Anal gland carcinoma (AGC) is a rare perianal invasive cancer composed of tubular glands lined by cuboidal epithelium. The clinical features and histogenesis of AGC are not well understood and its origin from anal glands is often difficult to prove. Little is known about immunophenotypic features of AGC that could be useful in establishing the diagnosis. This study evaluated the immunohistochemical profile of 2 cases of AGC in comparison to anal glands from 11 hemorrhoidectomy specimens. Sections from the specimens were routinely processed and immunostained using commercial antibodies to cytokeratin (CK) 7, CK20, CK5/6, p63, CDX2, smooth muscle actin, calponin, heavy chain smooth muscle myosin, p53, and p16. In case 1 of AGC, radiation and chemotherapy preceded an abdominoperineal resection. In biopsies from this case, the neoplastic anal glands had a tubular pattern, whereas most glands in the resection specimen exhibited mucinous features. The histologic pattern in case 2 was tubular. Normal anal glands showed immunoreactivity for myoepithelial and basal cell markers CK5/6 and p63 in basal and parabasal cell layers and for CK7 in superficial cell layers. In contrast, both cases of AGC were negative for CK5/6 and p63 and were diffusely positive for CK7. Normal glands and both cases of AGC were negative for the intestinal differentiation marker CDX2, CK20, smooth muscle actin, calponin, smooth muscle myosin heavy chain, p16, and p53. Our data suggest that loss of p63 and CK5/6 expression is a feature of AGC. Anal gland carcinoma shares negativity for CDX2 and CK7+/CK20– profile with normal anal glands. No evidence of myoepithelial cells was found in normal or malignant anal glands. These data may be useful in establishing the diagnosis of AGC.

(Arch Pathol Lab Med. 2007;131:1304–1311)
A 47-year-old male patient presented with a perianal abscess to an outside hospital in April 2005. The abscess was treated with incision and drainage. A fistula developed postoperatively. In May 2005, he presented again with bowel obstruction. Anoscopy showed a perianal mass in the midline measuring 1.5 to 2.0 cm. Hartmann procedure was performed. Biopsy demonstrated a well-differentiated perianal adenocarcinoma consistent with AGC. Computed tomography scan of the abdomen and pelvis was unremarkable. The patient received preoperative chemotherapy (5-fluorouracil) and radiation therapy (5400 cGy) without effect. Abdominoperineal resection was performed at Long Island Jewish Medical Center in January 2006. Grossly, a firm circumferential anal mass was seen slightly elevating the anal mucosa, but not involving it, 1 cm above the perianal skin margin. On sectioning, the tumor measured 2.5 cm in greatest dimension and appeared to infiltrate perianal muscle (Figure 1). A fistulous opening was seen in the perianal skin penetrating into perianal tissue for 1.3 cm.

**AGC2.**—The case was presented by the California Tumor Tissue Registry (www.cttr.org) as a Monthly Study Slide in May 1971. The patient, a 53-year-old man with a 10-year history of hemorrhoids and rectal bleeding, was seen in October 1970. On rectal examination there was a 3 × 3 × 2-cm tender mass at the anal verge. A biopsy confirmed the presence of an AGC. An abdominoperineal resection was performed. At surgery, the prostate was nodular and biopsy showed a well-differentiated adenocarcinoma, which was different from the anal carcinoma. A subtotal prostatectomy was added to the procedure. Grossly, a nodular focally cystic 1-cm mass was seen in October 1970. On rectal examination there was a 3 × 3 × 2-cm tender mass in the distal parts of the glands. The distal epithelium had basal cuboidal cells and either superficial mucin-producing columnar cells (5 cases, Figure 2, B) or superficial cuboidal cells (5 cases). In 5 cases, anal glands were lined by transitional epithelium only and in 2 cases by 2- to 3-cell-thick epithelium only.

**Anal Gland Carcinoma.**—Biopsy specimens of AGC1 and the specimen from AGC2 had crowded and haphazardly arranged tubular glands in the connective tissue and markers of myoepithelial cells and basal and intermediate cells of stratified epithelia (p63 and CK5/6), tumor markers (p53 and p16), and markers of smooth muscle differentiation (smooth muscle actin, calponin, and smooth muscle myosin heavy chain). We demonstrated that normal anal glands express p63 and CK5/6, whereas AGC loses this immunoreactivity and maintains a CK7+ profile.

**MATERIALS AND METHODS**

**Case Selection**

Approval for the study was obtained from the institutional review board of the North Shore–Long Island Jewish Health System (Lake Success, NY). Histologic material from 2 cases of AGC diagnosed according to the criteria of Hobbs et al (see above) and from 11 hemorrhoidectomy specimens retrieved from the files of the Department of Pathology at Long Island Jewish Medical Center was studied. Eight biopsy blocks and 16 blocks from the resection specimen were available for case 1 of AGC (AGC1) and 1 representative block from the resection specimen was available for case 2 of AGC (AGC2). At least 1 block was available for each hemorrhoidectomy specimen. Tissues were fixed in 10% formalin and processed routinely.

**Immunohistochemistry**

For immunohistochemical analysis, 5-μm sections were cut from formalin-fixed, paraffin-embedded tissue blocks and were transferred to glass slides. Immunostaining was performed by the modified avidin-biotin complex method on a Dako-Automatist (DakoCytomation, Carpinteria, Calif) or a Ventana Benchmark immunostainer (Ventana, Tucson, Ariz) using commercially available antibodies (Table 1). The slides were incubated with antibodies at room temperature followed by buffer washes. Then sections were incubated with secondary biotinylated antibodies, followed by avidin-peroxidase complex, and reacted with diaminobenzidine and hydrogen peroxide using Dako-Cytomation EnVision+ System-HPR. All slides were counterstained with hematoxylin. For negative control, slides were treated with DakoCytomation N-Universal Negative Control Mouse antibodies. Immunostaining reactivity for each antibody was scored as positive or negative in the cell type under study. Prospective antigen and prostate-specific antigen; PSAP, prostate-specific acid phosphatase; SMA, smooth muscle actin; and SMMMC, smooth muscle myosin heavy chain.

**RESULTS**

**Case Histories**

**AGC1.**—A 47-year-old male patient presented with a perianal abscess to an outside hospital in April 2005. The abscess was treated with incision and drainage. A fistula developed postoperatively. In May 2005, he presented

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**Table 1. Antibodies Used in This Study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDX2</td>
<td>CDX2-88</td>
<td>BioGenex (San Ramon, Calif)</td>
<td>RTU</td>
</tr>
<tr>
<td>CK5/6</td>
<td>D3/16B4</td>
<td>Zymed (South San Francisco, Calif)</td>
<td>1:100</td>
</tr>
<tr>
<td>CK7</td>
<td>OV-TL</td>
<td>Dako (Carpinteria, Calif)</td>
<td>1:200</td>
</tr>
<tr>
<td>CK20</td>
<td>KS20.8</td>
<td>Dako</td>
<td>1:200</td>
</tr>
<tr>
<td>Calponin</td>
<td>CALP</td>
<td>Cell Marque (Hot Springs, Ark)</td>
<td>RTU</td>
</tr>
<tr>
<td>p16</td>
<td>G175-405</td>
<td>BD Biosciences Pharmingen (San Jose, Calif)</td>
<td>1:200</td>
</tr>
<tr>
<td>p53</td>
<td>DO-7</td>
<td>Dako</td>
<td>1:100</td>
</tr>
<tr>
<td>p63</td>
<td>4A4</td>
<td>NeoMarkers (Fremont, Calif)</td>
<td>1:50</td>
</tr>
<tr>
<td>PSA</td>
<td>Polyclonal</td>
<td>Ventana (Tucson, Ariz)</td>
<td>RTU</td>
</tr>
<tr>
<td>PSAP</td>
<td>PASE/4L</td>
<td>Ventana</td>
<td>RTU</td>
</tr>
<tr>
<td>SMA</td>
<td>1A4</td>
<td>Dako</td>
<td>1:200</td>
</tr>
<tr>
<td>SMMMC</td>
<td>SMMS-1</td>
<td>Cell Marque</td>
<td>RTU</td>
</tr>
</tbody>
</table>

*RTU indicates ready to use; CK, cytokeratin; PSA, prostate-specific antigen; PSAP, prostate-specific acid phosphatase; SMA, smooth muscle actin; and SMMMC, smooth muscle myosin heavy chain.
Figure 1. Cut surface of case 1 of anal gland carcinoma infiltrating perianal muscle and closely approaching radial surgical margin.

Figure 2. Histologic features of normal anal glands. A, Transitional epithelium of an anal gland (hematoxylin-eosin, original magnification ×100). B, The 2- to 3-cell-thick columnar epithelium of a distal branch of an anal gland (hematoxylin-eosin, original magnification ×400).

Figure 3. Histologic features of anal gland carcinoma. A, Haphazardly arranged tubular glands infiltrating beneath anal squamous mucosa in biopsy specimens from case 1 of anal gland carcinoma (AGC1) (hematoxylin-eosin, original magnification ×40). B, Neoplastic glands composed of cuboidal epithelium with pronounced cellular atypia and surrounding desmoplastic stroma from case 2 of anal gland carcinoma (AGC2) (hematoxylin-eosin, original magnification ×400). C, Carcinoma with mucinous features from the AGC1 resection specimen (hematoxylin-eosin, original magnification ×100).
smooth muscle below the uninvolved squamous mucosa of the anal canal (Figure 3, A). An erosion of squamous epithelium in the area of the tumor was seen in AGC2. Neoplastic glands were lined by cuboidal epithelium with atypical cells containing pleomorphic nuclei with dark basophilic chromatin in AGC1 or clumped and margined chromatin and prominent nucleoli in AGC2 (Figure 3, B). Clusters of cells and single pleomorphic cells were seen in desmoplastic stroma and between smooth muscle fibers. AGC2 had a significant inflammatory component. The resection specimen from AGC1 showed 2 histologic patterns. The predominant pattern consisted of glands with well-differentiated columnar cells producing abundant mucin (Figure 3, C). Stromal pools of extravasated mucin were present in some areas. The second pattern consisted of haphazardly arranged glands lined by cuboidal epithelium, similar to the biopsy specimen. These glands were seen in immediate proximity to mucinous glands. No dysplasia was noted in either of the AGC cases. Fistula curettings from the biopsy and sections of fistula from the AGC1 resection specimen revealed only granulation tissue.

**Immunohistochemistry**

**Normal Anal Glands.**—The immunohistochemical findings are summarized in Table 2. p63 nuclear staining was seen in basal and intermediate cell layers of normal anal gland epithelium in all 11 hemorrhoidectomy specimens (Figure 4, A). In the distal part of anal glands, p63 staining was present in at least 1 basal cell layer (Figure 4, B). The most superficial epithelial layer in all studied normal glands stained negatively for p63, CK7 staining was present in 10 of 11 cases and was positive for CK5/6 and p63. Colorectal epithelium was present in 8 of 11 specimens and was diffusely positive for CDX2 (Figure 5, E), positive for CK20 in surface epithelium and superficial glands, and negative for CK7.

**Anal Gland Carcinoma.**—Both cases of AGC, including both tubular and mucinous glands of AGC1, stained uniformly negatively for p63 (Figure 5, A) and CK5/6 (Figure 5, B), suggesting that malignant glands had lost basal and intermediate cell differentiation of normal anal gland epithelium. Both AGC1 (tubular and mucinous glands) and AGC2 were diffusely positive for CK7 (Figure 5, C) and were negative for CK20 (Figure 5, D). Both cases failed to stain with antibodies to CDX2 (Figure 5, E), p16, and p53 (not shown). Both cases also stained negatively for prostate markers prostate-specific antigen and prostate-specific acid phosphatase.

**COMMENT**

Anal adenocarcinomas constitute 3% to 4% of cancers arising in the anal canal, and the vast majority of them are of colorectal type. The remaining small group constitutes perianal (extramucosal) adenocarcinomas subdivided by the World Health Organization classification into AGC and adenocarcinoma arising in perianal fistulas. The latter are usually of mucinous type. However, perianal

**Table 2. Results of Immunohistochemical Staining***

<table>
<thead>
<tr>
<th></th>
<th>CK7</th>
<th>CK20</th>
<th>CDX2</th>
<th>CK5/6</th>
<th>p63</th>
<th>SMA</th>
<th>Calponin</th>
<th>SMMHC</th>
<th>p53</th>
<th>p16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal anal glands (n = 11)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AGC1</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AGC2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* CK indicates cytokeratin; SMA, smooth muscle actin; SMMHC, smooth muscle myosin heavy chain; AGC1, case 1 of anal gland carcinoma; and AGC2, case 2 of anal gland carcinoma.

**Table 3. Demographics and Clinical Features of Anal Gland Carcinoma**

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Age ± 2 SD, y</td>
<td>59.1 ± 23.3</td>
<td>63.0 ± 20.1</td>
</tr>
<tr>
<td>M/F ratio</td>
<td>3:1</td>
<td>4:1</td>
</tr>
<tr>
<td>Presenting symptoms</td>
<td>Rectal pain</td>
<td>Anal stenosis (3 cases)</td>
</tr>
<tr>
<td></td>
<td>Rectal bleeding</td>
<td>Bleeding (3 cases)</td>
</tr>
<tr>
<td></td>
<td>Anal stenosis</td>
<td>Pain (2 cases)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bowel obstruction (2 cases)</td>
</tr>
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</table>

**Table 4. Outcomes of Anal Gland Carcinoma by the Time of Publication**

<table>
<thead>
<tr>
<th>Available Data</th>
<th>Meta-analysis of 20 Cases (Wellman, 1962), No. (%)</th>
<th>Meta-analysis of 15 Cases (1963–Present), No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 cases</td>
<td>9 cases</td>
</tr>
<tr>
<td>Death from tumor</td>
<td>4 (22)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Recurrence or metastases</td>
<td>4 (22)</td>
<td>4 (45)</td>
</tr>
<tr>
<td>Well (up to 4.5 y)</td>
<td>6 (33)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Death unrelated to tumor</td>
<td>3 (17)</td>
<td>0</td>
</tr>
</tbody>
</table>
mucinous adenocarcinomas without fistula formation have been reported\textsuperscript{6,18,19} and transition from neoplastic mucinous glands to normal anal glands has been demonstrated\textsuperscript{20-22} thus establishing anal gland origin of some perianal mucinous adenocarcinomas. So far, immunohistochemical comparison of different types of perianal mucinous adenocarcinoma has not been reported.

The 2 cases of perianal carcinoma presented in this study fulfill the criteria of AGC, as proposed by Hobbs et al\textsuperscript{7} because the neoplastic tubules do not involve luminal

Figure 4. Immunohistochemical assessment of normal anal glands. A and B, Positive p63 staining of basal and intermediate cell layers in proximal and distal parts of normal anal glands, respectively (immunoperoxidase, original magnifications ×100 [A] and ×400 [B]). C and D, Positive cytokeratin 5/6 staining of basal and intermediate cell layers in proximal and distal parts of normal anal glands, respectively (immunoperoxidase, original magnifications ×100 [C] and ×400 [D]). E, Cytokeratin 7 staining of normal anal glands (immunoperoxidase, original magnification ×100); inset corresponds to the rectangular zone of interest and shows 1 to 2 basal cell layers negative for cytokeratin 7 staining (immunoperoxidase, original magnification ×400). F, A normal anal gland is negative for calponin staining, whereas smooth muscle fibers are positive (immunoperoxidase, original magnification ×100).
mucosa, are lined by cuboidal cells showing scanty mucin secretion, and are CK7+/CK20−. Interestingly, biopsies from AGC1 showed only tubular glands, whereas the majority of glands in the resection specimen, even the most superficial, were mucinous. The transition may have resulted from the long period between the initial biopsy and the abdominoperineal resection. Alternatively, the transition may have been induced by radiotherapy or chemotherapy. Transition to mucinous carcinoma has been recently reported in colon cancer following radiotherapy.23

Another, less likely explanation is the difference in the sampling size between the biopsy and the resection specimen.

The presentation of AGC1 with a perianal abscess and fistula raised the possibility of the origin of cancer in the fistula. However, carcinoma usually develops in fistulas of many, typically 20 and more, years of duration.24 Short duration (1 month) and benign morphology of the fistula argue against its role in the development of cancer in AGC1.

Figure 5. Immunohistochemical assessment of anal gland carcinoma. A, Anal gland carcinoma (AGC) is negative for p63 (tubular glands from the case 1 of AGC [AGC1] biopsy specimen). B, AGC is negative for cytokeratin (CK) 5/6 (mucinous glands from the AGC1 resection specimen). C, AGC is positive for CK7 (case 2 of AGC). D, AGC is negative for CK20 (mucinous glands from the AGC1 resection specimen). E, AGC is negative for CDX2. Negatively staining neoplastic mucinous glands are seen next to CDX2-positive normal colorectal glands (immunoperoxidase; original magnifications ×100 [A through E]).
Interestingly, CK7 was expressed in the surface layer of anal glands in our study, whereas CK5/6 and p63 were expressed in the basal layers. The distribution of keratins is consistent with functional zonation of anal gland epithelium, where the most superficial cell layer is mucin-secretory and the underlying layers are stratified.

Smooth muscle actin–positive spindle-shaped myoepithelial cells have been reported to surround normal anal glands, although the myoepithelial nature of these cells had not been corroborated by staining for cytokeratins.28 Our data are at variance with those findings and show that p63- and CK5/6-positive cells of normal anal glands do not stain for the smooth muscle markers smooth muscle actin, calponin, and smooth muscle myosin heavy chain. Neither normal nor malignant anal glands showed smooth muscle immunoreactivity arguing against the presence of myoepithelial cells in the anal glands.

In conclusion, ACG is an aggressive disease of older age and with male preponderance. Loss of expression of p63 and CK5/6 in AGC compared with normal anal gland mucosa and its negativity for CDX2 compared with colorectal carcinoma comprise an immunostaining panel that could be helpful in the diagnosis of AGC. Analysis of additional cases is needed to verify the immunophenotype of ACG.

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References

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