Pathology of Anal Cancer

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KEYWORDS

• Anal cancer • Anal squamous intraepithelial neoplasia • Squamous cell carcinoma • Human papilloma virus (HPV) • Molecular

KEY POINTS

- Anal cancer is an uncommon tumor, squamous cell carcinoma (SCC) being the most frequent histology corresponding to 80% of all cases.
- Human papilloma virus (HPV) infection plays a key role in anal cancer development, encoding at least three oncoproteins with stimulatory properties.
- SCC expresses CK5/6, CK 13/19, and p63. P16 is a surrogate marker for the presence of HPV genome in tumor cells.

INTRODUCTION

Anal cancer accounts for approximately 2.4% of gastrointestinal malignancies.[1] Although anal cancer is a rare tumor, its frequency is increasing, especially in high-risk groups.[2] Tumors in this location are generally classified as anal canal or anal margin.

Squamous cell carcinoma (SCC) is the predominant type of tumor and shares many features with cervical cancer. Oncogenic human papilloma virus (HPV) infection plays a major role in both tumors.[3] HIV infection is associated with a higher frequency of HPV-associated premalignant lesions and invasive tumors.[4]

Normal Anatomy of the Anus

The anal canal is the terminal part of the large intestine and is slightly longer in male than in female patients. It measures approximately 4 cm and extends from the rectal ampulla (pelvic floor level) to the anal verge, which is defined as the outer opening of the gastrointestinal tract. The anal verge is at the level of the squamous-mucocutaneous junction with the perianal skin.[5,6]

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The dentate line (also called pectinate) consists of the anal valves and bases of the anal columns. It represents the anatomic division of the rectum from the anal canal. The dentate line originates from the embryonic union of the ectoderm with the endoderm. The anal canal epithelium can be divided into three zones (Fig. 1). The upper part consists of colorectal mucosal type, followed by an anal transition zone (ATZ) that is composed of specialized epithelium that starts at the dentate line and extends from 0.5-1 cm. Finally, the distal anal canal consists of squamous epithelium, which may be partially keratinized.[5,6] The anatomic distribution is clinically significant because it is related to lymphatic drainage and different types of precursor epithelium.

The tumor can be accessed for biopsy using anoscopy, rectoscopy, or direct examination of anal and perineal exophytic lesions.

Histology Classification

Tumors of the anal canal were classified by the World Health Organization (WHO) in three main groups: epithelial, mesenchymal, and secondary tumors. Epithelial tumors were subdivided into malignant and premalignant lesions (Box 1).[5]

Human Papilloma Virus Infection

Evidence indicates an association between oncogenic HPV infection with premalignant and malignant lesions of the genital tract, including the anus.[7] The presence of HPV in anal cancer is variable and may be influenced by the methodology used for virus identification and by population characteristics. The HPV infection can be detected in tumor tissue using different techniques, including in situ hybridization (Fig. 2) and polymerase chain reaction (PCR).

Currently, 88% of anal SCC tumor samples are usually HPV positive, with different rates according to geographic location.[8] HPV16 infection is the most common, present in 86% of cases. In some cases, coinfection was found with multiple HPV types.[9]

HPV is a nonenveloped virus with double-stranded DNA in circular form, containing a genome of around 8000 base pairs.[7] HPV can remain housed in the nuclei of basal epithelial cells for decades after initial infection of the mucosa, which usually occurs through sexual contact.[10] There are more than 240 types described and the alpha human papillomavirus is usually related to mucosal infection.[11] The high-risk HPV genotypes (16 and 18) encode at least 3 oncoproteins with stimulatory properties: E5, E6,

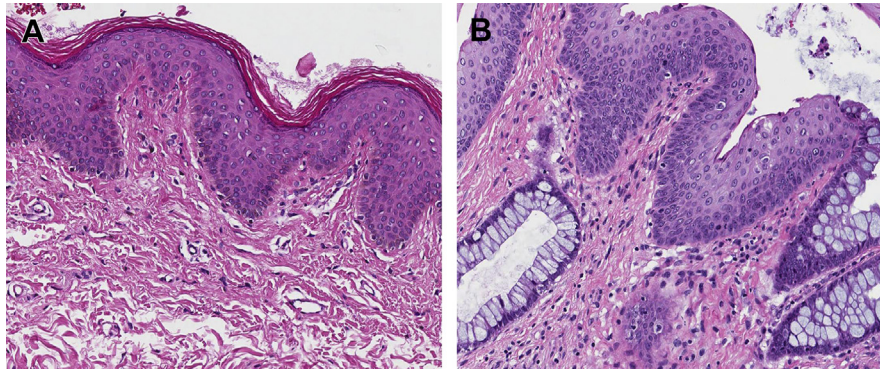

Fig. 1. Normal epithelium. (*A*) Squamous epithelium. (*B*) Anal transition zone epithelium showing in the left cuboidal or polygonal surface cells. In the right can be viewed colonic mucosa with an underlying crypt.

Box 1
World Health Organization histologic classification of tumors of the anal canal

- Epithelial tumors
  - Premalignant lesions
    - Anal intraepithelial neoplasia (dysplasia), low-grade
    - Anal intraepithelial neoplasia (dysplasia), high-grade
    - Bowen disease
    - Perianal squamous intraepithelial neoplasia
    - Paget disease
  - Carcinoma
    - SCC
    - Verrucous carcinoma
    - Undifferentiated carcinoma
    - Adenocarcinoma
    - Mucinous adenocarcinoma
  - Neuroendocrine neoplasms
    - Neuroendocrine tumor (NET)
      - NET G1 (carcinoid)
      - NET G2
    - Neuroendocrine carcinoma (NEC)
      - Small cell NEC
      - Large cell NEC
    - Mixed adenoneuroendocrine carcinoma
- Mesenchymal tumors
- Secondary tumors

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and E7. Integration of the viral DNA in the genome of the host cell is important for the progression of preneoplastic lesions to invasive carcinoma. During HPV integration, breakage of the E2 region of the viral genome occurs in the DNA of the infected cells, which causes the loss of suppressor function of the E2 protein. This results in an increased expression of the E6 and E7 proteins and their stimulation promotes invasiveness and keratinocyte immortalization.[10] E6 and E7 expression is required for the induction and maintenance of the transformed state of HPV-related neoplasm. The protein E7 interacts with Rb protein (pRb) and E6 is able to bind and inactivate P53.[12]

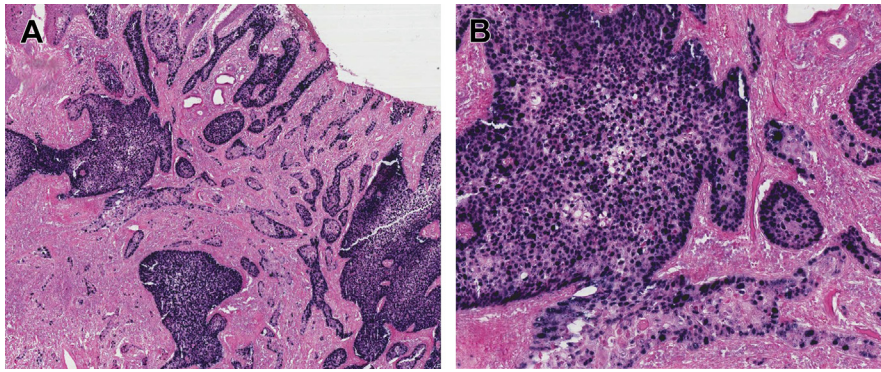

Fig. 2. In situ hybridization. (*A*) Note the positivity for high-risk HPV probe. (*B*) Diffuse staining with condensed and uniform signs in the nuclei of the cancer cells.

PREINVASIVE LESIONS

There are different terminologies used to describe premalignant lesions of the anal region. Squamous lesions were first described in the anal margin 50 years ago and they were initially classified as cutaneous lesions, such as Bowen disease and carcinoma in situ. Over time, a causal relationship was established between anal dysplasia and HPV perianal infection. However, terminologies of squamous precancerous lesions of anal canal continued to be diverse. The WHO classification (**Box 1**), for instance, has four items related to premalignant squamous lesions. There are two items grading intraepithelial neoplasia/dysplasia in low and high grade. In addition, two other categories, Bowen disease, and perianal squamous intraepithelial neoplasia. These last two probably designating the same lesion. To make things even more complicated, the American Joint Committee on Cancer (AJCC) definition for primary tumors classifies noninvasive squamous lesions as carcinoma in situ, Bowen disease, high-grade squamous intraepithelial lesion (HSIL), and anal intraepithelial neoplasias (AIN) 2-3. This shows the need to propose a clearer classification so that the work of clinicians and pathologists is more uniform.

Squamous Intraepithelial Lesions

Because HPV plays a causal role in perianal and anal neoplasia, anal squamous precancerous lesions should now be classified according to the same criteria and terminology as their cervical counterparts using the Lower Anogenital Squamous Terminology (LAST) system.[3]

A consensus process was organized by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology to recommend terminology unified across lower anogenital sites. The aim of this system of standardization was to create uniformity in histologic nomenclature for HPV-associated tumors that occurred in the anogenital tract. This permits a better use of current biomarkers and enables an efficient communication between specialties that establish the diagnosis and treatment of these lesions. The recommendation for classifying preinvasive lesions in the anal canal was similar to the cervical site. Hence, the same two-tiered nomenclature of intraepithelial lesion could be applied: low-grade squamous intraepithelial lesion (LSIL) and HSIL. The lesions are located above the basement membrane and may originate from the transitional or squamous epithelium of the anal canal. In the LAST system, condyloma is located with LSIL in the same category and Bowenoid papulosis is considered a special form of HSIL. Besides this, it does not discriminate cytologic from tissue specimens. Therefore, the terms LSIL and HSIL can be used in both materials. However, for histopathological diagnosis, the suffix AIN can be used; therefore, the lesion in the anal canal can be subcategorized as: LSIL-AIN 1 and HSIL-AIN 2–3.

LSIL-AIN 1 can be defined as a proliferation of metaplastic squamous cells with nuclear atypia in the lower third of the epithelium or demonstrating koilocytotic changes in a papillary lesion (**Fig. 3**). The histologic findings of HSIL-AIN 2-3 consist of a thickened proliferating epithelium containing atypical cells showing abnormal nuclear polarity, nuclear pleomorphism, and high nuclear hyperchromatism: cytoplasmic ratio and increased mitotic activity (**Fig. 4**).[5]

To date, knowledge of the long-term natural history of anal squamous intraepithelial lesion is still not well established. It is postulated that anal LSIL, corresponding to AIN 1, spontaneously regresses but may also progress to HSIL.[13] The overall progression rate of HSIL, corresponding to HSIL-AIN grade 2 or 3 invasive carcinoma, is 2% to 9% but can reach 50% in immunocompromised individuals (**Fig. 5**).[14]

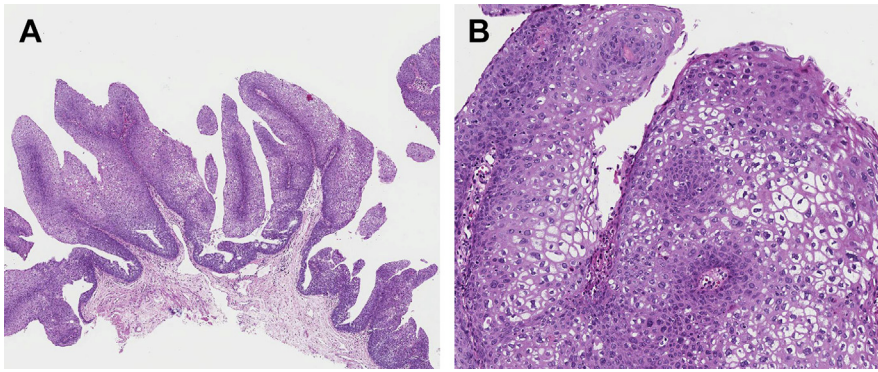

Fig. 3. Condyloma. (*A*) Note the exophytic polypoid feature with minimal stromal proliferation. Condyloma is equivalent to LSIL and AIN 1. (*B*) Enlarged nuclei and koilocytotic atypia.

Paget Disease

Primary anal Paget disease has a cutaneous origin and affects areas densely populated by apocrine glands arising from adnexal stem cells. Malignant cells produce mucus and infiltrate all layers of the epidermis. Histology reveals large cells with plentiful pale cytoplasm and large nuclei, and sometimes the tumor cells acquire a signet ring appearance.[5,15]

MALIGNANT NEOPLASMS

Even though cancer of the anal canal is rare, a variety of malignant neoplasms involve tumors in this location. Almost all of these are SCC. Other rare anal canal neoplasms include adenocarcinoma, neuroendocrine tumors, malignant melanoma, lymphomas, and various mesenchymal tumors.

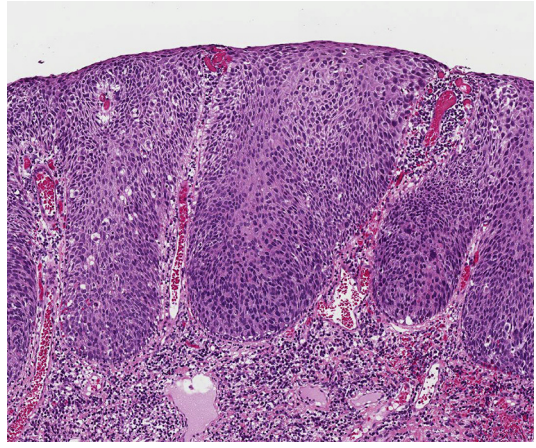

Fig. 4. High-grade squamous intraepithelial lesion–AIN 3. Note the abnormal basaloid cells having an increased nuclear cytoplasmic ratio extending to the entire thickness of the epithelium but restricted to the basement membrane.

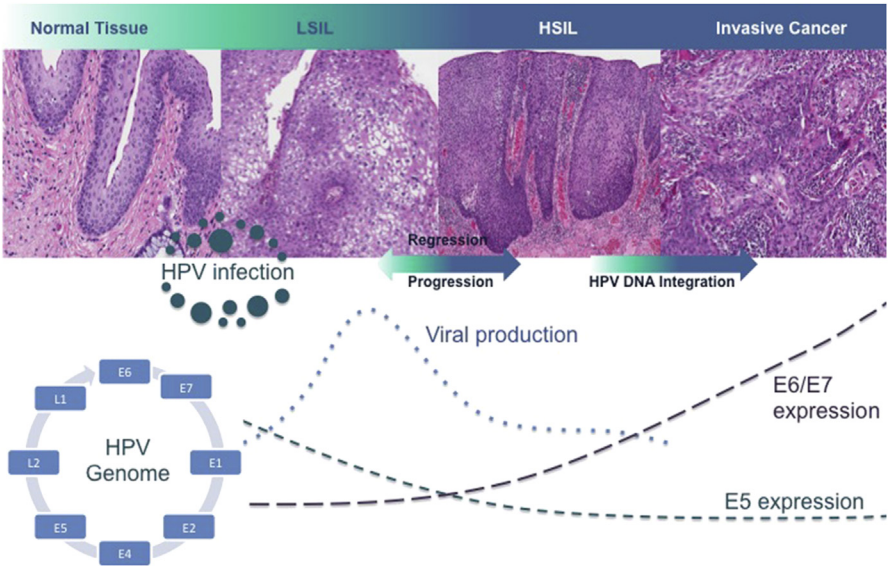

Fig. 5. Natural history of HPV infection and progression from normal tissue to anal cancer. Progressive changes in squamous epithelium through loss of normal apoptotic cell mechanism due to the presence of E6 and E7 oncoproteins. In SCC, virus genome integration occurs with loss of E2 regulatory region, resulting in uncontrolled E6/E7, p53, and pRb production.

Squamous Cell Carcinoma

This is the most common histology of anal cancer, corresponding to 80%.[16] The histologic classification of anal SCC was previously considered a complex issue, with several subtypes using a variable terminology. The current WHO classification recommends that the generic term SCC should be used for all squamous malignancies of the anal canal.[5] Tumors located in the anal canal predominantly develop at the transformation zone between the squamous and columnar epithelium of the anal canal[14]; and most tumors are composed of multiples features. The cells may have large pale eosinophilic squamous cells with or without areas of keratinization. Another pattern is tumor-cell islands with prominent palisading of nuclei. The cells can constitute tumor nests and differentiated tumors may present peripheral palisading or central keratinization.[5,15]

In the past, the basaloid subtype, also called cloacogenic carcinoma, was included in the WHO classification. It was withdrawn because either it was difficult to reproduce this diagnosis and prognosis, or the SCC treatment lacked correlation. Currently, the use of SCC is recommended to describe the main diagnosis, with a comment detailing particularities of each sample as a degree of differentiation, such as presence of mucinous, basaloid features and degree of keratinization (**Fig. 6**).[5]

STAGING

Two distinct categories of tumors arise in the anal canal region. Those that develop from the mucosa, called the anal canal tumors, and tumors that arise within the skin at or distal to the squamous mucocutaneous junction, termed as perianal cancers. This last type of tumor is staged and treated as skin cancer and not like anal canal cancer.[17] Staging of anal carcinomas should be determined in agreement with the criteria

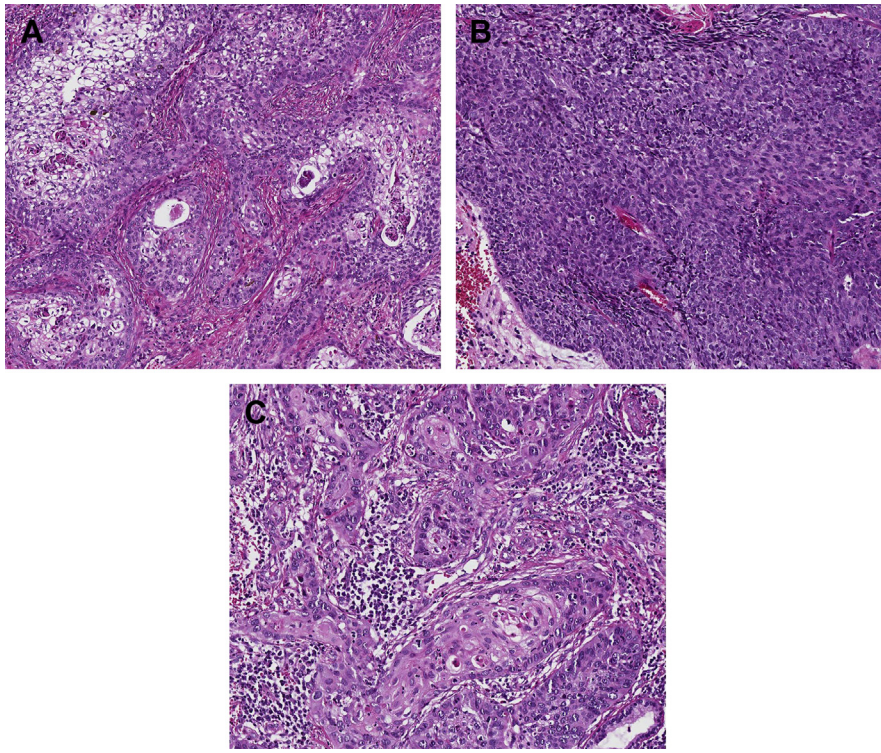

Fig. 6. SCC. (*A*) Note the clear cell features and abnormal keratinization. (*B*) The tumor shows basaloid features. (C) Nests infiltrated by keratinization.

of the AJCC (**Tables 1** and **2**).[17] Because most cases of anal cancer are treated using nonsurgical procedures, the role of staging based on the histopathological evaluation is limited to cases of resection in an early stage, salvage surgical treatment or sentinel lymph node procedure.[18]

Verrucous Carcinoma

The Buschke-Löwenstein tumor, or giant malignant condyloma, is an intermediate variant between condyloma and SCC. This tumor often presents as a large mass with cauliflower appearance and has an endophytic and exophytic growth. The lesion size can range from 1 to 30 cm and can initiate in the perianal skin, anal canal, or distal rectum.[19] Despite a benign histologic appearance with acanthosis and papillomatosis in regular arrangement of the epithelial layers, this tumor may present a locally destructive behavior by direct deeper invasion.[5]

Adenocarcinoma

This tumor can originate in the mucosa, anal glands, or anal canal fistulae, and may appear near the anal duct as a small pedunculated or ulcerated lesion,[15] or produce a submucosal mass. An association with Paget and Crohn disease is described. Generally, adenocarcinomas associated with congenital or acquired fistulae are mucin productive.

Table 1
Tumor-nodes-metastasis staging anal cancer

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ Bowen disease, high-grade squamous intraepithelial lesion (HSIL), anal intraepithelial neoplasia 2–3 (AIN 2–3)
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 5 cm in greatest dimension
T3	Tumor more than 5 cm in greatest dimension
T4	Tumor of any size invades adjacent organs; for example, vagina, urethra, bladder[a]
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in perirectal lymph nodes
N2	Metastasis in unilateral internal iliac and/or inguinal lymph nodes
N3	Metastasis in perirectal and inguinal lymph nodes and/or bilateral internal iliac and/or inguinal lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

[a] Direct invasion of the rectal wall, perirectal skin, subcutaneous tissue, or the sphincter muscles is not classified as T4.

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Table 2
Anatomic stage: prognostic groups

	Tumor (T)	Node (N)	Metastasis (M)
0	Tis	N0	M0
I	T1	N0	M0
II	T2	N0	M0
	T3	N0	M0
IIIA	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
	T4	N0	M0
IIIB	T4	N1	M0
	Any T	N2	M0
	Any T	N3	M0
IV	Any T	Any N	M1

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OTHER TUMOR TYPES

A variety of other tumor types may arise less frequently in the anal canal and make recognition more difficult.

Melanoma

Anal melanomas are a rare disease and account for 1% of anal and perianal lesions.[15] Two-thirds are found as pigmented lesions, usually ulcerated and bleeding. Similar to melanomas that arise in other mucous membranes, they are generally of the acrolentiginous type. Retrospective studies have reported poor prognosis, especially in patients with lymph node involvement or metastasis at diagnosis. Anal melanoma presents the same histologic appearance as that of cutaneous melanoma with S-100 expression. Positive c-kit was reported in 45%.[15,20] Differential diagnosis of Paget anal melanoma can be difficult if based solely on histologic features; therefore, association with immunohistochemical evaluation is useful.

Mesenchymal Tumors

Uncommonly lipomas, hemangiomas, leiomyomas and leiomyosarcomas, rhabdomyosarcomas, granular cell tumors, and Kaposi sarcoma may be located in the anal and perianal region.[5,15]

Basal Cell Carcinoma of the Anus

Basal cell carcinoma comprises 0.2% of anorectal neoplasms[21] and affects the skin of the anal region. There is no evidence of the carcinogenic role of HPV in this tumor but SCC with basaloid features may show overlapping of histologic findings with basal cell carcinoma.[5,21] Both tumors may have nests of oval cells with moderate quantity of eosinophilic to basophilic cytoplasm, peripheral nuclear palisading, and assorted mitotic activity. In a retrospective study of basal cell carcinoma, the nodular subtype of basal cell carcinoma was the most frequent and this tumor presented more retraction artifact and no atypical mitotic figures.[21]

Neuroendocrine Tumors

Anal neuroendocrine tumors are unusual and account for 1% of all anal cancers.[22] Anal small cell carcinoma has poor prognosis and generally begins in the upper part of the anal canal, grows fast, and promotes metastasis. Histologically, it may be confused with SCC due to basaloid features but immunohistochemistry is an important tool for differential diagnosis. Small cell carcinoma usually demonstrates positive chromogranin, leu7, neuron-specific enolase, synaptophysin, and neurofilament protein.[15]

Undifferentiated Tumors

These tumors do not have the characteristics that allow them to be classified as squamous or glandular. They usually have a high mitotic index and aggressive behavior.[15]

Secondary Tumors

Metastasis in the anal canal is extremely rare. Rectal and retrorectal cancer (chordomas, chondrosarcomas, and neurogenic tumors) can extend and invade the anus.[5,15]

IMMUNOHISTOCHEMISTRY OF ANAL CANAL CANCER

SCC expresses CK5/6, CK 13/19, and p63. CK7 is positive in adenocarcinoma and generally absent in SCC but tumors with adenoid cystic pattern can be CK7+. Classic

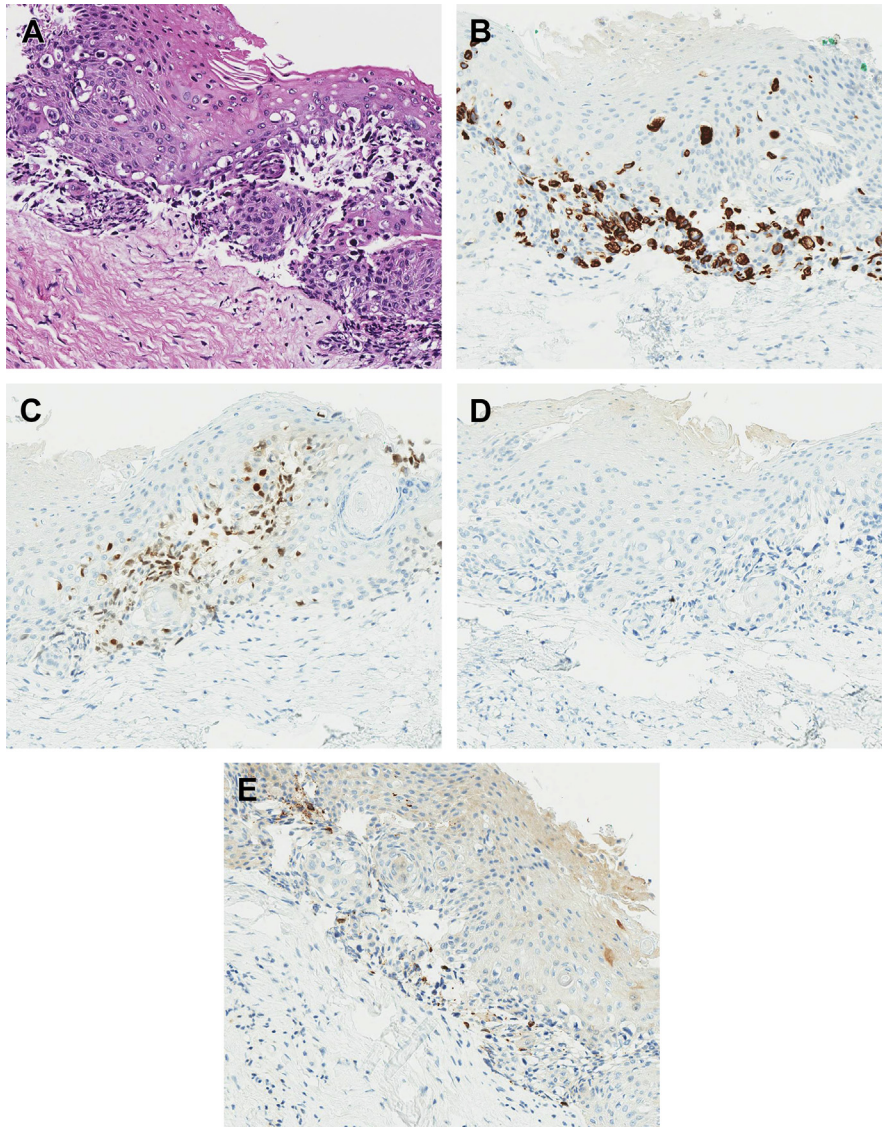

Fig. 7. Pagetoid extension of colorectal adenocarcinoma. Note the cells with clear cytoplasm within the squamous epithelium (*A*). These cells are positive for CK20 (*B*), Positive for CDX-2 (*C*), negative for GCDFP-15 (*D*), and negative for HMB45 immunostaining (*E*).

neuroendocrine markers are positive for neuroendocrine tumors, melanocytic markers for melanomas, and a panel of lymphoid markers for lymphomas. Primary perianal Paget disease is positive for CK and gross cystic disease fluid protein 15 (GCDFP15). However, when associated with a pagetoid extension of colorectal or urothelial adenocarcinoma, the pagetoid cells tend to coexpress CK20 and lack GCDFP-15 (**Fig. 7**).[23] The main differential diagnoses of anal canal tumors are summarized in **Box 1**.[5,15]

p16

In quiescent cells, pRb is active in the hypophosphorylated form. In its active form, it is able to bind and sequester E2F family transcription factors. The free form of E2F can stimulate activity of gene promoters that coordinate cell cycle transition from GAP (G)-1 to synthesis (S). In this way, active pRb is able to block cell cycle progression.[24,25]

The phosphorylation of pRb by cyclin dependent kinase (CDK) reduces its affinity for E2F factors and leads to cell cycle progression.[24,25] The CDKN2 is a tumor suppressor gene and encodes p16 protein (also called CDKN2A) that actively participates in the cell cycle, blocking activity of CDK involved in pRb phosphorylation. p16 expression is normally repressed by pRb-E2F complex but this process is inhibited by the presence of viral HPV E7 protein. Presence of p16 evaluated by immunohistochemistry is a surrogate marker for the presence of HPV genome in tumor cells in cervical and head and neck cancer.[26]

In SCC, p16 immunohistochemistry expression has shown better prognosis in retrospective series; however, these data need to be further investigated in larger prospective studies.[9,27] Besides this, p16 protein is a useful marker to confirm a diagnosis of HPV-related anal HSIL (**Fig. 8**, **Table 3**).[16]

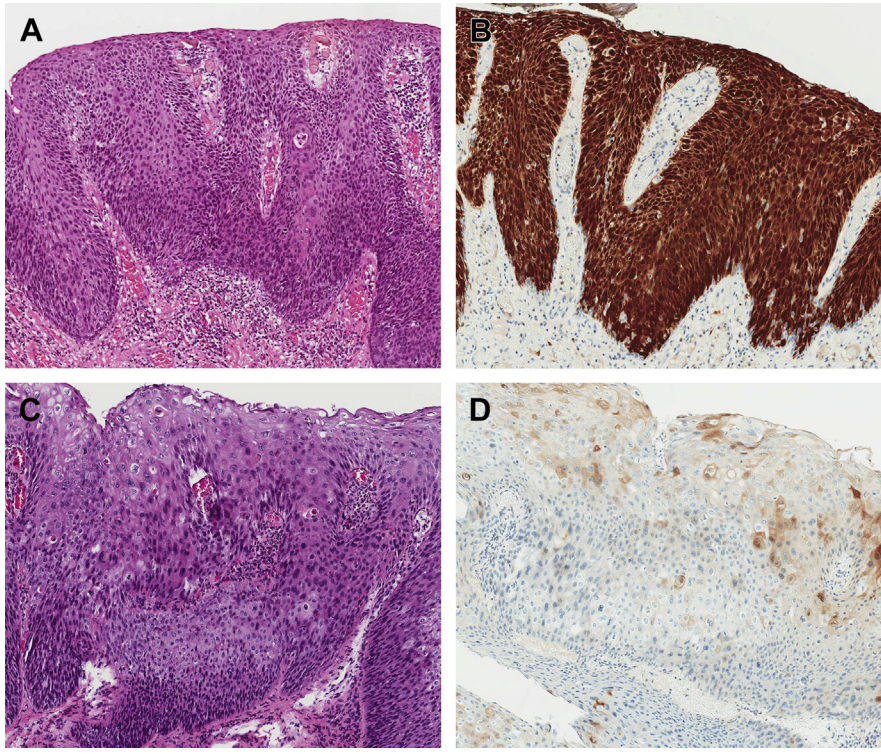

Fig. 8. p16 protein expression in intraepithelial lesions. (*A*) Hematoxylin-eosin stain (H&E) of HSIL-AIN3. Note the p16 immunostaining extending to the entire epithelial thickness (*B*). LSIL-AIN1 (*C*), note a mottled focal p16 expression (*D*), considering this case as a negative staining. This result argues against the diagnosis of HSIL (AIN 2-3).

Table 3
Immunohistochemistry of anal canal tumors

	SCC	Anal Gland Adenocarcinoma	Paget (Primary)	Paget (Secondary)	Melanoma	Neuroendocrine	Basal Cell Carcinoma
CK5/6	+	–	–	–	–	–	+
CK7/20	–/–	+/usually–	+/–	usually–/+	–	–/–	–/–
AE1/AE2	+	+	+	+	–	+	+
CAM5.2	+	+	+	+	–	+	…
GCDFP-15	–	–	+	–	–	–	–
CDX-2	–	usually–	–	+	–	–	–
CEA	–	–	+	+	–	–	–
Mucin	–	+	+	+	–	–	–
HMB45	–	–	–	–	+	–	–
MELAN-A	–	–	–	–	+	–	–
Vimentin	–	–	–	–	+	–	–
S100	–	–	–	–	+	–	–
Ber-EP4	–	+	+	+	–	–	+
p63	+	–	–	–	–	–	+
Chromogranin	–	–	–	–	–	+	–
Synaptophysin	–	–	–	–	–	+	–

Abbreviations: AE1/AE3, anti-pan cytokeratin [AE1/AE3]; Ber-EP4, anti-epithelial cell adhesion antibody [Ber-EP4]; CAM5.2, anticytokeratin [CAM5.2]; CDX2, caudal type homeobox 2; CEA, carcinoembryonic antigen; CK, cytokeratin; GCDFP-15, gross cystic disease fluid protein-15; HMB45, antimelanoma antibody (clone HMB45); MELAN-A, product of the MART-1 gene (recognized by antibody 103); p63, 63 protein; S-100, S-100 protein.

MOLECULAR FEATURES OF ANAL CANAL CANCER

There is paucity of data regarding the molecular profile of anal canal cancer due to the rarity of this disease. Genomic instability is important in cell transformation process and recognized as a risk factor for cancer development. Genetic aberrations in key genes can help tumor cells to obtain selective advantages and have demonstrated that HPV infection might increase DNA damage, endogenous mutations, and chromosome gain.

Recent studies have detected gain in chromosome 3q. In a study on 52 subjects with SCC or anal dysplasia, the gain in chromosome 3q in *PIK3CA* loci was frequent in high-grade dysplasia (53%) and cancer samples (78%) but was not seen in low-grade dysplasia or normal tissue, suggesting a possible involvement of this gene in the pathogenesis and progression of SCC.[28,29] Mutations in the *PIK3CA* gene in tumor samples are also described in retrospectives studies with variable frequency varying from 4% to 32%.[30–33] Discovered in the 1980s, the dimeric enzyme kinase lipid family, called phosphoinositide 3-kinases (PI3Ks), participates in regulating cellular functions such as survival, proliferation and differentiation.[34] Other studies reported chromosomal loses in 11q, 3p, 4p, 13q, 17p, 5p, and 18q.[35]

Mutations in the *EGFR*, *KRAS*, and *BRAF* genes are rare in SCC. Presence of *KRAS* mutations was described in 0% to 4%,[30,32,36–38] *BRAF* in 0% to 4.7%,[30,32,38] and *EGFR* in 0% to 3%.[36,37]

The prognostic role and impact of these mutations on the clinical outcome of SCC patients undergoing curative treatment are still controversial and require further investigation in prospective studies.

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