Immunohistochemical Localization of Survivin in Benign Cervical Mucosa, Cervical Dysplasia, and Invasive Squamous Cell Carcinoma

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Abstract

Survivin is an inhibitor of apoptosis protein (IAP) that is expressed in fetal development and in cancer. Survivin expression in premalignant lesions remains undefined. We obtained 73 samples of cervical squamous tissue, including 31 normal, 17 low- and 15 high-grade squamous intraepithelial lesions (LSILs, HSILs), and 10 squamous cell carcinomas (SCCs) from cone biopsy and hysterectomy specimens, and stained for survivin using an immunoperoxidase method. Nuclear staining was detected in normal mucosa, LSILs, and HSILs; staining intensity was greatest in cases with morphologic evidence of human papillomavirus (HPV) infection. In situ hybridization of serial sections demonstrated colocalization of HPV DNA and survivin. Cytoplasmic staining was observed in immature squamous metaplasia and in SCCs. Survivin expression in immature metaplastic squamous mucosa may reflect a role for survivin in normal squamous differentiation. However, the histologic correlation between nuclear staining and HPV infection suggests involvement of survivin in HPV-mediated disruption of normal cellular maturation.

The most common fatal malignancy of women in many developing countries is cervical cancer, while in the United States, there has been a tremendous reduction in cervical cancer mortality owing to the success of diagnostic cytopathology. The majority of cervical cancers are squamous cell carcinomas (SCCs), and most cases are preceded by intraepithelial precursor lesions that can be divided into low-grade squamous intraepithelial lesions (LSILs), including condyloma/mild dysplasia, and high-grade squamous intraepithelial lesions (HSILs), comprising moderate dysplasia, severe dysplasia, and carcinoma in situ. While high-grade lesions seem to progress to invasive cancer at a higher rate than low-grade lesions, it is not possible to determine the risk of progression in individual SILs. Molecular markers of malignant potential may one day have an important role in the detection of lesions that have the greatest potential for progression to cancer and may also have a role in increasing the sensitivity of current diagnostic techniques.

The 2 major classes of apoptosis inhibitors are the Bcl-2 family and the inhibitor of apoptosis protein family (IAP). The first IAP was identified in a baculovirus, and in recent years, many others have been identified in various mammalian species, including humans. Survivin seems to exert its primary antiapoptotic effect by binding to, and inhibiting, the proapoptotic group of proteases known as caspases (cysteine-containing aspartate-specific proteases), in particular, caspases 3 and 7. It also has been shown that survivin associates with microtubules of the mitotic spindle and that disruption of this interaction leads to a loss of survivin’s antiapoptotic function and increased caspase-3 activity. Survivin is unique among the human IAPs because it is expressed in most cancers but has been described only rarely in corresponding normal adult somatic tissues.
However, the role of survivin expression in malignant transformation has not yet been evaluated. Furthermore, the expression of survivin protein in cervical cancer has not been reported. The purpose of the present study was to determine whether survivin is expressed in benign, potentially premalignant, or malignant lesions of the cervical mucosa. For this purpose, the immunohistochemical localization of survivin protein was evaluated in normal cervical squamous mucosa, LSIL, HSIL, and invasive SCC.

Materials and Methods

Production and Characterization of the Antisurvivin Antibody

Antiserum to survivin was produced by immunization of New Zealand white rabbits with an amino-terminal survivin peptide sequence (PTLPPAWQPFLKDHRI) linked to keyhole limpet hemocyanin, as detailed by Ambrosini et al.11 Western blot analysis of the antiserum against 20 µg of total protein HeLa cell extract, using an immunoperoxidase method with enhanced chemiluminescence (Amersham Pharmacia, Buckinghamshire, England), showed reactivity of the antiserum with a single protein of approximately 16.5 kd, consistent with the published molecular weight of survivin. By contrast, reaction of the Western blot with preimmune serum showed no immunoreactivity.

Immunoglobulins from the immunized rabbit and the preimmune serum were purified by affinity chromatography on a peptide-sepharose matrix, coupled with Protein A (Pierce, Rockford, IL).

The specificity of the immunoglobulins for survivin was characterized by immunocytochemical evaluation of survivin expression in COS cells. A human complementary DNA (cDNA) corresponding to the full length of survivin was subcloned into the expression vector pSG5 (Stratagene, La Jolla, CA), to produce a survivin fusion protein bearing a C-terminal vesicular stomatitis virus glycoprotein epitope tag. The resultant plasmid vector was then expressed transiently into COS cells. Immunocytochemical analysis of the antisurvivin immunoglobulins against COS cells transfected with survivin cDNA using an immunoperoxidase detection method showed positive staining. By contrast, no staining was seen when COS cells transfected with expression vector lacking survivin cDNA were substituted for COS cells expressing survivin.

Tissue Samples

Benign cervical squamous mucosa, SILs, and cervical SCCs were obtained from cervical cone biopsy and hysterectomy specimens. Two pathologists (K.R.S. and M.F.) reviewed each case using standard criteria5,12 and selected a total of 73 histologic regions from 43 cases for the localization of survivin protein as follows: 31 nonneoplastic cervical squamous mucosa, 17 LSILs, 15 HSILs, and 10 SCCs. The LSILs included 7 cases with unequivocal histologic evidence of human papillomavirus (HPV) infection (condylomatous epithelium) and 10 cases with disordered epithelial maturation confined to the lower third of the squamous mucosa, with minimal evidence of HPV-associated nuclear atypia.

Immunohistochemical Localization of Survivin

Four-µm sections of formalin-fixed, paraffin-embedded tissue blocks were collected on glass slides (Superfrost Plus, VWR Scientific, West Chester, PA) and baked overnight at 60°C. Following passage through xylene and graded alcohols, the sections underwent a 15-minute treatment with 3.0% hydrogen peroxide. Antigen retrieval was performed by heating the slides for 10 minutes in a 0.1-mol/L concentration of citrate buffer (pH 6.0), in a Decloaking Chamber (Biocare Medical, Walnut Creek, CA). The slides then were cooled and incubated with antisurvivin antibody (1.8 ng/µL in phosphate-buffered saline, pH 7.4, with 1.0% bovine serum albumin) overnight in a humidified chamber at room temperature. Following reaction with the primary antibody, the sections were reacted with a peroxidase-labeled goat anti-rabbit antibody (Biosource International, Camarillo, CA) at 37°C for 30 minutes. Last, the sections were developed using 3,3'-diaminobenzidine (Dako, Carpinteria, CA), counterstained using hematoxylin, dehydrated in graded alcohols, and coverslipped. All cases that showed either focal or diffuse staining for survivin were recorded as positive for survivin expression. Negative controls were performed on all sections and consisted of substitution of preimmune immunoglobulin fraction for the survivin antibody.

HPV DNA in Situ Detection

Four-µm sections of formalin-fixed, paraffin-embedded tissue blocks were processed for HPV DNA in situ hybridization using probes for HPVs 6/11, 16/18, and 31/33, using a commercially available system, according to the manufacturer’s instructions (Rembrandt HPV DNA typing kit, Zymed, San Francisco, CA).

Results

Immunohistochemical localization of survivin protein revealed a reproducible pattern of staining in nonneoplastic cervical squamous mucosa, cervical dysplasia, and invasive squamous carcinoma Table II. The negative control sections were unstained in all cases.
Table 1
Survivin Detection in Cervical Mucosa

<table>
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<tr>
<th>Histologic Diagnosis</th>
<th>No. (%) of Survivin-Positive Cases</th>
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<tr>
<td>Benign cervical mucosa (n = 31)</td>
<td>22 (71)</td>
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<tr>
<td>Low-grade squamous intraepithelial lesion (n = 17)</td>
<td>14 (82)</td>
</tr>
<tr>
<td>High-grade squamous intraepithelial lesion (n = 15)</td>
<td>9 (60)</td>
</tr>
<tr>
<td>Squamous cell carcinoma (n = 10)</td>
<td>4 (40)</td>
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Survivin was detected in 22 (71%) of 31 sections of benign cervical squamous mucosa, and 2 distinct patterns of staining were seen. Mature squamous epithelium displayed a characteristic nuclear pattern of staining that was suprabasal, moderate in intensity, and confined to the lower half of the mucosa. By contrast, areas of immature squamous metaplasia displayed strong cytoplasmic staining with a distinct suprabasal, full-thickness distribution and a sharp demarcation from adjacent unstained mucosa Image 1A and Image 1B. The basal cell layer showed no staining for survivin, and minimal staining was detected in the endocervical glandular mucosa.

Survivin was detected in 14 (82%) of 17 sections of LSIL, including 13 cases with discrete nuclear staining and 1 case that showed only diffuse cytoplasmic staining. In cases that were characterized as LSIL solely on the basis of morphologic evidence of disordered squamous maturation, survivin was detected in 7 of 10 cases, and the intensity of nuclear staining was moderate, similar to that seen in mature squamous mucosa. By contrast, in cases of LSIL with nuclear and histologic features of HPV infection, strong nuclear staining was detected in all 7 cases. In these areas, survivin staining was especially intense in squamous cells with viral-associated nuclear atypia Image 1C and Image 1D.

Survivin was detected in 9 (60%) of 15 sections of HSIL. In cases that showed moderate dysplasia, survivin staining was predominantly cytoplasmic, suprabasal, and preferentially expressed in the lower levels of the squamous mucosa. In cases of severe dysplasia and carcinoma in situ, the staining was predominantly nuclear Image 1E and Image 1F.

Survivin was detected in 4 (40%) of 10 sections of SCC. The staining was almost exclusively cytoplasmic, and the intensity of staining was faint. Several cases that were predominantly unstained showed focal up-regulation of survivin in areas of keratinization Image 1G and Image 1H.

Three cases of condyloma with intense nuclear staining for survivin were analyzed for HPV DNA by in situ hybridization, using paired serial sections. Two cases tested positive for HPV 16/18, and the third showed a positive nuclear signal with the probe for HPV 31/33. Both survivin and HPV were preferentially expressed in the upper portions of the condylomatous epithelium. The distribution of survivin staining was similar to the distribution of HPV DNA nuclear positivity, and colocalization was identified in numerous epithelial cells Image 2A and Image 2B. Rare dyskeratotic cells showed cytoplasmic localization of survivin and were negative for HPV DNA (Images 2A and 2B).

Discussion
Survivin was detected by an immunohistochemical method in benign cervical squamous mucosa, cervical dysplasia, and invasive SCC, and the pattern of staining was distinctive within each diagnostic category. Intense staining was seen in immature metaplastic squamous epithelium and in cells that showed morphologic evidence of HPV infection. Mature squamous mucosa showed nuclear parabasal staining, while HSILs and SCCs showed heterogeneous staining distribution of a variable degree of intensity that diminished in correlation with the loss of morphologic features of well-differentiated squamous epithelium.

The immunohistochemical localization of survivin has been reported previously in human fetal tissues11,13 and in a variety of human tumors, while few studies have reported the expression of survivin in nonneoplastic adult somatic tissues. Specifically, survivin has been detected by immunohistochemical analysis in lung adenocarcinoma and squamous cell carcinoma, pancreatic adenocarcinoma, colonic adenocarcinoma, breast carcinoma, and high-grade non-Hodgkin lymphomas.11 To the best of our knowledge, the immunohistochemical localization of survivin protein in normal cervical mucosa, cervical dysplasia, and invasive squamous cell carcinoma has not been reported previously. However, Saitoh et al,14 using Northern blot analysis, found that in most cases, the levels of survivin messenger RNA in cervical cancer were 2 times greater than in normal cervical tissue.

Several reports indicate that survivin can be detected in benign and malignant cutaneous lesions. Chiodino et al and Grossman and coworkers16 demonstrated the detection of survivin in Bowen disease, a high-grade premalignant alteration of the cutaneous epithelium. Chiodino et al also showed that survivin is present in normal basal keratinocytes and in benign melanocytic lesions of the skin. In their study, faint cytoplasmic staining was found in normal basal keratinocytes, and a similar pattern also was seen in solar seborrheic keratoses. By contrast, there was stronger staining of cutaneous SCCs and malignant melanomas.15 Furthermore, 2 articles have documented marked up-regulation of survivin expression, as measured by immunoblotting, in nonneoplastic endothelial cells following stimulation by...
vascular endothelial growth factor. In addition, up-regulation of survivin was demonstrated by an immunohistochemical method in endothelial cells within areas of granulation tissue.

Studies have described the existence of alternatively spliced variants of survivin that seem to retain activity. Mahotka et al described the identification of 2 novel alternatively spliced survivin transcripts, designated survivin-DeltaEx3 (lacking exon 3) and survivin-2B (retaining part of intron 2 as a cryptic exon). The antipapoptotic function of survivin-DeltaEx3 was largely retained; however, there was a marked reduction of activity for survivin-2B. Conway et al showed that with progressive splicing, the survivin molecule seems to lose the carboxy coiled-coil and baculovirus repeat (BIR) domain. Since our antibody was developed against the amino-terminal end of the protein, it should detect all the known variants. However, the different patterns of cellular localization and staining intensity may reflect the localization of different spliced variants. It remains to be seen whether survivin-DeltaEx3 and survivin-2B spliced variants correspond with the various histologic patterns of localization.

In the present study, staining of paired serial sections demonstrated nuclear colocalization of survivin and HPV in cervical condylomata. The shift in the intracellular distribution of survivin could reflect specific nuclear translocation mechanisms or could result from an artifactual disruption caused by HPV infection. The colocalization of survivin and HPV suggests that HPV may have a direct or indirect effect on regulating the levels of survivin expression and the subcellular localization of survivin. Conversely, survivin may be recruited to participate in HPV-mediated dysregulation of the normal patterns of squamous differentiation. The molecular mechanisms that underlie these processes remain to be defined but could involve a direct or an indirect effect of HPV-encoded gene expression, potentially including HPV proteins E6, E7, or E2. Although an association between HPV infection and survivin expression has not been reported previously, studies have shown precedence for survivin nuclear translocation. Suzuki et al found that the nuclear translocation of survivin in HepG2 cells is dependent on both Fas stimulation and cell proliferation. Thus, it was hypothesized that this process could facilitate formation of a...
survivin/Cdkr complex, resulting in mitochondrial translocation of the protein p21 and interaction of p21 with procaspase 3, to suppress Fas-mediated apoptosis.

Survivin was detected in normal squamous mucosa, cervical dysplasia, and invasive carcinoma. Thus, the analysis of survivin expression is unlikely to have a potential role as a diagnostic or prognostic marker for cervical neoplasia. The patterns of expression of survivin, however, were distinct and reproducible within each diagnostic category and reflected the histologic diagnosis. The results of this study suggest that survivin may be involved in HPV-mediated dysregulation of normal squamous maturation through modulation of the processes of apoptotic cell death. The distribution of survivin in immature metaplastic squamous epithelial cells and in mature squamous epithelium also implicates survivin as a participant in normal squamous differentiation. Elucidation of the roles of survivin in HPV-mediated transformation and in the normal processes of squamous differentiation eventually may define the clinical relevance of the immunohistochemical localization of survivin in cervical tissues.

References


