Cervical adenoid basal tumors comprised of adenoid basal epithelioma associated with various types of invasive carcinoma: Clinicopathologic features, human papillomavirus DNA detection, and P16 expression

Anil V. Parwani MD, PhD, Ann E. Smith Sehdev MD, Robert J. Kurman MD, Brigitte M. Ronnett MD,*

Summary Adenoid basal tumors are uncommon cervical lesions that some pathologists consider invasive carcinomas but others consider “epitheliomas” due to their low-grade histological appearance and rarely documented malignant behavior. We report the clinicopathologic features of 10 tumors comprised of both typical low-grade adenoid basal tumors (epitheliomas) intimately associated with invasive carcinomas having infiltrative growth, increased cytological atypia and mitotic activity, and various types of differentiation, including adenoid basal/squamous, pure squamous, adenoid cystic, and small cell neuroendocrine. Tumors were evaluated for the presence of human papillomavirus (HPV) DNA and immunohistochemical p16 expression. The patients in the study group ranged in age from 45 to 81 years (mean, 65 years). Most of the patients presented with abnormal cervicovaginal smears. The initial diagnosis was made on specimens obtained by cervical biopsy, laser electrocautery excision procedure (LEEP), or cone biopsy in 8 patients. Two patients were incidentally diagnosed in hysterectomy specimens. All 10 patients had squamous intraepithelial lesions (9 high-grade, 1 low-grade). In all cases diagnosed in LEEP or cone biopsy specimens, the invasive carcinoma component was present in the excisional specimen and extended to the margins. Seven patients diagnosed on excisional or biopsy specimens who underwent hysterectomy had residual tumor in the cervix, ranging from microscopic foci to deeply invasive. No lymph node metastases were identified in 4 patients who were staged. Seven patients with follow-up were alive without evidence of disease after follow-up intervals of 8 to 84 months (mean, 45 months; median, 29 months). One patient with a component of small cell carcinoma died of other causes without evidence of disease at 18 months. HPV 16 DNA was detected in both the adenoid basal epithelioma and invasive carcinoma components in 9 tumors by in

Abbreviations: HPV, human papillomavirus; LEEP, laser electrocautery excision procedure; PCR, polymerase chain reaction.

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situ hybridization, and HPV 33 was detected by polymerase chain reaction in 1 tumor. All tumors expressed p16 diffusely. Adenoid basal tumors are high-risk HPV-related tumors that can be comprised of both a low-grade adenoid basal tumor, which can be designated as epithelioma, and invasive carcinomas of various types. The invasive component is usually evident in the excisional biopsy specimen, allowing for recognition of a tumor that needs further management.

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1. Introduction

Adenoid basal tumors of the cervix have been designated as “adenoid basal carcinomas” since their initial recognition, even though malignant behavior has rarely been observed [1-8]. They are unusual cervical tumors composed of nests of uniform cells displaying basaloïd, squamous, and glandular differentiation. The tumors are usually associated with squamous intraepithelial lesions, most often high-grade. Most affected patients are asymptomatic postmenopausal women who are diagnosed during follow-up of an abnormal cervicovaginal smear. Pure, typical tumors appear to behave in a benign fashion, leading the authors of one study to recommend the term “adenoid basal epithelioma” rather than adenoid basal carcinoma [9]. Although the cases described in that report were not associated with other types of invasive carcinomas (except for 1 case of a microinvasive squamous carcinoma), some other reports have described adenoid basal tumors associated with invasive carcinomas and carcinosarcomas [3,4,10-12]. In the present study we describe the clinicopathologic features of a series of adenoid basal tumors composed of a typical low-grade adenoid basal component (“epithelioma”) intimately associated with invasive carcinomas displaying one or more types of differentiation. We investigate the relationship between the adenoid basal epitheliomas and invasive carcinomas by assessing for the presence of HPV DNA and p16 expression in both tumor components. We then present a proposal to subclassify these tumors into adenoid basal epitheliomas and carcinomas.

2. Materials and methods

This study was approved by the Johns Hopkins Institutional Review Board. The files of the Gynecologic Pathology Consultation Service and Surgical Pathology Division, The Johns Hopkins Hospital were searched for cases designated as adenoid basal carcinoma, adenoid basal tumor, adenoid basal and squamous carcinoma, or adenoid basal epithelioma for the period 1984 to 2004. Of 14 potential cases of low-grade adenoid basal tumor associated with additional types of invasive carcinoma, 10 retrievable cases (9 consultation cases and 1 routine case) were identified. Typical low-grade adenoid basal tumors (n = 19) were excluded from the study.

2.1. Immunohistochemistry for p16

Formalin-fixed, paraffin-embedded tissue sections were used. Immunoperoxidase labeling was done with an automated BioTek-Tech Mate 1000 Staining System (Ventana/Biotek Solutions, Tucson, AZ) at room temperature with anti-p16(INK4a) (MTM Laboratories AG, Heidelberg, Germany) at a dilution of 1:500.

2.2. Human papillomavirus DNA detection by in situ hybridization

Formalin-fixed, paraffin-embedded tissue sections were used. Biotin-labeled HPV probe solutions (Dako, Carpinteria, CA) were applied to individual sections. These included a wide spectrum probe (cocktail of HPV 6, 11, 16, 18, 31, 33, 45, and 51) and separate type-specific probes for HPV 16 and HPV 18. Detection of hybridized probe was done by tyramide-catalyzed signal amplification using the Genpoint kit (Dako). Chromogenic detection was performed with diaminobenzidine/H2O2. Controls included tissue sections positive for HPV wide spectrum, the HeLa cell line for HPV 18, and the SiHa cell line for HPV 16. Biotin-labeled plasmid probes served as negative controls in each case. Cases with a discrete punctate reaction product specifically in tumor cell nuclei were interpreted as positive.

2.3. HPV DNA detection by polymerase chain reaction

To further analyze the tumors for the presence of HPV, polymerase chain reaction (PCR) for HPV DNA was performed on those specimens that were negative by in situ hybridization. Tissue sections were deparaffinized with xylene and resuspended and digested with buffer containing proteinase K. Amplifications were performed for betaglobin and for HPVs by the Roche line blot method [13]. This method uses biotinylated pooled primers for HPV and for betaglobin amplification. The amplified products are screened against more than 35 HPV probes and betaglobin probes immobilized on an extended filter strip. Specimens that are negative for betaglobin and HPV are considered unsatisfactory.

3. Results

3.1. Clinicopathologic features

The clinicopathologic features of the 10 cases are summarized in Table 1. The patients ranged in age from 45 to 81 years (mean, 65 years). Most of the patients presented with abnormal cervicovaginal smears. The initial
diagnosis was made on an excisional biopsy specimens obtained by laser electrocautery excision procedure (LEEP) or cone biopsy in 6 patients and in biopsy specimens in 2 patients. In 2 patients, the tumors were incidentally diagnosed in hysterectomy specimens removed for pelvic relaxation and uterine prolapse. The overlying cervical squamous mucosa demonstrated a squamous intraepithelial lesion in all cases (9 high-grade, 1 low-grade). Each tumor was comprised of typical low-grade adenoid basal tumor intimately associated with invasive carcinoma. The low-grade adenoid basal tumor components were characterized by discrete nests of tumor in which the surrounding stroma lacked a desmoplastic reaction. The epithelial nests displayed varying combinations of basaloid, squamous, and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinicopathologic Features of Cervical Adenoid Basal Tumors</th>
</tr>
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<tbody>
<tr>
<td>Case</td>
<td>Age</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
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<tr>
<td>2</td>
<td>71</td>
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<td>3</td>
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<td>10</td>
<td>65</td>
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<thead>
<tr>
<th>Tumor size, cm (maximum dimension/maximum depth)</th>
<th>Treatment</th>
<th>Margin status on hysterectomy</th>
<th>Lymph node status (positive/total)</th>
<th>Follow-up status, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2/0.3</td>
<td>Radical hysterectomy, lymphadenectomy</td>
<td>Negative</td>
<td>Negative (0/26)</td>
</tr>
<tr>
<td>2</td>
<td>0.6/0.4</td>
<td>Total abdominal hysterectomy, BSO; Radiation therapy</td>
<td>Positive</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>At least 0.8/0.5</td>
<td>Radiation therapy</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>2.5/1.4</td>
<td>Radical hysterectomy, BSO, lymphadenectomy</td>
<td>Positive, with tumor in parametria</td>
<td>Negative (0/19)</td>
</tr>
<tr>
<td>5</td>
<td>1.0/0.8</td>
<td>Total vaginal hysterectomy, RSO</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>0.4/0.2</td>
<td>Radical hysterectomy, lymphadenectomy</td>
<td>Negative</td>
<td>Negative (0/45)</td>
</tr>
<tr>
<td>7</td>
<td>3.3/1.4</td>
<td>Total abdominal hysterectomy, BSO, lymphadenectomy; Radiation therapy</td>
<td>Negative</td>
<td>Negative (0/14)</td>
</tr>
<tr>
<td>8</td>
<td>1.0/0.6</td>
<td>Hysterectomy</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>1.3/1.3</td>
<td>Radical hysterectomy, BSO, lymphadenectomy</td>
<td>Negative</td>
<td>Negative (0/41)</td>
</tr>
<tr>
<td>10</td>
<td>1.6/0.8</td>
<td>Total vaginal hysterectomy</td>
<td>Negative</td>
<td>ND</td>
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Abbreviations: ASCUS, atypical squamous cells of undetermined significance; AGUS, atypical glandular cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; BSO, bilateral salpingo-oophorectomy; RSO, right salpingo-oophorectomy; ND, not done; NA, not available; NED, no evidence of disease; DOC/NED, died of other causes with no evidence of disease. * Pap smear reported as abnormal, not further specified.
glandular differentiation with minimal atypia and mitotic activity (Figs. 1 and 2). The low-grade adenoid basal tumor was connected to the overlying squamous intraepithelial lesion in 3 cases. In all tumors, the low-grade adenoid basal tumor component merged with areas of invasive carcinoma (Fig. 3). These areas of invasive carcinoma were distinct from the low-grade adenoid basal tumor components in that the carcinomatous components exhibited greater nuclear atypia and increased mitotic activity compared with the low-grade components and had infiltrative patterns. Thus there was little or no stromal response to the low-grade components, but in the carcinomatous components the surrounding stroma exhibited a more spindled or myxoid appearance, often with inflammatory cells, consistent with stromal desmoplasia (Fig. 4). The invasive carcinomatous components in the tumors exhibited varying degrees of adenoid basal and squamous differentiation. Adenoid basal differentiation was characterized by cells in the nests with scanty cytoplasm, peripheral palisading, and focal gland formation (Figs. 4 and 5), and squamous differentiation was manifested by increased amounts of dense eosinophilic cytoplasm in cells within the central portions of these nests (Fig. 6). Thus some invasive components had pure adenoid basal differentiation, some displayed combined adenoid
basal and squamous differentiation, and some had pure squamous differentiation (Figs. 3 to 6). A focus of lymphatic invasion was identified in 1 case (Fig. 4). One tumor also had a component of adenoid cystic carcinoma, solid variant (Figs. 7 and 8). In another tumor, most of the invasive component exhibited features of a high-grade small cell neuroendocrine carcinoma, characterized by cells with hyperchromatic nuclei, nuclear molding and streaming, and minimal cytoplasm (Fig. 9). This component was focally positive for cytokeratin and expressed neuroendocrine markers. In the 6 cases diagnosed on excisional biopsy specimens, the invasive carcinoma extended to the margins of those specimens (Fig. 7).

Most patients were treated with some form of hysterec-
tomy, often with bilateral salpingo-oophorectomy and lymph node dissection. All those treated by surgery had residual tumor in the cervix, ranging from just residual adenoid basal tumor in the case with microinvasive squamous carcinoma (in the excisional biopsy specimen) to variable amounts of residual invasive carcinoma. The amount of residual carcinoma ranged from microscopic foci to deeply invasive tumor. For those cases in which maximum dimensions could be obtained, the tumors ranged in size from 0.4 to 3.3 cm in greatest dimension, with depth of invasion ranging from 0.2 to 1.4 cm. Most cases had negative margins on the hysterectomy specimens, and all cases with lymph node dissections had no evidence of lymph node metastases. However, 1 case had positive margins with unknown lymph node status, and another case had involvement of the parametria with a positive margin but negative lymph nodes. Seven of 8 patients with available follow-up were alive without evidence of disease after follow-up intervals of 8 to 84 months (mean, 45 months; median, 29 months); 2 cases were recent, with very limited follow-up. One patient with a component of small cell carcinoma died of other causes, without evidence of disease on imaging studies, at 18 months.

3.2. Immunohistochemical p16 expression and HPV detection by in situ hybridization and PCR

All tumors exhibited diffuse expression of p16 (cytoplasmic, often with nuclear as well) in both the adenoid basal epithelioma and invasive carcinoma components (Fig. 10). HPV 16 DNA was detected in both the adenoid basal epithelioma and invasive carcinoma components in 9 tumors (Fig. 11). The positive reactions were characterized by discrete punctate reactivity confined to basal and squamous differentiation, and some had pure squamous differentiation (Figs. 3 to 6). A focus of lymphatic invasion was identified in 1 case (Fig. 4). One tumor also had a component of adenoid cystic carcinoma, solid variant (Figs. 7 and 8). In another tumor, most of the invasive component exhibited features of a high-grade small cell neuroendocrine carcinoma, characterized by cells with hyperchromatic nuclei, nuclear molding and streaming, and minimal cytoplasm (Fig. 9). This component was focally positive for cytokeratin and expressed neuroendocrine markers. In the 6 cases diagnosed on excisional biopsy specimens, the invasive carcinoma extended to the margins of those specimens (Fig. 7).

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tumor nuclei. In 1 tumor (case 9), HPV DNA was not detected by in situ hybridization, but PCR demonstrated the presence of HPV 33.

4. Discussion

Adenoid basal tumors are uncommon tumors of the cervix that have been designated as “adenoid basal carcinomas” in most reports [1-8]. More recently, the designation “adenoid basal epithelioma” has been suggested as a more appropriate term, because most reported cases have been associated with benign behavior [9]. Of 39 cases reported in 7 studies, only 1 patient died of tumor [8], but it appears that the tumor in that fatal case had unusual histological features and may have been a high-grade invasive basaloid carcinoma rather than a typical adenoid basal tumor [1,2,6,8,9,14,15]. In addition, several studies have emphasized the need to distinguish adenoid basal tumors from adenoid cystic carcinomas of the cervix because the latter are associated with a distinctly unfavorable prognosis [3,6,8,16].

Based on the collective experience in the literature, with specific reference to points made in the study suggesting the term “epithelioma” and the cases in the current study, several observations can be made. First, based on the previous studies, it appears that pure, typical adenoid basal tumors (without malignant cytological features and unassociated with other forms of invasive carcinoma) lack many of the features of a malignant neoplasm and virtually always behave in a benign fashion, even when present deep within the cervical stroma [9]. Second, based on the cases in our study and other reports in the literature, some adenoid basal tumors can be associated with an unequivocal invasive carcinoma displaying the microscopic features of carcinoma, including increased cytological atypia, mitotic activity with abnormal mitotic figures, and deeply infiltrative growth of these cytologically malignant components. In addition, the invasive components can display patterns of differentiation indicating specific subtypes of invasive carcinoma with established malignant behavior, including squamous, adenoid cystic, and small cell neuroendocrine carcinomas.

Although no lymph node metastases or tumor-related deaths were observed in our study, all of the tumors contained a carcinomatous component displaying malignant cytological features; in addition, many deeply invaded the cervical stroma, and some extended to margins or involved the parametrial soft tissue. Notable cytological atypia and deeply infiltrative growth are pathological features associ-
ated with carcinomas rather than benign tumors; hence, use of the term “carcinoma” is appropriate for tumors exhibiting these features despite the lack of documented malignant behavior in the reported tumors.

We suggest the following approach to diagnosing the spectrum of tumors exhibiting adenoid basal differentiation. Pure low-grade adenoid basal tumors lacking appreciable cytological atypia, mitotic activity, and an infiltrative pattern in a desmoplastic stroma (ie, without evidence of invasive carcinoma of adenoid basal/squamous, pure squamous, adenoid cystic, or other type) that are confined to an excisional specimen with clearly negative margins can be designated as “adenoid basal epitheliomas.” This terminology has been recommended by other investigators and is more appropriate than the term “adenoid basal hyperplasia,” because it acknowledges that these lesions are neoplasms that may be precursors of the associated invasive carcinomas occasionally encountered [9,17]. Tumors composed of both typical low-grade adenoid basal tumor (epithelioma) and an invasive, cytologically malignant component exhibiting adenoid basal/squamous, pure squamous, and/or adenoid cystic differentiation can be diagnosed as invasive carcinomas based on their morphological features. Those with mixed differentiation can be subclassified according to the patterns of differentiation (such as mixed adenoid basal/squamous carcinoma, or mixed squamous and adenoid cystic carcinoma). Many tumors with the features of carcinoma may well behave in a benign fashion after complete excision based on the experience in the literature and this study, but the designation “carcinoma” is appropriate in view of their morphological features. Thus most adenoid basal tumors can be classified as either epithelioma or carcinoma when either the entire lesion is available for evaluation or the microscopic features of malignancy are present.

Tumors extending to the margins of an excisional specimen but lacking cytological features of malignancy and the histological features of typical invasive squamous, adenoid basal, or adenoid cystic carcinoma pose some difficulty, in that adequacy of treatment cannot be determined. Accordingly, in practice such tumors can be designated as adenoid basal epithelioma with a comment that the possibility of an invasive carcinomatous component cannot be excluded due to incomplete evaluation in the setting of a positive margin. In such cases another excisional specimen or hysterectomy is required to exclude an invasive component and assure adequate treatment.

Other investigators have demonstrated by both in situ hybridization and PCR that cervical adenoid basal carcinomas, adenoid cystic carcinomas, and small cell carcinomas contain HPV DNA (most often HPV 16) [4,5,7,18,19].

Fig. 8  Adenoid cystic carcinoma. Higher magnification of the tumor in Fig. 7 illustrates the typical appearance of the solid form of adenoid cystic carcinoma.

Fig. 9  Small cell neuroendocrine carcinoma associated with adenoid basal epithelioma. Small cell carcinoma component (lower portion) is composed of undifferentiated cells with scanty cytoplasm and hyperchromatic nuclei displaying nuclear molding and streaming. Small nests of adenoid basal epithelioma (upper portion) are seen adjacent to the carcinoma.
study confirms these observations and demonstrates that both the adenoid basal epithelioma and invasive carcinoma components contain high-risk HPV. In addition, the finding of high-risk HPV DNA in both components suggests that the carcinomas arose from the adenoid basal epitheliomas and lends further support to the concept that the epitheliomas are neoplastic precursor lesions [17]. Immunohistochemical detection of p16 expression is associated with high-risk HPV infection in cervical intraepithelial lesions and invasive carcinomas and can serve as a surrogate marker of high-risk HPV infection in the appropriate pathological setting [20,21]. The diffuse expression of p16 in all tumors in our study, including 1 tumor in which HPV DNA was not detected by in situ hybridization, indicates that immunohistochemical expression of p16 can be used as a surrogate marker of high-risk HPV infection in these tumors when in situ hybridization reactions do not detect HPV due to technical reasons or when in situ hybridization and PCR techniques are not available in the laboratory. The finding of various subtypes of high-risk HPV-related invasive cervical carcinomas intimately associated with adenoid basal epitheliomas in our cases lends support to the concept that adenoid basal tumors are of putative reserve cell origin, by demonstrating the potential of these invasive components to undergo divergent differentiation [3,4].

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References


