Expression of Human Telomerase Reverse Transcriptase in Vulvar Intraepithelial Neoplasia and Squamous Cell Carcinoma

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Context.—Human telomerase reverse transcriptase (hTERT), an enzyme that enables cells to overcome replicative senescence and to divide indefinitely, is overexpressed in many cancers and their precursor lesions.

Objective.—To test whether hTERT expression is related to neoplastic progression and resistance to apoptosis in vulvar epithelia.

Design.—Immunohistochemical study of hTERT expression in 101 formalin-fixed, paraffin-embedded archival vulvar epithelia consisting of normal squamous vulvar epithelia (n = 25), lichen sclerosus (n = 10), high-grade classic vulvar intraepithelial neoplasia (n = 16), differentiated vulvar intraepithelial neoplasia (n = 20), and vulvar invasive squamous cell carcinoma (n = 32) and related to survivin and p53 expression. Immunostaining for all factors was scored for moderate and strong interstices with regard to quantity to determine upregulation and overexpression (score 0, 0% immunoreactive cells; score 1+, <5% immunoreactive cells; score 2+, 5% to 50% immunoreactive cells; score 3+, >50% immunoreactive cells). Score 3+ was considered as overexpression.

Results.—Nuclear hTERT immunoexpression was closely related to survivin reactivity, increased from normal vulvar squamous epithelia to lichen sclerosus and to high-grade classic vulvar intraepithelial neoplasia, differentiated vulvar intraepithelial neoplasia, and invasive keratinizing squamous cell carcinoma (P < .001), and followed the morphologic distribution of atypical squamous epithelial cells. Overexpression of hTERT was comparable to that seen for p53 in invasive keratinizing squamous cell carcinoma (P = .62); significant differences were calculated for differentiated vulvar intraepithelial neoplasia (P = .003) and high-grade classic vulvar intraepithelial neoplasia (P = .001).

Conclusion.—Human telomerase reverse transcriptase is upregulated in vulvar intraepithelial neoplasia and invasive keratinizing squamous cell carcinoma compared with nonneoplastic squamous epithelia of the vulva as an apparently early and preinvasive event in the neoplastic transformation, with development of cellular longevity and resistance to apoptosis by survivin activation as associated features, independent of the etiology of vulvar intraepithelial neoplasia.


Invasive squamous cell carcinoma (ISCC) is the most common type of all invasive vulvar cancers. Vulvar intraepithelial neoplasias (VINs) are precancerous changes, defined as proliferative intraepithelial squamous lesions that display abnormal growth, exhibiting lack of cellular maturation and crowding of cells. According to the grade of dysplasia, they are designated as VIN grade 1, 2, or 3. The vast majority of VIN lesions are considered grade 2 or 3, with a distinction between these 2 grades being highly subjective. Basaloid and warty (condylomatous) or classic subtypes of VIN are associated with relatively younger women, and evidence of human papillomavirus (HPV) nucleic acids. A third, “differentiated” subtype of VIN (d-VIN) is associated with relatively older women and a low index of HPV nucleic acids. The morphologic and etiologic heterogeneity of VIN is consistent with a dual pathogenesis of vulvar squamous cell carcinomas. Warty or basaloid carcinomas are related to the presence of HPV, genital warts, and warty and basaloid VIN, and occur in younger women. In contrast, keratinizing ISCC is frequently found in women older than 55 years, is usually unrelated to HPV infection, but may be associated with differentiated VIN and lichen sclerosus (LS). This type of ISCC accounts for 65% of vulvar invasive ISCC and marks another pathway in the development of these tumors.1

Telomeres are specific DNA-protein complexes, located at the ends of eukaryotic chromosomes and are essential for chromosome stability.2 In human chromosomes, they consist of thousands of copies of the nucleotide sequence 5’-TTAGGG-3’ ranging from 5 to 20 kb in length. With each cell division, telomeres are progressively shortened...
because of the inability of the DNA polymerase complex to replicate the 5’ end of the lagging strand. This shortening of telomeres may function as a mitotic clock, by which normal cells count their divisions and eventually signal their senescence. Telomerase is a ribonuclein complex that extends and maintains telomeres. By activation of this enzyme cells are able to overcome replicative senescence and to divide indefinitely. This view is supported by observations that telomerase is frequently activated in many kinds of cancers or cancer cell lines but not in most normal tissues. There are 2 major subunits of the telomerase enzyme complex that contribute to in vitro enzymatic activity. Human telomerase RNA component is an intrinsic RNA component that contains a template region and binds to TTAGGG repeats in telomeres. Human telomerase reverse transcriptase (hTERT) is a catalytic subunit with reverse transcriptase activity. Human telomerase RNA component is constitutively present in normal and cancer cells. Experimentally, telomerase activity can be induced by introduction of the hTERT gene into telomerase-negative normal cells. Telomerase activity and hTERT mRNA expression are closely associated in human cancers.

Survivin is an inhibitor of apoptosis protein and a key regulator of programmed cell death and cell cycle progression. It inhibits cell death in response to several apoptotic stimuli via caspase-dependent and independent mechanisms. In contrast to embryonic development, survivin is undetectable in most normal adult differentiated tissues. It is frequently overexpressed in human cancers, has been—in predominantly nuclear localization—shown to be an unfavorable prognostic marker correlating with metastases and poor prognosis in several malignancies, and has been related to angiogenesis and chemoresistance. Expression of survivin is deregulated by gene amplification, hypomethylation, and increased transcription.

p53 is a tumor suppressor gene effective at the G1 phase of the cell cycle that mediates growth arrest, initiates repair, or induces apoptosis. Mutation of p53 leads to an impaired function and results in stabilization of the protein, allowing its immunohistochemical detection.

Despite its relevance in neoplastic growth, hTERT expression has not been evaluated earlier over a range of vulvar squamous epithelial lesions. The present study is the first to assess whether hTERT upregulation and overexpression is of relevance in different kinds of VIN and in the progression to ISCC. We further hypothesized that hTERT expression may be related to the development of resistance to apoptosis. Thus, we evaluated the expression of hTERT in relation to survivin and p53 in normal vulvar epithelia (NE), the inflammatory condition of vulvar LS, high-grade VINs of the classic type (HG-VIN) as well as d-VIN, and ISCC of the vulva using immunohistochemistry to identify any differences in this spectrum, and, consequently, to test whether these factors would contribute to the understanding of vulvar squamous neoplasia.

**MATERIALS AND METHODS**

**Tissue Specimens**

A total of 101 vulvar tissues originating from 67 preoperatively untreated patients were included in this study. Each patient contributed one tissue block only. This study was carried out in accordance with local ethical guidelines and all patients consented to the use of the tissues. The specimens consisted of vulvar biopsies and local excisions as well as partial and total vulvectomies collected from the files of the authors’ institutions and were obtained between 1992 and 2010. In 11 cases of ISCC a lymph node dissection was performed, with metastatic disease in 1 case. If present in the studied slides, normal epithelia as well as lower-grade lesions adjacent to higher-grade changes were evaluated, too. However, all cases of LS were independent without other associated lesions. The histologic diagnoses were as follows: NE (n = 25), LS (n = 10), classic HG-VIN (n = 16), d-VIN (n = 18), and ISCC (n = 32). Six cases of d-VIN were isolated; the others were associated with ISCC. The specimens were fixed in formalin and embedded in paraffin. Formalin fixation did not exceed 24 hours. In each case, all original hematoxylin-eosin-stained sections as well as the clinical histories were reviewed and a representative tissue block was chosen for immunohistochemical staining. Seven patients died of recurrent disease.

The classification of VIN as well as the grading and staging of the ISCC was performed as described previously. All ISCCs were keratinizing. Applying the recent International Federation of Gynecology and Obstetrics (FIGO) staging, most cases of ISCC were staged as FIGO I (n = 31), IA, n = 2; IB, n = 29) and 1 as FIGO IIb. In accordance with the recommendations of the Gynecologic Oncology Group, grading of ISCC depended upon the extent of undifferentiated cells, with grade 1 tumors (n = 11) displaying no undifferentiated cells, grade 2 tumors (n = 12) containing an undifferentiated component of less than 30%, and grade 3 cancers (n = 9) with a higher percentage of undifferentiated tumor cells. The mean age of patients with ISCC was 73.4 years (range, 49–90 years). The HG-VIN lesions covered by this study correspond to the classic type. Differentiated VIN was recognized by defined criteria. Basal and midlayer keratinocytes were large and had abundant eosinophilic cytoplasm, abnormal nuclei, and prominent nucleoli. The rete ridges were elongated and branching, with occasional keratin pearls. Previously, these lesions have been shown to be p53 reactive. Hyperplastic epithelial changes adjacent to ISCC not meeting these criteria were not diagnosed as d-VIN.

**Immunohistochemistry**

The same paraaffin-embedded tissues that were used for the original hematoxylin-eosin-stained sections were used for immunohistochemistry. They were cut at 3 µm. Subsequently, the sections were deparaffinized in xylene and rehydrated via graded ethanol. A standard immunohistochemical technique was performed using a Ventana BenchMark XT immunostainer with an affinity-purified rabbit anti-human antibody to hTERT (1:400; Rockland, Philadelphia, Pennsylvania), a prediluted rabbit anti-human monoclonal antibody to survivin (clone EP2880Y, Biogenex, San Ramon, California), and a monoclonal mouse anti-human antibody to p53 (1:200; clone DO-7, DAKO, Carpinteria, California). Heat epitope retrieval as provided by the immunostainer was done in a Tris-based buffer supplied by the manufacturer (CC1 cell conditioning solution; Ventana Medical Systems, Illkirch Cedex, France) at pH 7.5 for 60 minutes for all antibodies used. The antibodies were incubated at 37 °C to hTERT as well as p53 for 40 minutes and to survivin antibody for 60 minutes. The enzymatic reactivity was visualized with 3-3′ diaminobenzidine (incubation time 8 minutes). A human tonsil served as external positive control for hTERT, and a case of skin with squamous cell carcinoma and adjacent normal epidermis for survivin and p53. For negative controls serial sections of the same specimens were used, omitting the primary antibody from the staining protocol and substituting it with commercially available nonimmune IgG serum (DAKO). The immunohistochemical slides were evaluated and interpreted by one of the authors (H.B.) without knowledge of the clinical data in a blinded manner. Necrotic tissue areas were not considered in the interpretation of immunostaining.

Immunoreactivity for both antibodies was scored as described previously for survivin immunostaining in vulvar neoplasia.

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only moderate and strong staining intensities were considered eligible for evaluation. Strong intensity corresponded to the intensity of the positive controls. To assess upregulation, sections were scored semiquantitatively as follows: 0, 0% immunoreactive cells; 1+, less than 5% immunoreactive cells; 2+, 5% to 50% immunoreactive cells; 3+, more than 50% immunoreactive cells. Finally, for the purpose of this study, a score of 3+ was defined as indicative of overexpression of the factors investigated. The slides were screened at low power for any staining; higher magnification (×100) was used to determine immunohistochemical scores.

Statistical analysis was carried out using GraphPad Prism software (version 4.0; San Diego, California). The χ² test and Fisher exact test were used to analyze the immunoreactivity of the studied factors. If applicable, tests were 2-tailed. Degrees of freedom (df) were calculated as appropriate. Survival analysis was done by the log-rank test. Because only 1 tumor exceeded stage I, analysis for stage was not performed. Statistical significance was accepted at the P < .05 level.

RESULTS

hTERT and Survivin Immunohistochemistry

Human telomerase reverse transcriptase and survivin immunoreactivity were exclusively observed in nuclear patterns. The staining pattern and scoring overlapped largely for both factors, with the exception of NE, for which 20% of cases showed a score of 2+ for survivin. In NE and LS, staining was noted in parabasal/basal epithelial layers (Figure 1). In d-VIN (Figure 2) and HG-VIN (Figure 3), the staining pattern followed the extension of atypically proliferating epithelia; for d-VIN, these proliferations never exceeded 50%, leading to no case with score 3+. In the ISCC group, immunoexpression was accentuated in immature cancer cells in the periphery of horn pearls and at the margins of invasive tumor nests, and was seen diffusely in sheets of nonkeratinizing tumor cells (Figure 4). Immunoscores were upregulated from NE (hTERT and survivin, respectively: score 0/1+, 100% and 80%; score 2+, 0% and 20%, LS (score 0/1+, 60%; score 2+, 40% for both factors), HG-VIN (score 0/1+, 0%; score 2+, 44% for both factors), and d-VIN (score 0/1+, 11%; score 2+, 89% for both factors) to ISCC (score 0/1+, 0%; score 2+, 44% for both factors). Because score 3+ was defined by immunostaining in more than 50% of lesional cells was observed exclusively in neoplastic vulvar epithelia, overexpression of hTERT was noted for 0% of NE, 0% of LS, 56% of HG-VIN, 0% of d-VIN, and 56% of ISCC cases.

Immunoreactivity for both factors increased significantly from NE to LS and to HG-VIN, d-VIN, and ISCC (P < .001, χ² test, df = 4; Table 1). Significant overexpression was noted in HG-VIN versus d-VIN (P = .001, Fisher exact test), and in ISCC versus d-VIN (P < .001, Fisher exact test). Overexpression increased with tumor grade (P = .02, χ² test, df = 2; Table 1). Upregulation was observed in d-VIN versus LS (P = .01, Fisher exact test). Immunoscores did not differ significantly for hTERT without or with associated ISCC (P = .53, Fisher exact test); the two 0/1+ score cases were seen adjacent to ISCC. With regard to overexpression, survival analysis revealed no significant differences for both factors, respectively, (P = .96, log-rank test, df = 1).

p53 Immunohistochemistry

If present, p53 reactivity was noted with exclusively nuclear staining in diffuse patterns, obscuring nuclear details. The staining intensity corresponded to the intensity of the positive control; because p53 positivity was recognized by staining obscuring nuclear details, weak intensities were excluded from the scoring procedure, and we did not separate moderate and strong intensities, which appeared very subjective in our materials. In d-VIN, reactivity was seen in the lower epithelial layers with atypical keratinocytes with enlarged nuclei in the lower epithelial layers in a differentiated vulvar intraepithelial neoplasia. There is superficial parakeratosis and elongation of rete ridges, with evidence of occasional small horn pearls (original magnification ×100).

Figure 1. Occasional nuclear immunostaining for human telomerase reverse transcriptase in cells of the basal/parabasal layers in this case of nonneoplastic vulvar squamous epithelium with changes of lichen sclerosus displaying a homogenous dermal stroma and an atrophic epidermis (original magnification ×100).

Figure 2. Nuclear human telomerase reverse transcriptase immunostaining is confined to atypical keratinocytes with enlarged nuclei in the lower epithelial layers in a differentiated vulvar intraepithelial neoplasia. There is superficial parakeratosis and elongation of rete ridges, with evidence of occasional small horn pearls (original magnification ×100).
Association of hTERT/Survivin and p53 Immunoscores in Vulvar Squamous Neoplastic Epithelia

To test whether there is a relation of hTERT/survivin and p53, we compared immunoscores in ISCC and d-VIN, respectively (Table 2). Overexpression of hTERT/survivin was comparable to that seen for p53 in ISCC (score 3+, 56% versus 47%, P = .62, Fisher exact test), indicating a correlation by a lack of statistically significant differences of immunoscores. Significant differences for hTERT/survivin and p53 overexpression were calculated for d-VIN (0% versus 45%, P = .003, Fisher exact test), consistent with a lack of a correlation in this type of preinvasive and intraepithelial lesions. For HG-VIN, conditions were different because there were no cases with p53 overexpression (56% versus 0%, P = .001, Fisher exact test), providing evidence for hTERT/survivin overexpression independent of p53-related mechanisms (Table 2).

COMMENT

Telomerase activity in the course of malignant transformation has been discussed under different aspects. It has been debated whether telomerase levels simply mirror the fraction of proliferating cells or whether they are independently upregulated by aggressively growing cells.10 Besides defects in the regulation of the cell cycle, a
supplementary mechanism granting immortality is needed for malignant tumor growth. Activation of telomerase as a marker of unlimited cell proliferation has been demonstrated in most human cancers.12

To our knowledge, hTERT expression has not been investigated so far over a range of vulvar neoplastic tissues. Wada et al13 localized hTERT protein in multifocal VIN and reported on hTERT nuclear staining in HG-VIN correlating with squamous maturation and the degree of nuclear atypia; normal mucosa revealed faint nuclear staining of parabasal cells and lower intermediate layer squamous cells. These findings are in agreement with our notions on hTERT upregulation and overexpression in HG-VIN. In contrast, NE showed only weak and occasional basal/parabasal immunoreactivities. Expression of hTERT in d-VIN, which does not display full-thickness atypia, followed the morphologic distribution of atypical cells; thus, the highest scoring consistent with upregulation was 2+ in 89% of these cases.

In this study, hTERT expression was exclusively seen in neoplastic nuclei, consistent with its phosphorylation that correlates with translocation from the cytoplasm to the nucleus, reflecting relevance for telomerase activity.2 We observed a progressive increase of hTERT expression from nonneoplastic to neoplastic and finally invasive vulvar squamous epithelia similar to those noted for proliferation markers like Ki-67 and topoisomerase IIx in a previous study.4 A comparable increase of hTERT expression was reported by Saha et al15 for cervical intraepithelial neoplasia. They concluded that this phenomenon represents an early genetic abnormality in cervical pathogenesis, which may be assumed for vulvar neoplasia, too.

We observed nuclear survivin staining throughout all cohorts, which followed the distribution of atypically proliferating cells. Survivin exists in a cytoplasmic and a nuclear subcellular pool, consistent with its function in the regulation of both cell viability and cell division. The nuclear pool of survivin is considered to be involved in proliferation.3 Indeed, expression patterns were similar to those of different proliferation markers (Ki-67, topoisomerase IIx) in vulvar tissues as noted previously.2 Several studies deal with survivin immunohistochemistry in squamous cell carcinomas of different origins. Nuclear survivin expression has been detected in the majority of esophageal squamous cell carcinomas, where it has been considered an unfavorable prognostic marker.5 Similarly, survivin was overexpressed in oral squamous cell carcinoma, with only sporadic expression in the basal and parabasal layers of normal oral mucosa.14 Branca et al15 noted that expression of survivin was related to the grade of cervical intraepithelial lesions with direct relationship between the increasing grade of lesion and the intensity of survivin staining; survivin expression was not detected in normal or metaplastic squamous epithelium. The biological role of survivin in vulvar squamous lesion is well explained by its described function as an inhibitor of apoptosis. As such it has been characterized as an inhibitor of caspases and provides a cytoprotective step downstream of the mitochondrial pathway of apoptosis.4 More complex aspects include its participation in the spindle assembly checkpoint and kinetochore-microtubule attachment and a function in cellular stress response with different heat shock proteins as chaperones, thereby contributing to cellular homeostasis. In the biology of neoplasia, survivin displays multifaceted functions for advantage in cell proliferation, survival, and adaptation. It maintains viability of aneuploid cells, bypassing cell-cycle checkpoints and promoting resistance to microtubule-targeting agents.10 As a consequence, survivin is under consideration as a factor in tumor therapy, with experimental evidence that silencing is a universal requirement

### Table 1. Immunohistochemical Expression of Human Telomerase Reverse Transcriptase (hTERT), Survivin, and p53 in Normal and Neoplastic Squamous Epithelia of the Vulva*

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<td></td>
<td>0/1+</td>
<td>2+</td>
<td>3+</td>
<td>0/1+</td>
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<tr>
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<td>2</td>
<td>16</td>
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<tr>
<td>ISCC</td>
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<tr>
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Abbreviations: d-VIN, differentiated vulvar intraepithelial neoplasia; HG-VIN, high-grade vulvar intraepithelial neoplasia, classic type; ISCC, invasive squamous cell carcinoma of the vulva; LS, lichen sclerosus; NE, normal vulvar squamous epithelia.

* Data are grouped by immunohistochemical score (0/1+, 2+, or 3+).

### Table 2. Overexpression of Human Telomerase Reverse Transcriptase (hTERT)/Survivin and p53 in Vulvar Neoplasia*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. Cases</th>
<th>hTERT/Survivin, No.</th>
<th>p53, No.</th>
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<tr>
<td></td>
<td>0/1+/2+</td>
<td>3+</td>
<td>0/1+/2+</td>
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<tr>
<td>HG-VIN</td>
<td>16</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>d-VIN</td>
<td>18</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>ISCC</td>
<td>32</td>
<td>14</td>
<td>18</td>
</tr>
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</table>

Abbreviations: d-VIN, differentiated vulvar intraepithelial neoplasia; HG-VIN, high-grade vulvar intraepithelial neoplasia, classic type, and taken together; ISCC, invasive squamous cell carcinoma of the vulva.

* Data are grouped by immunohistochemical score (0/1+/2+ or 3+).
for successful tumor suppression in humans. Likewise, Liu et al\textsuperscript{16} recently noted that survivin gene silencing sensitized prostate cancer cells to selenium growth inhibition.

Previously we characterized the immunoexpression of survivin in vulvar tissues and found increasing scores from NE via VN to ISCC.\textsuperscript{6} Herein we observed a close correlation of overexpression of hTERT and survivin. Different studies provided evidence for a relation of these factors. Shan et al\textsuperscript{17} reported on an overexpression of survivin and hTERT that strongly correlated with recurrence and local invasion of extramammary Paget disease lesions. The expression levels of survivin were positively related to those of hTERT in non–small cell lung carcinomas.\textsuperscript{18} Yuan et al\textsuperscript{19} found that repression of hTERT resulted in the rapid down-regulation of survivin in telomerase-immortalized fibroblasts and tumor cell lines, but not in cells immortalized via an alternative lengthening of telomeres mechanism. The authors concluded that these observations have important therapeutic implications, as telomerase and survivin are both broadly expressed in human cancers. Furuya et al\textsuperscript{20} reported that survivin enhanced the expression of hTERT in colon cancer cells by promoting the expression of specificity protein 1 (Sp1)– and c-Myc–mediated gene transcription. They suggested that the interaction between survivin and aurora-B kinase may be essential for survivin to increase hTERT expression. Endoh et al\textsuperscript{21} noted that overexpression of survivin enhanced telomerase activity by upregulation of hTERT. The DNA-binding activities of Sp1 and c-Myc to the hTERT core promoter were increased in survivin gene transfectant cells. Further, knockdown of survivin by a small inhibitory RNA decreased Sp1 and c-Myc phosphorylation. Endoh et al\textsuperscript{21} suggested that survivin participates not only in inhibition of apoptosis, but also in prolonging cellular lifespan. A relation of survivin and hTERT was suggested by Zhang et al\textsuperscript{22} in gastric carcinoma cells; they detected down-regulation of survivin expression by Western blot and inhibition of telomerase activity by telomerase repeat amplification protocol by upregulation of p27kip1. Barbosa et al\textsuperscript{23} recently investigated survivin and telomerase expression in the uterine cervix of women with human papillomavirus–induced lesions, noted a significant increase for both factors associated with the severity of the lesion, and considered that as a mechanism promoting cell proliferation and survival. Another significant correlation of expression of survivin and hTERT was observed in colorectal adenocarcinomas, with a lack of an association to survival. The authors\textsuperscript{24} interpreted their findings on prevalence of survivin expression and the correlation to hTERT activity as considerable in gene targeting therapy for colorectal carcinoma. Indeed, combined inhibition of survivin and hTERT with antisense oligodeoxynucleotides and small interfering RNAs showed antitumoral activity in bladder cancer cells.\textsuperscript{25} Wang et al\textsuperscript{26} introduced a tumor-specific RNA interference system in HeLa cells of human cervical carcinoma. They showed that survivin promoter–driven small interfering RNA expression vector targeting hTERT may have potential use in radiosensitization therapy with a targeted tumor gene silencing effect.

We noted p53 overexpression in the cohorts of d-VIN and ISCC exclusively, p53 is an important factor to the understanding of the 2 pathways leading to squamous cell carcinoma of the vulva.\textsuperscript{1} In the HPV-independent pathway keratinizing ISCC may develop through a d-VIN. It has a different set of genetic alterations than those in the first pathway, including p53 alterations.\textsuperscript{1} A strong correlation between high p53 expression and DNA aneuploidy has been observed for d-VIN and ISCC recently, providing evidence of the malignant potential of d-VIN.\textsuperscript{25} Strong staining for p53 with a high labeling index was commonly found in d-VIN, which may be of value in confirmation of these lesions, and supports the theory that d-VIN is the most likely precursor of conventional keratinizing SCC, the most common type of vulvar invasive carcinoma.\textsuperscript{26} In our study, overexpression of hTERT was closely correlated with that of p53 in ISCC. However, these immunoscores differed increasingly in d-VIN, indicating a relevance of the correlation between hTERT and p53 between these lesions during the development of an invasive phenotype. Accordingly, the coexpression of immunodetectable and overexpressed p53 with hTERT in vulvar ISCC reflects impaired mechanisms of apoptosis in the HPV-independently developed pathway of vulvar carcinogenesis. In contrast, the lack of p53 overexpression together with frequent high hTERT scores characterizes HG-VIN.

Immunoscoring for p53 poses problems in vulvar squamous lesions. Indeed, high cutoff levels thought to reflect p53 mutations have been proposed for different tumors. We used a relatively low cutoff of more than 50% to define overexpression. This is based on the observation by us and others that p53 staining is related to the abundance of immature squamous cells seen in d-VIN and ISCC, which is in contrast to other neoplasms with p53 mutations.\textsuperscript{27,28} Thus, p53 reactivity decreases dramatically in maturing cells of the middle and upper epithelial levels in d-VIN as well as in maturing and keratinizing cells of ISCC, which may lead to some variability of staining intensities. Interestingly, it has been shown that mutated p53 protein is degraded as a function of keratinocyte maturation. One study noted p53 expression in 5% to 95% of ISCC.\textsuperscript{27} This should be taken into consideration when choosing a scoring system in these lesions, which, with few exceptions, are associated with TP53 mutations.\textsuperscript{29} Of note, p53 immunohistochemistry reflects p53 mutations inaccurately. Mutations of the p53 gene can occur upstream of the protein segment targeted by immunohistochemistry. Such truncated mutated p53 protein will go undetected by this method.\textsuperscript{30} Consequently, our study focuses primarily on the evaluation of p53 protein overexpression.

In this study, the inflammatory condition of LS showed low to moderate immunoscores (1+, 2+) for hTERT. Additionally, hTERT was upregulated in d-VIN compared with LS. Expression of hTERT did not link LS to neoplastic epithelial changes clearly; there were no patterns of overexpression as defined by a score 3+ in this case cohort. This observation is supported by van der Avoort et al\textsuperscript{27} who compared DNA ploidy measurements and high p53 expression in neoplasias of the vulva and suggested that d-VIN has a higher malignant potential than LS and thus is a more likely precursor of squamous cell carcinoma. Interestingly, immunoscores for hTERT did not differ statistically between d-VIN without and with associated ISCC. However, to avoid a bias in distinguishing it from d-VIN by immunohistochemical field effects, we only evaluated LS without association to neoplasia.
This study for the first time to our knowledge presents evidence for upregulation and/or overexpression of hTERT in VIN and ISCC compared with normal squamous epithelia of the vulva. This notion apparently reflects an early and preinvasive event in the neoplastic transformation of vulvar squamous epithelia, with development of cellular longevity and resistance to apoptosis as associated features. The pattern of hTERT expression closely mirrors the histologic features of the different kinds of VIN. In contrast, such a trend was noted for p53 in d-VIN only. Accordingly, expression of hTERT is a common event independent of the etiology of VIN as well as p53 abnormalities, characteristic of atypically proliferating squamous epithelia in HG-VIN as well as d-VIN. However, the diagnostic value in individual cases is limited. Overlapping expression patterns and immuno-scores of hTERT in d-VIN and keratinizing ISCC limit an application as a marker to distinguish between these lesions or to predict a potential progression of intraepithelial neoplasia. Because of its frequent upregulation and overexpression in neoplastic vulvar squamous lesions, further studies should evaluate the relevance of hTERT as a parameter with possible therapeutic implications.

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References


