CD44s (85.7%) when compared with the WD, MD, and IM (in the tumor areas with certain degree of differentiation), which expressed this molecule in 100% of the cases ($P = .330$). The C localization of CD44s prevailed in IM tumors (57.1%) and IU (42.9%). IM tumors had no M staining, and 14.3% of the IUs showed staining only in M, equivalent to 15.4% of WD. MD (80.8%) showed greater M/C expression. The WD (84.6%) combined the expression between C (30.8%) and M/C (53.8%). None of these differences was statistically significant (Table 2). The reactivity for the CD44 antigen was more evident in cases of endocervical and endometrioid adenocarcinomas (75%), followed by 50% of the papillary and villoglandular ones ($P = .060$). The localization of CD44 expression had cytoplasmic tendencies in 64.7%. These data showed no statistically significant differences ($P = .479$). See Table 3. In exocervical epithelium, CD44s immunoexpression was observed to be more intense at intermediate layers, whereas it progressively disappeared at superficial layers. M staining was fine and granular but very intense. In the underlying stroma, the cytoplasm from the fibroblasts and the endothelial cells were CD44s immunopositive (Figure 2C). The benign glandular cells generally showed intense staining in both basal and apical portions. However, some cells showed a C staining; in some cases, the stain showed a greater intensity than in the epithelial cells (Figure 2D).

CD44v3. Immunoreactivity for CD44v3 was exhibited by 97.5% of cervical tumors and 81.8% of the controls. When comparing the antigenic expression between both tumors and controls, statistically significant differences ($P < .000$) were observed; staining was irregular, with different degrees of reactivity and different cellular localizations. We found that 96.8% of SCCs and 100% of adenocarcinomas responded positively to the CD44v3 antibody. The greatest proportion of cases of both histological types (49) showed staining between + and ++, without significant differences ($P = .230$). 60 SCC cases showed CD44v3 expression in C and M/C in the same proportion for both localizations (48.4%). For the group of adenocarcinomas, the antigenic localization was mainly C (89.5%), with statistical differences between both

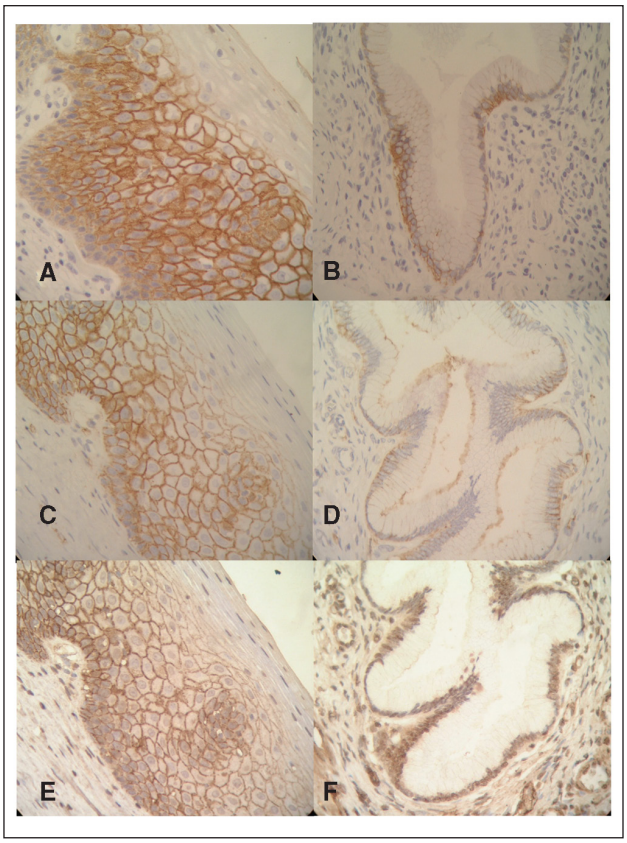

Figure 2. Immunoreactivity of CAMs in benign exocervical and endocervical tissue; E-cadherin: A, B, 20×; CD44s: C, D, 20×; CD44v3: E, F, 20×.

histopathological groups ($P < .000$). No case of cervical cancer showed CD44v3 staining in M. Membrane staining showed a thick, brown, and sharp-edged appearance; a fine–granular homogeneous appearance was observed in the cytoplasm. Staining intensity varied between moderate (if it was cytoplasmic) and intense (if it was membranous) as can be observed in Figures 1K to 1N. The comparison of CD44v3 localization between tumors and controls showed statistically significant differences ($P < .000$). In controls, the localization was predominantly M, whereas in tumors, expression of this protein in M/C and C was observed. No case showed M expression (Figures 1K to 1N and 2E to 2F). CD44v3 expression was significantly different ($P < .008$) in IUs; it was expressed in >5% of the neoplastic cells in 75% of the cases. However, in the remaining cases, CD44v3 was expressed in 100% of neoplastic cells. The cytoplasmic tendency prevailed, showing independent or combined form with M expression. We found C localization only in 42.9% of IM carcinomas and in 50% of IUs, whereas in M/C it was 57.1% and 25%, respectively. In MD, 48.1% showed CD44v3 in C and 51.9% in M/C. WD showed mainly C localization (53.8%) without significant differences (Table 2). CD44v3 immunoexpression occurred in 100% of the histological subtypes of cervical adenocarcinoma; 100% of the villoglandular and mucinous adenocarcinomas showed CD44v3 expression in <50% cells, followed by the endometrioid (60%), papillary (50%), and endocervical, which were less reactive (25%). The rest of the cases showed +++ reactivity, with statistically significant differences ($P < .012$). Localization was predominantly C in 17/19 (89.5%) of the cervical adenocarcinomas. See Table 3. CD44v3 immunoexpression of benign exocervical cells was observed at some deep layers. It was more marked at intermediate layers, whereas it progressively disappeared in high intermediate and superficial layers. Membrane staining appeared to be fine and granular, though very intense. In the underlying stroma, the cytoplasm of the fibroblast and the endothelial cells showed immunoreactivity (Figure 2E). In general, the benign endocervical cells showed intense M staining in both basal and apical portions. However, in some others, a fine brown line was seen surrounding the cell. Cytoplasms were negative. Stroma cells and all endothelial cells showed C staining with greater intensity than the epithelial cells (Figure 2F).

Though E-cadherin, CD44s, and CD44v3 expressions in this series of cervical carcinomas are quantitatively equivalent to benign cervical tissue, they show predominantly cytoplasmic localization, which suggests that there is retention of adhesion proteins in the cytoplasm of malignant cervical cells rather than in the regular membrane.

Discussion

In this study, CAM immunoexpression was examined in a series of 81 cervical cancers and compared with that in benign cervical tissue using immunohistochemistry. The loss of cell cohesiveness is characteristic of invasive neoplasia and high-grade intraepithelial lesions of the cervix.[24] The progressive reduction of CAM expression in neoplastic cervical tissue indicates a possible participation in the epithelial stratification process and in the maintenance of the adult tissue structure.[12,24] Cell adhesion alterations, especially in E-cadherin, are frequent in cervical cancer and have been related to the development and progression of this disease.[17] In this study, we found abnormal immunohistochemical expression in CAMs, not only with respect to lowered or absent expression but also with respect to expression tending toward inadequate cytoplasmic localization. Substantial qualitative alterations (cytoplasmic or membranous localization) occur during the development of the cervical lesions and quantitative alterations (changes in expression) in the main molecules

involved in cellular adhesions, such as E-cadherin, CD44, and their isoforms.[14]

E-cadherin expression in malignant cervical cells and dysplastic cell membrane was decreased in 50% of the cell components in 905 cases according to van de Putte et al.[16] Similarly, it has been reported that E-cadherin is distributed abnormally in the cytoplasm of 58% of the preinvasive lesions and in 71% of cervical cancer cases as compared with benign epithelial tissue.[16] In this study, the most significant finding was the decrease of membrane immunoreactivity and the progressive increase of the cytoplasmic localization, possibly related to the destruction of the histological structure as a consequence of the neoplastic transformation.

It is possible that the tendency toward CAM cytoplasmic localization may be because of a pathway that provokes its functional inactivation: (1) in cases of cervical cancer, the pRb protein pathway is blocked by the direct interaction of E7 high-risk HPV, being a possible origin of E-cadherin alternation[8]; (2) the exaggerated activation of the ubiquitin-proteasome pathway could also explain the cytoplasmatic retention and rapid degradation of the CAMs, thus, avoiding their transfer to the cellular membrane[13,25-27]; (3) the mutations and methylations of the genes that encode the CAMs could be another cause of these alterations.[2]

The antigenic expression of the CD44 family and its isoforms is found to be markedly disturbed in different neoplastic gynecological tissues, including cervical cancer[5,28,29] associated with the progression and metastasis of tumors.[30,31] The CD44 family[11,32] and E-cadherin[33] can be found to be greatly altered in the lesser differentiated carcinomas. In this study, significant differences in CD44v3 expression and E-cadherin were observed in the IM and IU carcinomas with respect to those carcinomas with a greater degree of differentiation. In the histological subtypes of the adenocarcinomas, no significant difference was found, although these proteins were likewise highly altered. This suggests that the loss of epithelial differentiation and the cohesiveness are related to the functional alteration of the cell adhesion. We also observed reduced significant expression in the adenocarcinomas in comparison with SCCs probably resulting from the degree of aggression and the prognosis of each histological type of tumor.[34] It is possible that the behavior of each histological tumor type is related to the degree of CAM alteration, as in the case of neuroendocrine cervical carcinoma, where the loss of adhesion by BRG-1,[29] a regulator of CD44 expression, is involved.

With respect to the isoforms from the CD44 family, the v3 has been little studied in cervical neoplasia. In this study, CD44v3 expression was markedly altered in both histological tumor types related to E-cadherin and CD44s. Thus, we suggest that this protein could serve as a marker in invasive cervical neoplasia.

Analysis of the immunoreactivity of CAMs in cervical neoplastic tissue shows that there are marked alterations, when compared with the immunoreactivity in benign cervical tissue, seen during carcinogenesis and kept in the invasive lesion.[31,33-35] Benign cervical tissue included in this study responded as expected against the antibodies in the study. This coincided with the findings in other reports where it has been compared with malignant tissue.[30,34-36]

It is worth insisting that different results from diverse studies may be a result of variations of technical types (different antigenic retrieval methods and specificity of the primary antibodies used) as well as the consideration of different parameters for the interpretation of the immunohistochemical results.[14]

In conclusion, our findings confirm that in cervical cancer, the cellular interaction is found to be markedly altered by loss or decrease of the membrane antigenic expression and by predominantly cytoplasmic localization when compared with benign epithelial cervical tissue. These findings reaffirm that the loss of cell cohesiveness occurs in the malignant phenotype. We suggest that these proteins could serve as markers for invasive cervical neoplasia.

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Declaration of Conflicting Interests

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