

# Spindle Cell Epithelioma of the Vagina Shows Immunohistochemical Staining Supporting Its Origin From a Primitive/Progenitor Cell Population

Henry Skelton, MD; COL Kathleen J. Smith, MC, USA

● Spindle cell epitheliomas of the vagina (SCEVs) coexpresses epithelial and mesenchymal markers and were first described as a “mixed tumors of the vagina.” However, unlike mixed tumors of other organs, which are believed to originate from myoepithelial cells, SCEVs neither immunohistochemically nor ultrastructurally show features of myoepithelial cells. The present expanded battery of immunohistochemical stains is presented on this rare tumor, including cytokeratin AE1/AE3, CK7, CK20, S100 protein, epithelial membrane antigen,  $\alpha$ -smooth muscle actin, desmin, CD34, CD99, Bcl-2, vimentin, estrogen and progesterone receptors, and Ki-67. There was minimal expression of  $\alpha$ -smooth muscle actin and negative staining with S100 protein, with coexpression of cytokeratins and vimentin and expression of estrogen and progesterone receptors, as previously reported in SCEVs. In addition, diffuse expression of CD34, CD99, and Bcl-2 immunohistochemical stains was found, which has not previously been reported. The coexpression of CD34, CD99, and Bcl-2 in SCEVs is consistent with its origin from a primitive/progenitor cell population.

(*Arch Pathol Lab Med.* 2001;125:547–550)

Spindle cell epitheliomas of the vagina (SCEVs) or “mixed tumors of the vagina” have some important differences from mixed tumors that arise in other areas, such as skin, salivary glands, breast, mediastinum, and trachea.<sup>1–3</sup> All the latter tumors appear to arise from myoepithelial cells.<sup>2,3</sup> However, an origin of myoepithelial cells was questioned in the largest review of SCEVs by Branton and Tavassoli.<sup>1</sup> They pointed out that the myoepithelial cells do not occur either in the vagina or in vaginal glandular inclusions of mullerian or mesonephric origin.<sup>1</sup> They noted that SCEVs are well circumscribed but not encapsulated and are characterized by proliferation of spindle

cells admixed with clusters of cells with an epithelioid appearance and sometimes with squamous differentiation.<sup>1</sup> In addition, SCEVs express both cytokeratin and vimentin and ultrastructurally show tonofilaments not only within recognizable aggregates of squamous cells but also within the spindle cell population.<sup>1</sup> However,  $\alpha$ -smooth muscle actin expression occurs mainly in the supportive stromal myofibroblastic cell and not in the tumor cells.<sup>1,4,5</sup> This finding and lack of expression of S100 protein, another marker of myoepithelial cells, suggest that the cell of origin is not a myoepithelial cell.<sup>4</sup>

An additional case of a SCEV with a broader spectrum of immunohistochemical stains is presented that supports the origin of these tumors from a progenitor cell population.

## REPORT OF A CASE

The patient was a 32-year-old woman who had not seen a physician for at least 7 years. She presented with a complaint of a mass in the vagina. She reported no pain or other symptoms. She had no prior history of tumor or gynecologic problems. During a gynecologic examination, a circumscribed firm nodular mass was found in the lower posterior vaginal wall. The lesion was surgically removed and submitted for pathologic examination. Six months after excision, the site was healed with no evidence of recurrence.

## Histopathologic Findings

The biopsy specimen measured approximately 2 × 4 cm in greatest dimensions and appeared completely excised on the sections examined. Biopsy sections showed a well-circumscribed but not encapsulated tumor (Figure 1). The margins were expansile and located within a few millimeters of the overlying epithelium (Figure 2).

The tumor was composed of a spindle cell population, with some more epithelioid cells, but no component that showed definitive epithelial features or squamous differentiation. The spindle cells were small, with sparse, faintly granular cytoplasm and poorly defined cell borders (Figures 1 and 2). The nuclei were dark, slightly vesicular, and ovoid and had fine chromatin. In some areas the spindle cells showed no pattern, whereas in other areas the cells formed cords and had a more epithelioid appearance (Figure 2). In some areas, there was hyaline matrix material between tumor cells (Figure 2). Small vessels were seen throughout the tumor, some of them branched.

## Immunohistochemical Stains

The tumor expressed vimentin (BioGenex, San Ramon, Calif), CD34 (QBEND 10, BioGenex), CD99 (Signet, Dedham, Mass), and Bcl-2 (Dako, Carpinteria, Calif) diffusely (Figures 3 through

Accepted for publication September 18, 2000.

From the Department of Dermatology, National Naval Medical Center, Bethesda, Md (Dr Smith); and Laboratory Corporation of America, Herndon, Va (Dr Skelton).

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army, the Department of the Navy, or the Department of Defense.

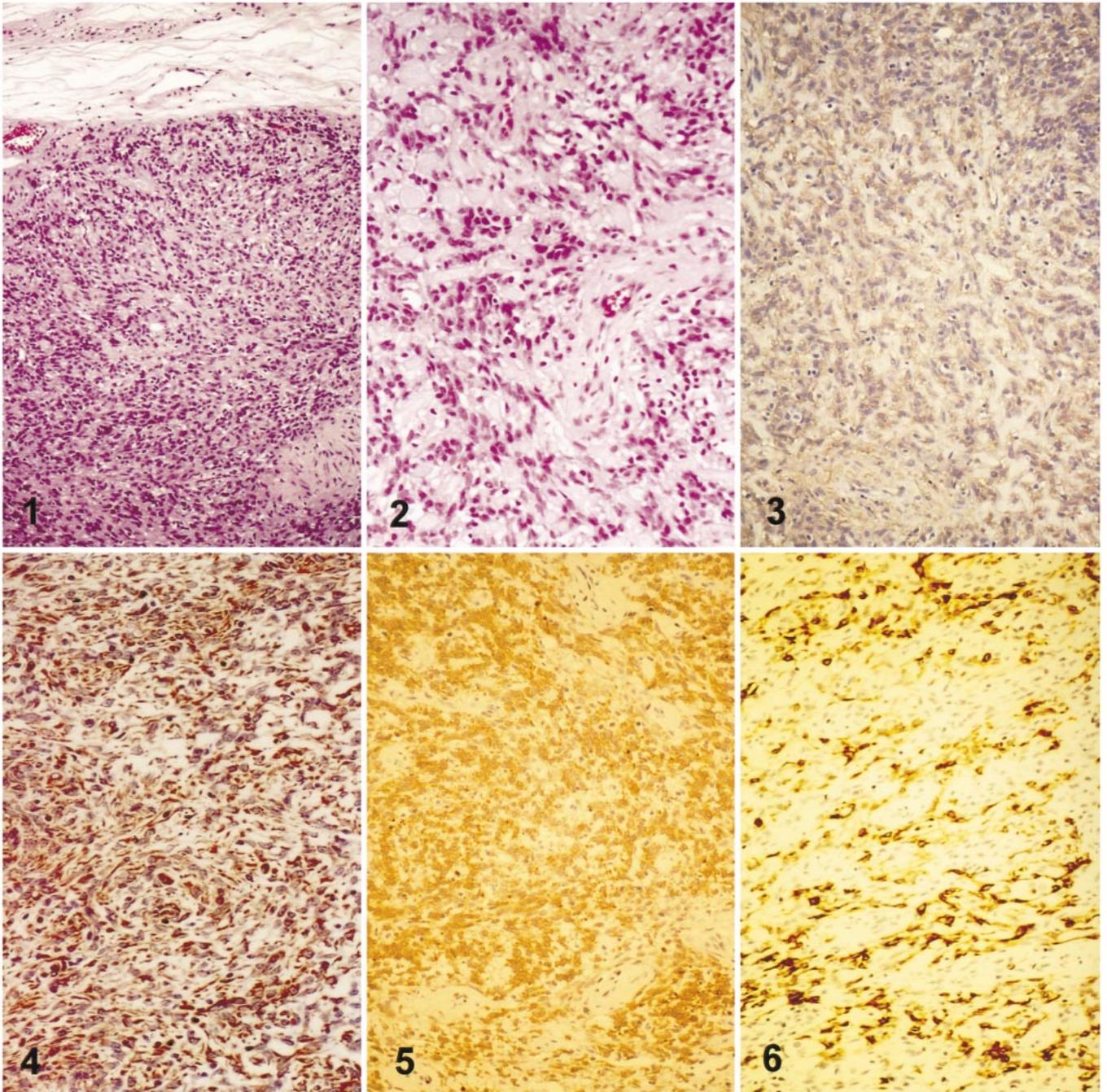
Reprints: COL Kathleen J. Smith, MC, USA, Department of Dermatology and Pathology, National Naval Medical Center, Bethesda, MD 20889-5600 (e-mail: HSkell@aol.com).

# Report Documentation Page

Form Approved  
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE <b>APR 2001</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2001 to 00-00-2001</b>	
4. TITLE AND SUBTITLE <b>Spindle Cell Epithelioma of the Vagina Shows Immunohistochemical Staining Supporting Its Origin From a Primitive/Progenitor Cell Population</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Department of Dermatology, National Naval Medical Center, Bethesda, MD</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>4</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			



**Figure 1.** Biopsy specimen showing a well-circumscribed but not encapsulated tumor with numerous small vessels throughout, some branched. There are spindle cells and more epithelioid cells. The cells were small, with sparse, faintly granular cytoplasm and poorly defined cell borders. Nuclei are dark, slightly vesicular, and ovoid and have fine chromatin. In some areas there is no pattern, whereas in other areas the cells formed cords and are more epithelioid in appearance. In some areas, there is prominent hyaline matrix material between tumor cells (hematoxylin-eosin, original magnification  $\times 100$ ).

**Figure 2.** Higher-power view of the biopsy specimen shown in Figure 1 (hematoxylin-eosin, original magnification  $\times 200$ ).

**Figure 3.** Immunohistochemical staining for CD34 showing diffuse staining of tumor cells (original magnification  $\times 100$ ).

**Figure 4.** Immunohistochemical staining for CD99 showing diffuse staining of tumor cells (original magnification  $\times 200$ ).

**Figure 5.** Immunohistochemical staining for bcl-2 showing diffuse staining of tumor cells (original magnification  $\times 200$ ).

**Figure 6.** Immunohistochemical staining for CK7 showing positive staining in some of the tumor cells (original magnification  $\times 200$ ).

5). The tumor cells stained intensely but not diffusely in areas with AE1/AE3 (Dako) and CK7 (BioGenex) (Figure 6), CK20 (Dako) and  $\alpha$ -smooth muscle actin (Dako) showed minimal staining, and epithelial membrane antigen (Dako) showed weak expression in scattered cells. Desmin (Dako) and S100 protein (BioGenex) were negative. Estrogen and progesterone receptors (Dako) showed moderately intense expression in most tumor cells. Ki-67 (MIB-1, Dako) showed scattered nuclear expression throughout the tumor that averaged approximately 4 to 5 positive cells per high-power  $\times 40$  field averaging 10 random fields.

### COMMENT

Tumors that show both epithelial and mesenchymal features are uncommon but occur in various anatomic sites. The origin of mixed tumors from most sites has been ascribed to myoepithelial cells.<sup>2,3</sup> These mixed tumors may show variable amounts of epithelial and mesenchymal components, which not uncommonly show chondroid and osseous differentiation.<sup>2,3</sup> They not only coexpress vimentin and cytokeratin but, at least in areas, coexpress  $\alpha$ -smooth muscle actin and/or S100 protein.<sup>2,3</sup> In addition, they commonly coexpress other intermediate filament proteins such as glial filament acidic protein.<sup>3</sup>

However, SCEVs are distinctive because they express little if any  $\alpha$ -smooth muscle actin and S100 protein or intermediate filaments other than cytokeratin and vimentin. In addition, SCEVs appear to lack myoepithelial differentiation ultrastructurally, and they lack the spectrum of morphologic mesenchymal tissue (ie, chondroid and bone) seen in other mixed tumors.<sup>1</sup>

Although most SCEVs do express hormonal receptors, the significance of this is not known, since estrogen and progesterone receptors are commonly expressed in the area even in vaginal fibroepithelial polyps.<sup>6</sup> In the present case of SCEV, there was diffuse expression of immunohistochemical stains CD34, CD99, and Bcl-2 not previously reported.

CD34 is a 115-kd, heavily glycosylated transmembrane protein first identified on hematopoietic stems and committed progenitor cells.<sup>7,8</sup> Within the hair follicles, cells that coexpress CD34 and cytokeratin are found within the outer root sheath, where the follicular stem cells are believed to reside.<sup>7</sup> Although CD34<sup>+</sup> expression is seen in a diverse group of benign and malignant mesenchymal cell populations, its expression appears characteristic of mesenchymal progenitor cell populations.<sup>4,8</sup>

CD99 is the product of the *MIC2* gene and is a glycoprotein that is involved in  $\beta_2$ -integrin-independent cell adhesion, which depends on activation of protein kinase and reorganization of the cytoskeleton.<sup>9</sup> Although the spectrum of malignant and benign tumors that express CD99 is ever expanding, it is characteristic of primitive neuroectodermal tumors within the Ewing family.<sup>9,10</sup> These tumors show cytogenetic defects that result in rearrangements of the *EWS* gene, with *ETS* transcription factor genes leading to markedly increased expression of CD99.<sup>11</sup> In addition, lymphoblastic leukemia or lymphoma of precursor lymphocytes of both B- and T-cell phenotype commonly express CD99.<sup>9,10</sup> Most of these primitive, precursor, or progenitor cell populations also express Bcl-2. Again, Bcl-2 expression is not specific, but even in adult mammalian tissues Bcl-2 has a restricted distribution and is expressed mainly in progenitor cells and long-lived cells in various organs.<sup>12</sup> Bcl-2 is an antiapoptotic molecule that moderates mitochondrial pore formation, and it has been shown that CD99 also has a role in modulating apopto-

sis.<sup>13</sup> Resistance to apoptosis is a characteristic of progenitor cell populations. Thus, coexpression of CD34, CD99, and Bcl-2 without immunohistochemical or ultrastructural evidence of myofibroblastic differentiation is consistent with the origin of SCEVs from more primitive, less differentiated cells than other mixed tumors.

In recent years, an ever-widening spectrum of CD34<sup>+</sup> tumors has been described; some have features similar to those in SCEVs, and these CD34<sup>+</sup> tumors were considered in the differential diagnosis. Solitary fibrous tumors (SFTs) are uncommon soft tissue neoplasms that are believed to be of mesenchymal origin. Although they were first described in the pleura, they have since been described arising in other serosal surfaces and many other sites.<sup>14</sup> Morphologically, SFTs are well-circumscribed but not encapsulated tumors composed of bland spindle-shaped cells with several architectural appearances, including a storiform pattern, fascicles of spindle cells with a wavy, neural appearance, a patternless pattern, abundant myxoid matrix in areas, densely cellular fascicles of spindle cells with a herringbone pattern, and SFTs with atypical multinucleated giant cells admixed with the spindle cell proliferation.<sup>14</sup> Mitoses and areas of necrosis are not identified except in the densely cellular variants, where there may be some nuclear atypia and scattered mitotic figures. The SFTs are characterized by prominent vascularity, often with branching vessels as seen in hemangiopericytomas and SCEVs.<sup>1,14</sup> Although scant collagen may be seen in SFTs, more advanced degrees of collagenization with spindle cells separated by strands of ropelike collagen may be seen. Although this collagenized pattern is distinctive, it may be mistaken for the eosinophilic matrix material seen in SCEVs. In addition, SFTs show strong CD34 reactivity and CD99 expression, and they are negative for S100 protein, HMB-45, and  $\alpha$ -smooth muscle actin, but they show no morphologic, immunohistochemical, or ultrastructural evidence of epithelial derivation. Similarly, hemangiopericytomas and schwannomas may show diffuse staining for CD34 but lack morphologic, immunohistochemical, and ultrastructural evidence of an epithelial origin. In addition, schwannomas express S100 protein and are encapsulated.

Mixed epithelial-mesenchymal tumors of female genital tract are rare.<sup>15</sup> By definition, all these tumors contain intimately admixed epithelial and mesenchymal elements by standard light microscopic examination.<sup>15</sup> They may occur as both benign (adenofibroma and adenomyoma variants, an intermediate variant [atypical polypoid adenomyoma]) and malignant variants (adenosarcoma, carcinosarcoma [malignant mixed mesodermal tumor, malignant mixed müllerian tumor], and carcinofibroma).<sup>15</sup> Unlike SCEVs, mixed epithelial-mesenchymal tumors occur in the uterus as polypoid, noncircumscribed, usually solitary masses.<sup>15</sup> Clinically, they present with vaginal bleeding.<sup>15</sup> Adenofibromas show epithelium that is usually of proliferative endometrial type; however, a flattened single cell cuboidal cell can be seen admixed with a benign variably fibrous stroma.<sup>5</sup> Adenomyomas are characterized by an intimate admixture of benign endometrial glands without architectural complexity and a stroma composed of smooth muscle and fibrous tissue.<sup>15</sup> Adenosarcomas show an admixture of a benign epithelial component and a sarcomatous component, whereas carcinosarcomas are characterized by an admixture of malignant epithelial and mesenchymal elements, and carcinofibromas are composed of a malig-

nant epithelial component and benign fibrous component.<sup>15</sup> The malignant components of these tumors show an aggressive infiltrative growth pattern and metastatic potential.<sup>15</sup>

Rare malignant mixed tumors of the vagina do occur. They show a biphasic pattern of glands and spindle cells, which resemble synovial sarcoma.<sup>16</sup> These tumors lack the circumscription seen in SCEV with more cytologic atypia and a higher mitotic rate.<sup>16</sup> Malignant mixed tumors of the vagina have been proposed to arise from the Gartner ducts or related mesonephric rests.<sup>16</sup>

The origin of SCEVs has been proposed to be from a possible embryonic remnant.<sup>1</sup> Although the embryologic development of the vagina is controversial, the hymenal ring appears to be derived from the urogenital sinus.<sup>1,17,18</sup> The greatest controversy exists on the relative contributions of the mullerian ducts and urogenital sinus to the development of the vagina. Some people believe that the proximal two thirds to four fifths of the vagina is derived from the mullerian ducts, whereas the urogenital sinus forms the remaining distal portion.<sup>1</sup> Still others believe that the mullerian ducts descend to the level of the hymenal ring, fuse, and then are invaded distally by urogenital sinus tissue that ultimately forms vaginal epithelium.<sup>17,18</sup>

The second theory suggests that foci of primordial vaginal epithelium may migrate and develop aberrantly, resulting in abnormal growths later in adult life.<sup>17</sup> The expression of both epithelial and mesenchymal markers is well known with mullerian-derived tissue.<sup>19</sup> Mullerian-derived tumors express cytokeratin AE1/AE3, vimentin, and CK7 with weak to minimal CK20 staining.<sup>19</sup> Wolffian adnexal tumors, so-called female adnexal tumor of probable wolffian origin, also show varying morphologic structures with solid (spindle cells), tubular (lined by columnar cells), retiform, and multicystic (spaces lined by cuboidal and attenuated cells) patterns. Wolffian adnexal tumors also coexpress vimentin and AE1/AE3 and have been shown to express CK7 with minimal expression of CK20.<sup>19</sup> Since urothelial epithelium expresses both CK7 and CK20 and the tumor we examined showed only CK7 staining, this may favor a mullerian or possible wolffian origin. Ki-67 expression showed only a small percentage of the cells were cycling, and to date none of these tumors have metastasized.<sup>20</sup> Complete surgical excision is considered curative.

Most mixed tumors demonstrate an orderly sequence of transformation or metaplasia from an epithelial to a mesenchymal phenotype and in many cases show the devel-

opment of relatively mature epithelial and/or mesenchymal elements. The SCEVs on the other hand appear to arise from a primitive/progenitor cell that coexpresses CD34, CD99, Bcl-2, cytokeratin, and vimentin and shows little or no mature epithelial or mesenchymal components.

#### References

1. Branton FA, Tavassoli FA. Spindle cell epithelioma, the so-called mixed tumor of the vagina. *Am J Surg Pathol.* 1993;17:509-515.
2. Ballance WA, Ro JY, El-Naggar AK, Grignani DJ, Ayala AG, Romsdahl MG. Pleomorphic adenoma (benign mixed tumor) of the breast. *Am J Clin Pathol.* 1990;93:795-801.
3. Wilk M, Kesper D, Uerlich M, Biwer E, Wimheuer R. Chondroid syringoma: immunohistologic indications of myoepithelial differentiation. *Hautarzt.* 1994;45:324-329.
4. Nakajima T, Watanabe S, Sato Y, et al. An immunoperoxidase study of S-100 protein in normal and neoplastic tissues. *Am J Surg Pathol.* 1982;6:715-727.
5. Wach F, Hein R, Kuhn A, Landthaler M, Krieg T, Eckert F. Immunohistochemical demonstration of myoepithelial cells in sweat gland carcinomas. *Br J Dermatol.* 1994;130:432-437.
6. Hartmann C, Sperling M, Stein H. So-called fibro-epithelial polyps of the vagina exhibiting an unusual but uniform antigen profile characterized by expression of desmin and steroid hormone receptors but no muscle-specific actin or macrophage markers. *Am J Clin Pathol.* 1990;93:604-608.
7. Schirren CG, Burgdorf WH, Sander CA, Plewig G. Fetal and adult hair follicle: an immunohistochemical study of anticytokeratin antibodies in formalin-fixed and paraffin-embedded tissue. *Am J Dermatopathol.* 1997;19:335-340.
8. Silverman JS, Tamsen A. Fibrohistiocytic differentiation in subcutaneous fatty tumors: study of spindle cell, pleomorphic, myxoid, and atypical lipoma and dedifferentiated liposarcoma cases composed in part of CD34+ fibroblasts and FXIIIa+ histiocytes. *J Cutan Pathol.* 1997;24:484-493.
9. Kasinrerk W, Tokrasinwit N, Moonsom S, Stockinger H. CD99 monoclonal antibody induce homotypic adhesion of Jurkat cells through protein kinase and protein kinase C dependent pathway. *Immunol Lett.* 2000;10:33-41.
10. Park CK, Shin YK, Kim TJ, Park SH, Ahn GH. High CD99 expression in memory T and B cells in reactive lymph nodes. *J Korean Med Sci.* 1999;14:600-606.
11. Gyure KA, Prayson RA, Estes ML. Extracerebellar primitive neuroectodermal tumors: a clinicopathologic study with bcl-2 and CD99 immunohistochemistry. *Ann Diagn Pathol.* 1999;3:276-280.
12. Baer R. Bcl-2 breathes life into embryogenesis. *Am J Pathol.* 1994;145:7-10.
13. Sohn HW, Choi EY, Kim SH, et al. Engagement of CD99 induces apoptosis through a calcineurin independent pathway in Ewing's sarcoma cells. *Am J Pathol.* 1998;153:1937-19345.
14. Suster S, Nascimento AG, Miettinen M, Sichel JZ, Moran CA. Solitary fibrous tumors of soft tissue. *Am J Surg Pathol.* 1995;19:1257-1266.
15. Silverberg SG, Kurman RJ. Mixed epithelial-nonepithelial tumors. In: *Tumors of the Uterine Corpus and Gestational Trophoblastic Disease.* Washington, DC: Armed Forces Institute of Pathology; 1992:153-179. *Atlas of Tumor Pathology*; 3rd series, fascicle 3.
16. Kurman RJ, Norris HJ, Wilkinson E. Mixed epithelial and mesenchymal tumors. In: *Tumors of Cervix, Vagina, and Vulva.* Washington, DC: Armed Forces Institute of Pathology; 1992:164-165. *Atlas of Tumor Pathology*; 3rd series, fascicle 4.
17. Merlob P, Bahari C, Liban E, Reisner SH. Cysts of the female genitalia in the newborn infant. *Am J Obstet Gynecol.* 1978;132:607-610.
18. Ulfelder H, Robboy SJ. The embryologic development of the human vagina. *Obstet Gynecol.* 1976;126:769-776.
19. Carano KS, Soslow RA. Immunophenotypic analysis of ovarian and testicular mullerian papillary serous tumors. *Mod Pathol.* 1997;10:414-420.
20. Han AC, Duszak R. Coexpression of cytokeratins 7 and 20 confirms urothelial carcinoma presenting as an intrarenal tumor. *Cancer.* 1999;86:2327-2330.