Journal of Clinical and Translational Pathology **2022** vol. 2(4) | 149–158
DOI: 10.14218/JCTP.2022.00027

Review Article

A Review and Update on Epithelial Tumors of the Anal Canal

Xiaoyan Liao*

Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY, USA

Received: October 26, 2022 | **Revised:** November 26, 2022 | **Accepted:** December 1, 2022 | **Published:** December 13, 2022

Abstract

Despite its short length, the anal canal is an anatomically and histologically complex organ that can harbor many benign and malignant conditions. The trend for anal cancer has been on the rise, affecting predominantly women. In recent years, new concepts have emerged regarding anal tumor origin, pathogenesis, classification, and molecular characterization. Particularly, the role of human papillomavirus (HPV) has been increasingly recognized for its important role in anal carcinogenesis, not just for squamous lesions, but also for non-squamous neoplasia. Understanding different mechanisms of tumorigenesis are essential for proper tumor classification, which will allow more accurate diagnosis, proper clinical management, and optimal patient outcomes. This review aims to provide an overview of the normal anatomy, histogenesis, and pathogenesis of the anal canal, as well as to update on current knowledge of epithelial tumors associated with HPV.

Citation of this article: Liao X. A Review and Update on Epithelial Tumors of the Anal Canal. J Clin Transl Pathol 2022; 2(4):149–158. doi: 10.14218/JCTP.2022.00027.

Introduction

Located at the caudal end of the gastrointestinal tract and closely associated with the genitourinary and gynecologic organ systems, the anal canal is a complex tubular structure measuring only 3.0-5.0 cm. Despite the short length, it can give rise to various tumors and tumor-like lesions.[1] Depending on different cell origins, the tumor types can be categorized as epithelial tumors including squamous cell carcinoma, neuroendocrine neoplasms, adenocarcinoma, mesenchymal tumors, melanoma, and lymphoma.[2] Malignant neoplasm in the anal canal is very rare. It is estimated that in 2022, there will be 9,440 new cases of anal cancer in the United States, and 1,670 patients will die from the disease, accounting for 0.5% of all cancer incidence and 0.3% of total cancer-related deaths, respectively.[3] Women are predominantly affected, comprising more than two-thirds of the patient population.[3] Although anal cancer is rare, the trend is rising with an average of 2.2% increase each year. Since the publication of the 5th edition of WHO tumor classification of the digestive system in 2019, new concepts have emerged regarding tumor origin, pathogenesis, classification, molecular findings, and treatment regimens in the anus. Similar to tumors of the head & neck and gynecologic tract, the role of human papillomavirus (HPV) has been well established in anal carcinogenesis, calling for a unified tumor classification scheme based on HPV status.[4] This review aims to provide a comprehensive understanding of the normal anatomy, histogenesis, and pathogenesis of the anal canal, with a focus on the current knowledge of epithelial tumors, especially those associated with HPV.

Embryology, anatomy, and histology

The embryogenesis of the anal canal is closely related to an embryonic structure called "cloaca" at the 4th week of gestation (Fig. 1a). The cloaca is the dilatation of the caudal end of the hindgut connected to the allantois (primitive urogenital sinus), a common opening through which the digestive, urinary and reproductive systems meet and exit. There is also a tailgut at this stage. At the 5th week, the tailgut regresses, the urogenital sinus separates to form its opening, and the outer body wall folds for the ectoderm to form an anal pit roofed by a membrane that ruptures at the 7th week to establish continuity between the upper and lower anal canal, so that the upper 2/3 is of endoderm origin, while the lower 1/3 is of ectoderm origin.

Anatomically, the so-called "surgical anal canal" starts at the apex of the anal sphincter complex, or anorectal ring, and ends at the anal margin or anal verge (Fig. 1b). The anorectal ring is a palpable landmark during digital rectal exams, and it is very important to preserve this structure during surgery for fecal continence. The anal verge, also called "anus" or "perianus", coincides with the palpable intersphincteric groove. The anal margin refers to the transition from the squamous mucosa to the perianal skin, which is defined as being within 5 cm of the anal verge. Unlike the non-keratinizing anal squamous mucosa, the perianal skin is hair-bearing and keratinizing squamous epithelium vested with epidermal appendages (hair follicles, apocrine and sweat glands). The squamous anus in between the dentate line and the anal margin is distinct from the rectal mucosa grossly and thus referred to as an "anatomic anus".

Histologically, the anal canal is composed of 3 zones with

Keywords: Anal canal; Human papillomavirus; Dysplasia; Squamous cell carcinoma; Neuroendocrine carcinoma; Anal adenocarcinoma.

Abbreviations: AIN, anal intraepithelial neoplasia; BLT, Buschke-Löwenstein tumor; HIV, human immunodeficiency virus; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; ICP, inflammatory cloacogenic polyp; LSIL, low-grade squamous intraepithelial lesion; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; SRUS, solitary rectal ulcer syndrome; SqCC, squamous cell carcinoma; VC, Verrucous carcinoma.

***Correspondence to**: Xiaoyan Liao, Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Ave, Box 626, Rochester, New York 14642, United States. ORCID: https://orcid.org/0000-0002-5052-0632. Tel: +1 585-276-7156, Fax: +1 585-273-3637, E-mail: xiaoyan_liao@urmc.rochester.edu

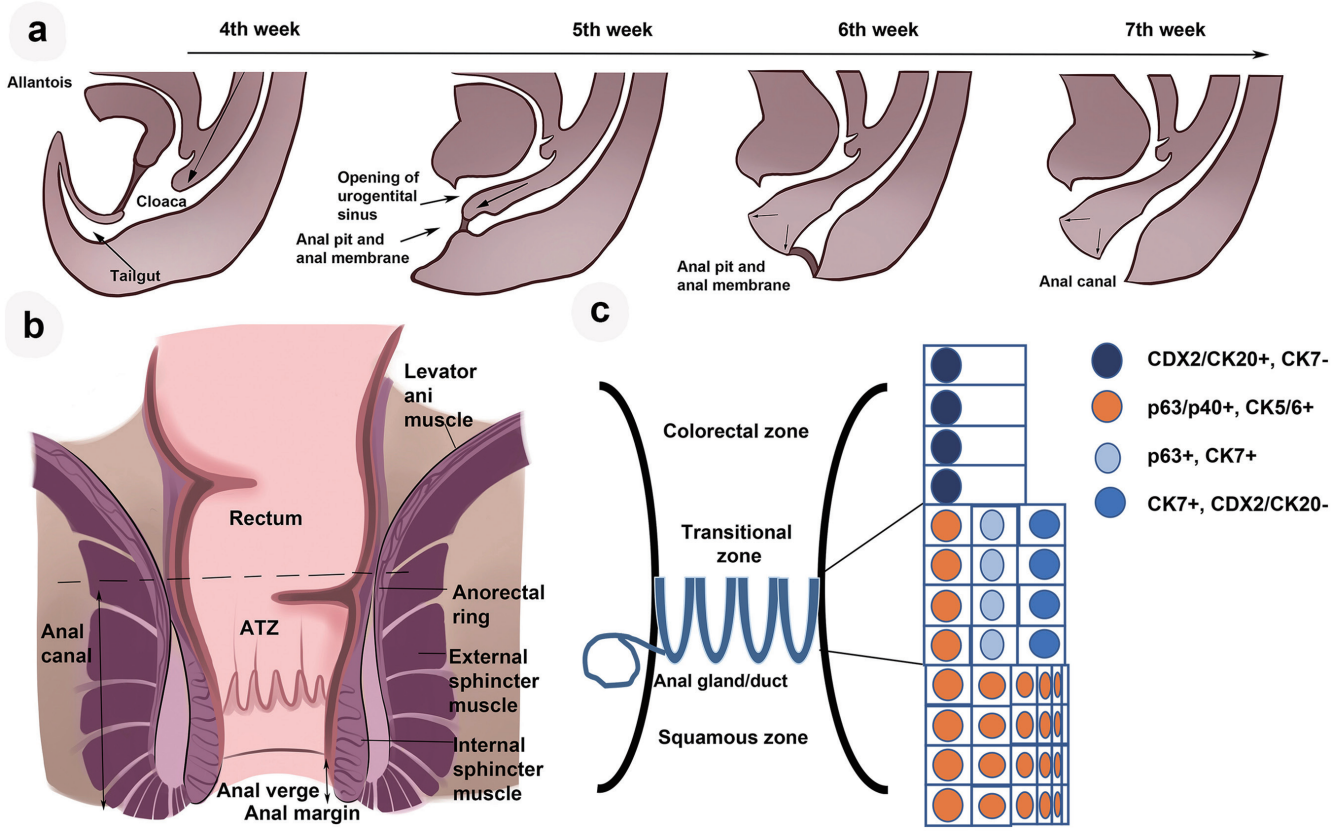

Fig. 1. Embryogenesis, normal anatomy, and histology of anal canal. (a) The development of the anal canal is originated from an embryologic structure called cloaca at the 4th week of gestation. In the 5th week, the urogenital sinus separates to form its opening and the ectoderm invaginates to form the anal pit, which is roofed by the anal membrane in the 6th week. In the 7th week, the anal membrane ruptures to establish continuity between the upper and lower anal canal. (b) Anatomically, the surgical anal canal starts at the apex of the anal sphincter complex, a palpable landmark during a digital rectal exam, and ends at the anal margin or anal verge (Digital reproduction from uptodate: https://www.uptodate.com/contents/image?imageKey=ONC%2F62539&source=graphics_gallery&topicKey=3730). (c) Histologically, the anal canal is composed of 3 zones with 3 types of epithelial cells: the colorectal zone covered by colonic epithelium (CK7-, CK20+, CDX2+), the transition zone and anal gland (duct) covered by a multilayered transitional epithelium (CK7+, CK20-, CDX2-), and the squamous zone covered by squamous epithelium (p40+, p63+, CK5/6+).

3 types of the epithelium (Fig. 1c).[5,6] The colorectal zone is covered by colonic epithelium with an immunophenotype of CK7(−), CK20(+), and CDX2(+). The transition zone is covered by a multilayered epithelium composed of basal, parabasal, and epithelial cells.[6] The basal and parabasal cells are positive for squamous markers CK5/6 and p63, while the epithelial cells are positive for CK7 and CK19, but negative for CK20 and CDX2. The squamous zone is covered by squamous epithelium immunoreactive to p40, p63, and CK5/6. The anal glands reside in the wall of the anal canal and secrete via the anal ducts into the anal crypts along the dentate line. Both anal glands and ducts share similar histomorphology to the transitional epithelium with the same immunophenotype, which is important to keep in mind when dealing with anal adenocarcinoma.[5]

Benign tumor-like conditions of the anal canal

The most common benign lesion of the anal canal is hemorrhoid, which can occur either distal (external) or proximal (internal) to the dentate line. Hemorrhoids are caused by elevated abdominal pressure resulting in an abnormal dilation of the venous plexus. Histology shows engorged, thick-walled vessels with or without thrombosis (Fig. 2a). Fibroepithelial polyp (FEP), the second most common benign acquired lesion in the anal canal, is a polypoid hypertrophied anal papilla similar to a skin tag. FEP is characterized by loose fibrous stroma containing thin-walled capillary vessels (Fig. 2b). While both hemorrhoid and FEP are benign reactive conditions, they can co-exist with other disease processes, such as squamous dysplasia,[7] and many malignant lesions may misguide clinically as hemorrhoid.[8]

Inflammatory cloacogenic polyp (ICP) and solitary rectal ulcer syndrome (SRUS) are similar lesions in different locations.[9,10] ICP arises in the anal transitional zone with associated squamous or transitional epithelium, while SRUS occurs in the rectum not associated with squamous or transitional epithelium. Both lesions are caused by trauma or abnormal muscle contraction resulting in prolapse. Patients are those middle-aged presenting with rectal bleeding, diarrhea, pain, or cramps. Endoscopy shows ulcerated and indurated areas on the anterior or anterolateral wall of the distal rectum or anal canal. Histology shows the upward extension of the hypertrophic/splayed muscularis mucosae obliterating the lamina propria (prolapse) and downward displacement of hyperplastic irregular crypts into the submucosa or muscular propria (proctitis cystica profunda). The surface can show villous hyperplasia and is often eroded and covered with inflammatory exudate (Fig. 2c–d). ICP/SRUS can mimic tubulovillous adenoma or malignancy, causing diagnostic chal-

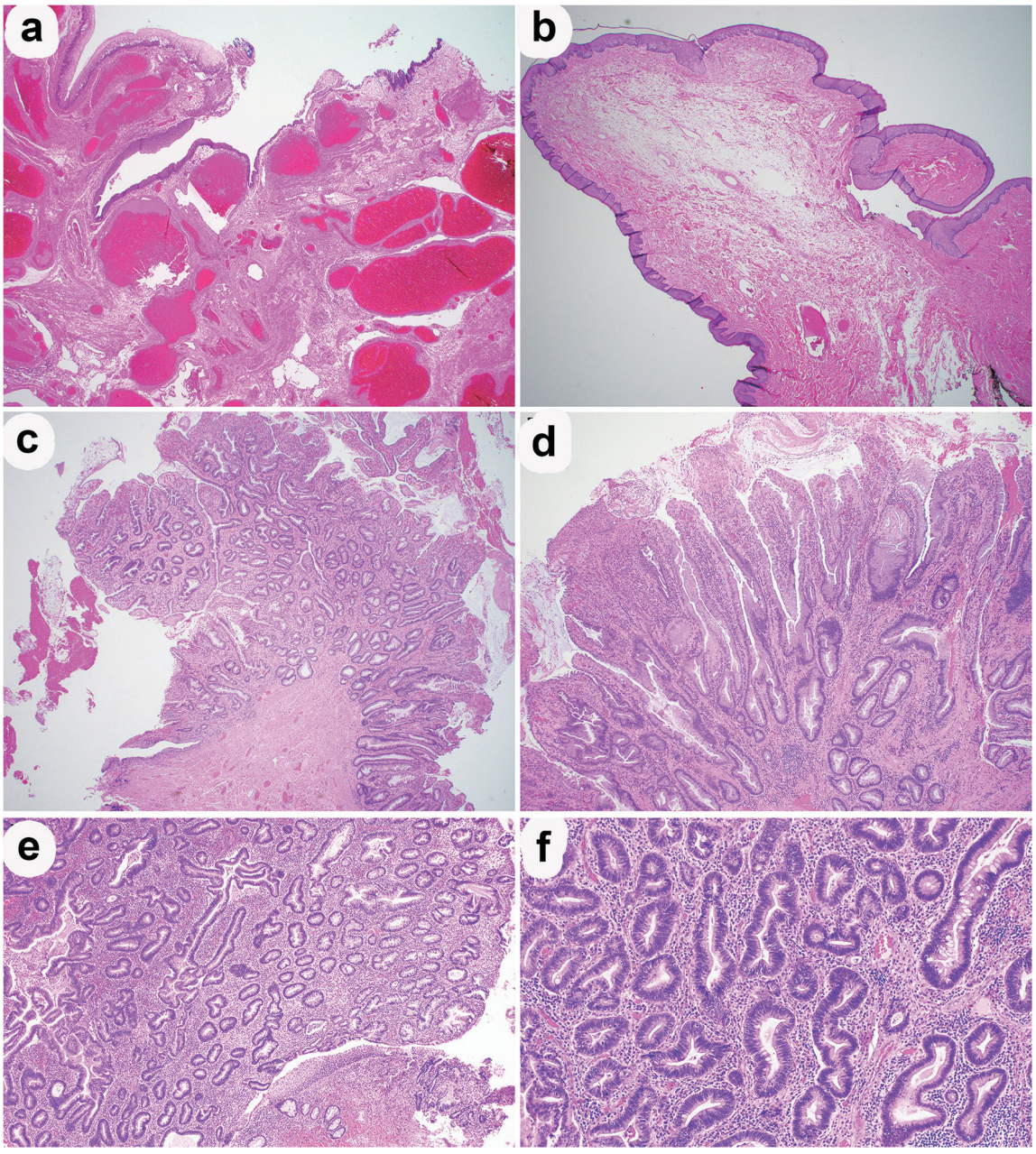

Fig. 2. Benign tumor-like conditions in the anal canal. (a) Hemorrhoid. (b) Fibroepithelial polyp. (c-d) Inflammatory cloacogenic polyp in comparison to a well-differentiated adenocarcinoma (e-f). Magnification: a-c, e: 40×. d, f: 200×

lenges. The key to avoiding over-diagnosis is to recognize surface maturation, mucin depletion, small uniform nuclei, evenly spaced glands with lobulated architecture, and background prolapse-related changes. Occasionally, a well-differentiated adenocarcinoma at the anal canal may also mimic ICP (Fig. 2e–f).

HPV-associated anal squamous dysplasia and precancerous lesions

Human papillomavirus (HPV) is etiologically linked to a variety of benign and malignant epithelial lesions. Condyloma acuminatum is the most common HPV-associated low-grade benign squamous neoplasm, usually caused by low-risk (LR) serotypes HPV 6 and 11, but also high-risk (HR) serotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), or a mixture of more than 1 serotypes.[11–13] Histologically, condyloma acuminatum is characterized by papillary proliferation of hyperkeratotic squamous cells with koilocytic changes and wrinkled nuclei described as "resinoid". Dysplasia is usually mild and confined to the lower 1/3 epithelium. Occasionally, old condylomata may show little to no koilocytosis and minimal dysplasia, which can be difficult to distinguish from a papilloma or sebaceous keratosis (Fig. 3a).

The giant condyloma acuminatum, also called Buschke-Löwenstein tumor (BLT), is a variant of condyloma acumi-

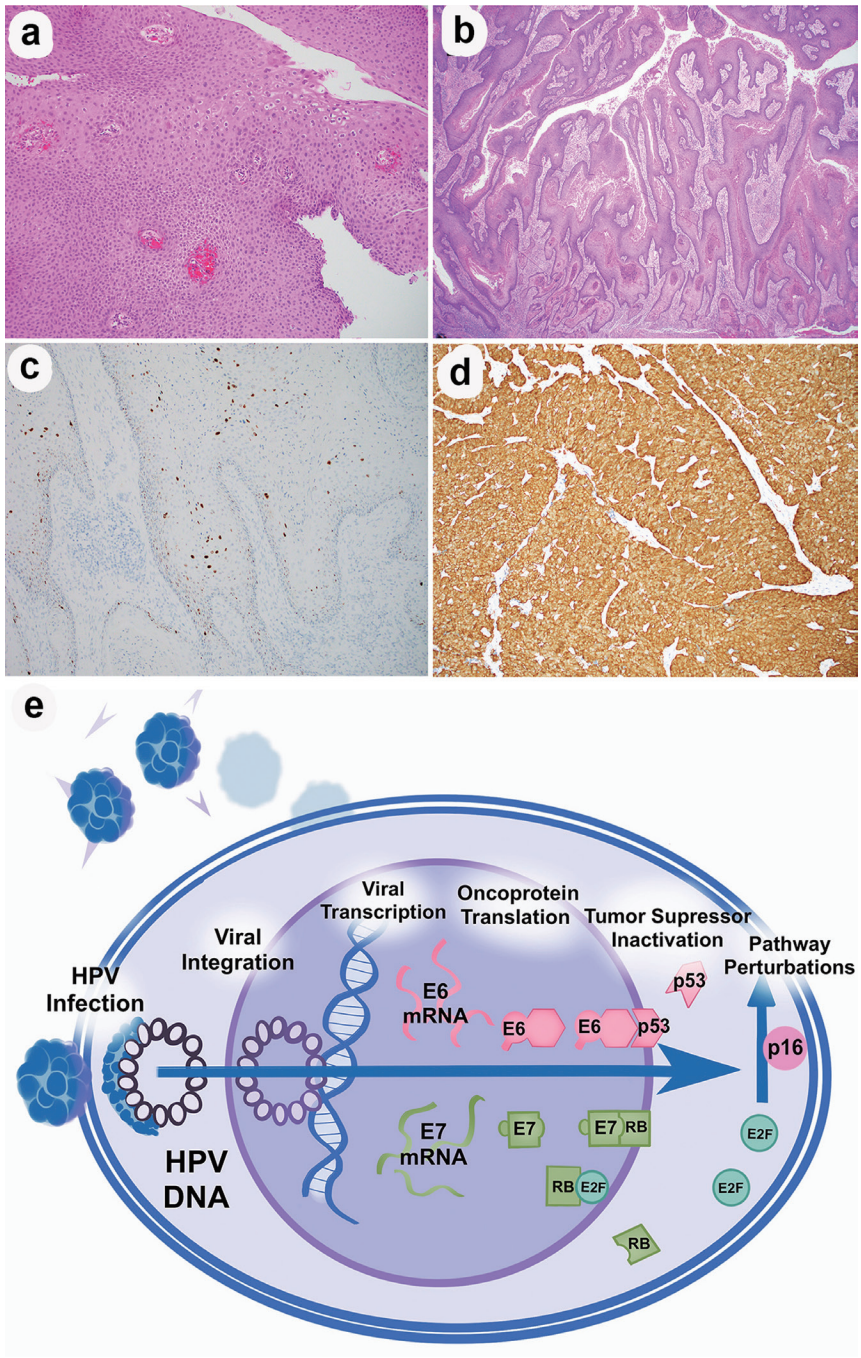

Fig. 3. Condyloma and HPV-related signaling pathway. (a) A near burnt-out condyloma with rare koilocytes. (b) A giant condyloma acuminatum with invasion. This case is positive for High-risk HPV by DNA in-situ hybridization (c) and diffuse, continuous, strong, cytoplasmic, and nuclear staining of p16 (d) resulting in a "block" pattern of positivity. (e) A schematic illustration of HPV carcinogenesis (reproduced with permission of Dr.William Westra). In HPV-infected cells, the viral oncoproteins E6 target p53, and E7 target pRb for ubiquitination. pRb normally binds transcription factors E2F, preventing its activation of cell proliferation. E7 releases E2F from pRb, which upregulates p16 as a regulatory feedback attempt to inhibit further cell proliferation. Magnification: a, b: 40×. c, d: 100×

natum first described as a penile neoplasm by Buschke and Löwenstein in 1925.[2] BLT is defined as a large (≥5 cm), exophytic, HPV-induced squamous proliferation that is broadly destructive (Fig. 3b). Zhang *et al* published the largest series of BLT in 2020 describing the clinicopathologic features of 38 BLTs collected from multiple medical centers, where half of the cases showed invasion.[14] The invasive BLTs are frequently positive for HR HPV, and histologically demonstrate dyskeratosis, neutrophilic microabscess, Keratin cyst, and abnormal mitotic figures. The prognosis is relatively good with rare cases of lymphovascular invasion, perineural invasion, or lymph node metastasis. Only one patient died of disease progression.[14] The differential diagnosis for BLT is verrucous carcinoma, an extremely well-differentiated verruciform squamous

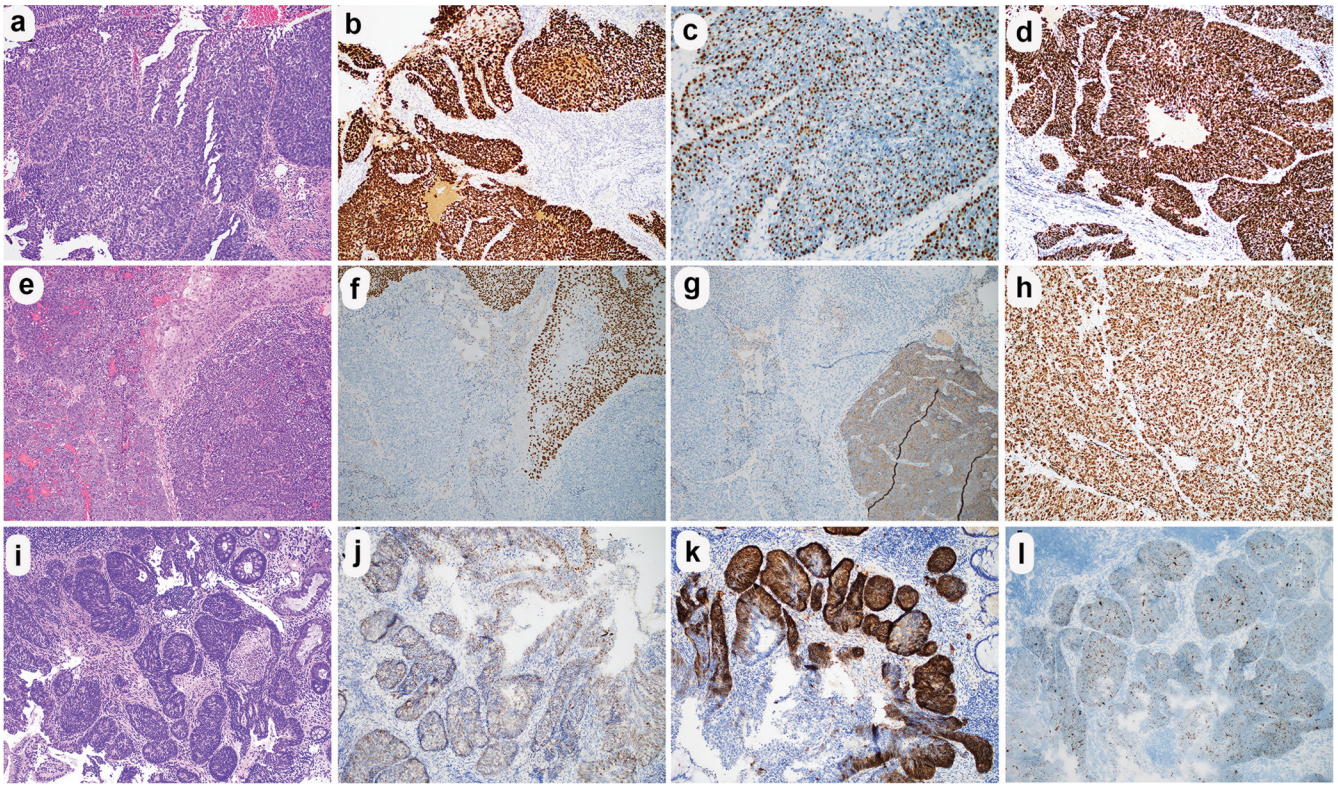

Fig. 4. Comparisons between anal squamous cell carcinoma (SqCC) and neuroendocrine carcinoma (NEC). (a-d) A basaloid SqCC is characterized by marked nuclear hyperchromasia, scant cytoplasm, and peripheral palisading. The tumor cells are diffusely positive for p40 (b), HR HPV (c), with >90% Ki67 labeling index (d). (e-g) An example of NEC with basaloid morphology (e) and mixed squamous dysplasia on the surface is shown by p63 expression (f). There is squamous carcinoma in-situ elsewhere (not shown). (g) The invasive component is mixed with an NEC component on the right that is diffusely positive for synaptophysin, and a poorly differentiated carcinoma on the left that is negative for both squamous and NE markers. (h) HR HPV is diffusely positive. (i-k) A hybrid tumor intermediate between neuroendocrine and squamous differentiation. The tumor is basaloid by morphology (i). It is weakly positive for p63 (j), but negative for p40 and CK5/6 (not shown), diffusely positive for CD56 (k), but negative for synaptophysin and chromogranin (not shown). HR HPV is diffusely positive (l). Magnification: a, e, i: 40×. b, d, f-h, j-l: 100×

cell carcinoma (SqCC) characterized by bulbous deep borders but a lack of koilocytosis. Historically, VC was considered in the spectrum of BLT/giant condyloma, but it is now recognized as a different entity unrelated to HPV.[2,15,16]

Besides condyloma, HPV also causes flat anal dysplasia or anal intraepithelial neoplasia (AIN), which can be graded as mild (AIN1), moderate (AIN2), severe (AIN3) based on the thickness of dysplasia, cytological atypia, squamous maturation, and mitotic activity. The terminology for AIN is now unified using the two-tiered grading system of Lower Anogenital Squamous Terminology (LAST 2013),[17] where low-grade squamous intraepithelial lesion (LSIL) includes AIN1 and condyloma, while high-grade squamous intraepithelial lesion (HSIL) refers to AIN2 and AIN3. The anal canal is the usual location for AIN and carcinoma in-situ, but those can also occur in the perianal skin. Multicentricity is not uncommon as more than 10% of women with anal HPV also had cervical HPV.[18]

Grading anal dysplasia is usually straightforward by histology alone. In challenging cases, immunohistochemistry for p16 may be useful in aiding the diagnosis. p16 is a tumor suppressor protein overexpressed in HR HPV-associated cancer and precursor lesions as a regulatory feedback attempt to inhibit HPV-induced cell proliferation (Fig. 3c–e).[19] p16 overexpression is strictly defined as continuous, strong, cytoplasmic and nuclear staining in the abnormal cells, resulting in a "block" pattern of positivity (Fig. 3d). The surrogate use of p16 has been well-studied in cervical biopsies, where it is recommended to help to distinguish HSIL from mimickers, AIN1 from AIN2, or resolving professional disagreements in interpretation particularly if the differential diagnosis involves HSIL.[17] The use of p16 immunostain in anal lesions is not clear. Liu and colleagues evaluated p16 immunoreactivity in 1,000 anal biopsies and found high sensitivity of p16 block positivity in diagnosing AIN2 (89%) and AIN 3 (95%), but the specificity is relatively low as 37% LSIL/AIN1 exhibited block staining.[20] Nevertheless, morphology is still the key to grading dysplasia. In our practice, we rarely rely on p16 immunohistochemistry in anal biopsies. Challenging cases may be presented at consensus conferences which have been proven a helpful approach to settling interobserver disagreements.[21]

Anal squamous cell carcinoma (SqCC)

Similar to the gynecologic tract where HPV is associated with carcinomas of the cervix and endocervix but not the endometrium,[22] the anal canal is particularly prone to HPV infection and developing malignancy. SqCC is the most common type of carcinoma in the anal canal, arising either above or below the dentate line.[2] It is mostly HPV-associated (>90%), often HPV 16, which underlies 90% of cases.[4,23,24] HIV-infected population is at particular risk for those lesions.[4] Histologically, HPV-driven SqCC frequently shows basaloid morphology (Fig. 4a–d), similar to those in oropharyngeal SqCC.[2]

In contrast, HPV-negative SqCC is rare, comprising <10% of anal SqCC. They occur in the context of long-standing chronic inflammation such as Crohn's disease or fistula and often show heterogeneous morphology with mixed keratinizing and non-keratinizing squamous cells.[25]

The current WHO tumor classification does not subclassify anal SqCC based on HPV status, assuming that they are often HPV dependent. WHO also does not recommend to use of the term "basaloid variant", given that morphology can be heterogenous and bears unknown significance.[2] Graham *et al* in 2016 revisited 37 basaloid SqCC diagnosed between 1994–2014. After 10 of those cases were reclassified either to basal cell carcinoma, melanoma, or neuroendocrine carcinoma, the remaining 27 basaloid SqCC were female predominant, and all HPV positive.[26] The authors also described 4 histology patterns of basaloid SqCC, the transitional cell carcinoma-like, basaloid with peripheral palisading, the adenoid cystic carcinoma-like, and the mucinous microcystic with mixed mucin-producing cells, which help to recognize the lesion but do not have any prognostic significance or therapeutic implication.

Because HPV status is important to subclassify SqCC of head&neck and gynecologic tract, it has been advocated to use a unified diagnostic terminology and nomenclature for SqCC of the anal canal. Zhu *et al*. compared a total of 96 HPV-positive and 20 HPV-negative anal SqCC and found that anal SqCC can be subclassified based on HPV and *TP53* mutation status, where the HPV positive/*TP53* wild-type SqCC were associated with better survival than HPV-negative/*TP53* mutant SqCC, while the HPV-positive/*TP53* mutant and HPV-negative/TP53 wild-type SqCC fell in between. The authors strongly recommended testing HPV and *TP53* status for all anal SqCC.[25] Similarly, a European study at the same time analyzed a total of 372 anal SqCC in HIV-negative patients (mean age: 63.4, F:M = 2.51). After standard treatment, 25.5% had disease progression, persistent disease, or recurrence, and 27.4% died of disease. Negative HPV/p16 status and aberrant p53 expression even predicted patient survival better than tumor size and nodal status. They also found that Cisplatin-based chemoradiotherapy was superior in HPV-positive SqCC than radiotherapy alone and other concurrent chemoradiation therapies,[27] an important finding that needs to be validated in future studies.

Neuroendocrine neoplasms (NEN) of the anal canal

The 2019 World Health Organization (WHO) classification of digestive system tumors has made substantial changes regarding the nomenclature and grading schemes for all neuroendocrine neoplasms, including the well-differentiated neuroendocrine tumors (NET) and the poorly differentiated neuroendocrine carcinoma (PD NEC).[2] Previously, NET was graded into G1 (<2 mitoses/2mm^2 and Ki-67 <3%) and G2 (2-20 mitoses/2mm^2 and Ki-67 3–20%), but now also G3, which is well-differentiated by morphology but has high mitosis (>20 mitoses/2mm^2) or Ki-67 (>20%) equivalent to NEC. PD NEC by definition is a high-grade malignancy with a solid growth pattern, abundant mitoses (>20 mitoses/2mm^2 or Ki-67 >20%), and frequent necrosis, morphologically classified as small cell or large cell types. Previously termed mixed adenoneuroendocrine carcinoma (MANEC) is now renamed as mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) to incorporate those where the non-neuroendocrine component is not adenocarcinoma, or the neuroendocrine component is a low-grade NET. For MiNEN, each component must account for >30% of the whole neoplasm. However, a minor (i.e., <30%) NEC component should be mentioned. This diagnosis must exclude non-neuroendocrine neoplasms with a scattered expression of neuroendocrine markers with no identifiable neuroendocrine cell morphology.

The gastrointestinal tract harbors many neuroendocrine cells residing in the colorectal-type mucosa. For NET, the small intestine is the most common localization by frequency followed by the rectum.[28] Some reported that the incidence of rectal NET is approximately 0.17% during a screening colonoscopy, representing 12–27% of all NETs and 20% of gastrointestinal NETs.[29] Rectal NETs are often diagnosed incidentally as polyps, almost exclusively G1, and generally have an excellent prognosis.[30,31] Review of our institutional archived pathology database identified 101 patients with rectal NETs and only 1 anal NET in the past 10 years (unpublished data). Little is known about NET tumorigenesis, but at least for now, there are no studies to show an etiologic link to HPV infection.

Although both NET and NEC share the expression of neuroendocrine markers (synaptophysin, chromogranin, CD56, and INSM1), they appear to originate from completely different pathways. A handful of earlier studies showed that similar to SqCC, small cell NECs of the anus are almost exclusively driven by HR HPV with a striking female predominance.[32,33] A preliminary study at our institution showed similar findings (unpublished data). Morphologically, NECs are very similar to basaloid SqCC and sometimes only immunohistochemistry can reliably make the distinction. Most of the anorectal NECs can be classified as small cells or large cell NEC, but they often show heterogeneous morphology with mixed small and large cells. Like SqCC, anal NECs often co-exist with squamous dysplasia, mix with a component of basaloid SqCC, or a poorly differentiated carcinoma that does not express any neuroendocrine or squamous cell markers (Fig. 4e–h). When the non-neuroendocrine component exceeds >30% of the tumor volume, a diagnosis of MiNEN may be more appropriate, although even a small component of PD NEC will likely drive the poor clinical course in those patients. Occasionally, a hybrid tumor intermediate between neuroendocrine and squamous differentiation may also be encountered (Fig. 4i–l), which may be designated as "amphicrine" although the term is not adopted by the current WHO tumor classification. Treatment of anal NEC may follow the same cisplatin-based chemotherapy regimen plus radiation as for its pulmonary counterpart. Unfortunately, there is a frequent lymph node and distant metastasis at the time of diagnosis; even after aggressive treatments, the prognosis is dismal with a 5-year survival rate <20% (Table 1).[33–35]

Anal adenocarcinoma

The etiologic role of HPV in anal adenocarcinoma has not been well understood until recently.[4,36] Anal adenocarcinoma is very rare, comprising <1% of all anal cancer. The current WHO tumor classification described 2 subtypes, those originating from the mucosa of intestinal type, and those extra mucosal which may be associated with an anal gland (duct), a pre-existing anal fistula, or other non-fistulating glandular structures in the anal canal (acquired or congenital malformations or embryological remnants).[2] They can also be classified as primary or secondary if the tumor undergoes metastasis or direct extension from adjacent organs, such as the rectum, prostate, urinary bladder, and gynecologic tract. Immunohistochemically, those of intestinal origin are CK7(−), CK20(+), and CDX2(+), while those of anal gland (duct) origin are CK7(+), CK20(−), and CDX2(−). Primary anal gland (duct) carcinoma is extremely rare. The largest case series published so far included 5 cases of anal gland

Table 1. Comparisons between anal squamous cell carcinoma (SqCC) and poorly differentiated neuroendocrine carcinoma (PD NEC)

Features	SqCC	PD NEC
Prevalence	Rare, 0.2-1.7/100,000	Very rare, <1/100,000
Epidemiology	Female predominant	Female predominant
Precursor	Squamous dysplasia or carcinoma in-situ	Squamous dysplasia or carcinoma in-situ
HPV	Frequently HR HPV positive	Frequently HR HPV positive
Sub-classification	Morphology: not recommended; HPV: dependent or independent; p53: Normal or aberrant	Morphology: small cell, large cell, MiNEN, amphicrine(?); HPV (?): dependent or independent
Histology	Basaloid, palisading, vesicular chromatin mixed keratinizing and/or non-keratinizing; Variable mitosis or Ki67	Basaloid, nuclear molding, fine chromatin; Can be pure or mixed with SqCC; High mitosis or Ki67
Immunohistochemistry	At least one squamous marker strongly positive (p40, CK5/6, p63)	At least two NEC markers (Synaptophysin, chromogranin, CD56, INSM1) strongly positive
Prognosis	Stage dependent: 5-year survival rate ~65%	Generally poor: 5-year survival rate ~13%

(duct) carcinomas, all CK7(+), CK20(−), p16(−), and HPV-unrelated.[37]

Despite the earlier studies not relating anal adenocarcinoma to HPV infection, more and more evidence emerge to support the role of HPV in anal canal carcinogenesis. Notably, Herfs *et al*.[38] studied 74 primary anal canal adenocarcinomas and proposed a dualistic model where they could be classified into two types, those with an immunophenotype of the anal gland/transitional epithelium and those of colorectal epithelium. In this largest case study, 11 of 26 of those anal gland/transitional types were positive for HPV, while none were positive for HPV in colorectal-type adenocarcinoma. The differences in etiology led to distinct immune profiles, mutational landscapes, and prognosis, supporting the use of HPV status as a classification scheme for anal adenocarcinoma.

Following this seminal study on the role of HPV in anal adenocarcinoma, the John Hopkins group described a novel group of HPV-related adenocarcinoma in the lower anogenital tract, including 3 from the anal canal. This unique subtype of adenocarcinoma is morphologically characterized by papillary, villiform, and villoglandular architecture, resembling HPV-related endocervical adenocarcinoma.[39] Interestingly, we recently also reported a similar case in a 71-year-old female, who developed frequent local recurrence and perianal Paget's disease.[40] In addition to the adenocarcinoma component showing similar papillary and villiform/villograndular architecture, there is also squamous differentiation that would technically qualify as an adenosquamous carcinoma (Fig. 5). Judged by location, morphology, and immunophenotype (CK7+, CK20−, CDX2+/−), we concluded that the main tumor likely originated from the transitional epithelium/anal gland (duct). Yet, beyond that, HR HPV was also detected in the adjacent squamous mucosa with HSIL and colorectal epithelium with dysplasia (Fig 5g–h), the latter accidentally proves that the colorectal epithelium can also be infected by HPV leading to carcinogenesis, despite controversial opinions in earlier studies.[41–44]

The finding of an HPV-related anal adenocarcinoma resembling HPV-relate endocervical adenocarcinoma calls again for a unified classification scheme for the lower anogenital tract. This is not the first time that HPV-related anal adenocarcinoma shares similarities to those in the gynecological tract. It has been recognized that stratified mucin-producing intraepithelial lesions (SMILE), a well-known entity with overlapping features of HSIL and adenocarcinoma in-situ (AIS) described in the cervix,[45] can also occur in the anal canal, particularly at the transitional zone.[46] Similar to the gynecologic tract, invasive SMILE has also been reported in the anal canal in published case reports,[46,47] further supporting the hypothesis that like the cervical transitional zone, the transitional epithelium also serves as the progenitor cells for HPV-related adenocarcinoma in the anal canal.[38]

Based on the new data and my observations, a much-simplified tumor classification for anal adenocarcinoma is proposed (Fig. 6): the colorectal type, and the transitional type. The colorectal type can arise from the distal rectum (exophytic), entrapped rectal mucosa in a fistula (endophytic), and likely HPV unrelated (except on rare occasions). The transitional type includes those from the transitional zone and anal gland (duct) and can be further classified as HPV-independent and HPV-dependent. The HPV-dependent may be exophytic and morphologically similar to a usual type endocervical adenocarcinoma, an adenosquamous carcinoma, or invasive mucin-producing carcinoma. In contrast, HPV-independent anal gland (duct) carcinoma is more likely to be endophytic without surface involvement. All adenocarcinomas can be mucinous or non-mucinous, and cause perianal Paget's disease.

Perianal Paget's disease

Paget's disease refers to a malignant neoplasm with an intraepithelial location. It was named after Sir James Paget, an English surgeon and surgical pathologist who first described the inflammatory cancerous condition around the nipple in 1874. The first case of extramammary Paget's disease (EMPD) affecting the penis and scrotum was described by Radcliffe Crocker in 1889. Clinically, it presents as an erythematous, ulcerated lesion of eczematoid appearance. Two types of EMPD were described, the primary type is thought to originate from the apocrine glands of perianal skin and is exclusively intraepithelial, while the secondary type is often associated with an underlying invasive carcinoma. Local recurrence is very common (multifocal, ill-defined), and the prognosis is dependent on the presence/absence of an invasive component and if there is regional lymph node metastasis. The perianal Paget's disease (PPD) is often secondary to an invasive colorectal type adenocarcinoma, in which a combination of CDX2 and GCDFP-15 can help distinguish primary vs. secondary PPD.[14] It is worth mentioning that PPD can be associated with any type of epithelial tumor, including NEC,[48]

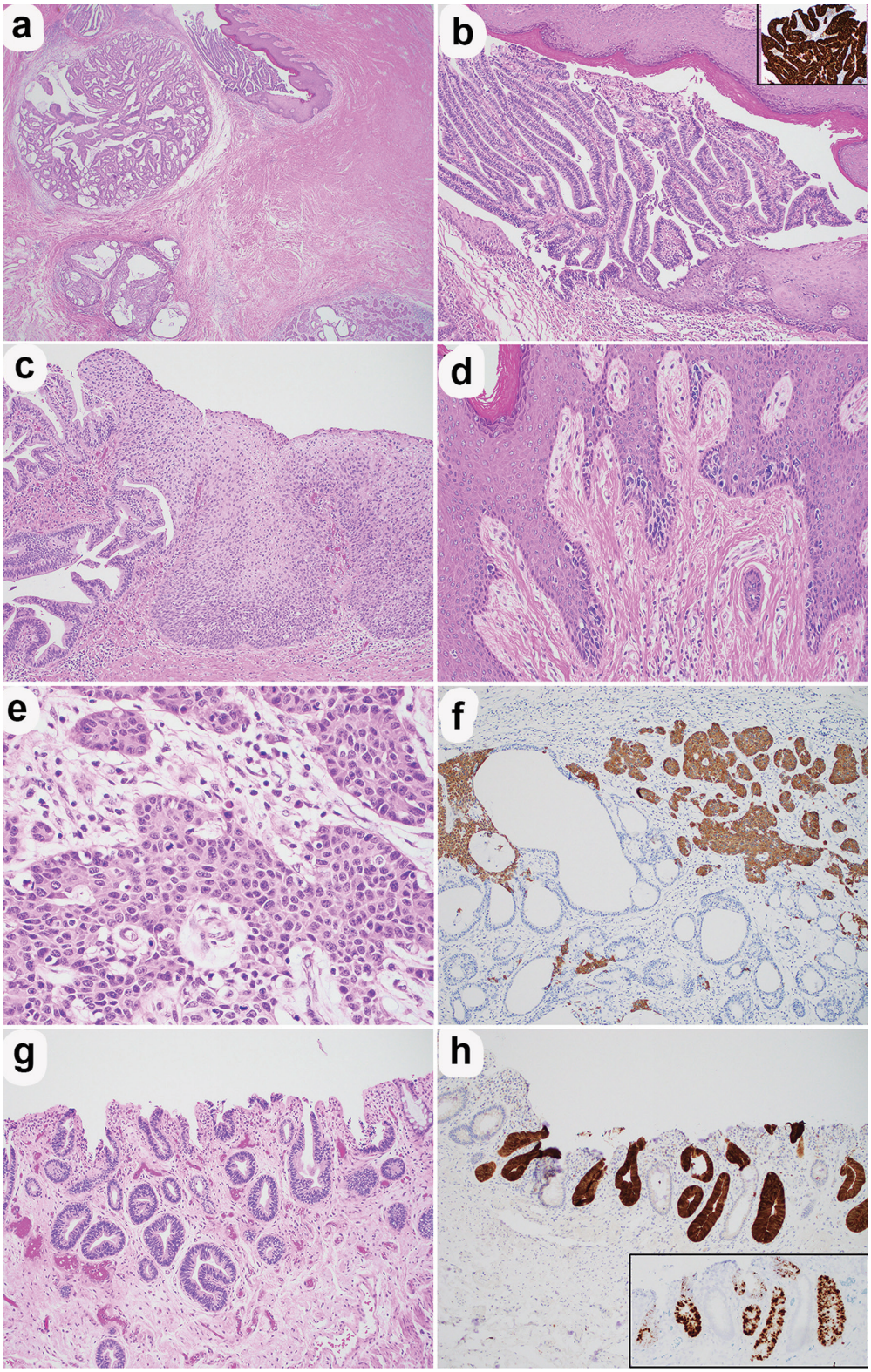

Fig. 5. A unique case of HPV-associated adenocarcinoma in the anal canal. (a) Low power magnification shows the lesion starts at the transitional zone with an exophytic villoglandular configuration resembling endocervical adenocarcinoma (b). The entire lesion is diffusely CK7 positive (inlet in b) and showing downward invasion likely involving the anal gland (duct). The adjacent squamous cell harbors high-grade dysplasia (c) with Pagetoid spreading (d). The invasive carcinoma shows focal squamous differentiation with solid growth (e) and diffuse CK5 expression (f). The lesion also involves the adjacent colonic mucosa with dysplastic changes (g), confirmed by diffuse strong p16 expression (h) and HR HPV in-situ hybridization (inlet in h). Magnification: a, 20×, b-d, f-h: 100×, e: 200×

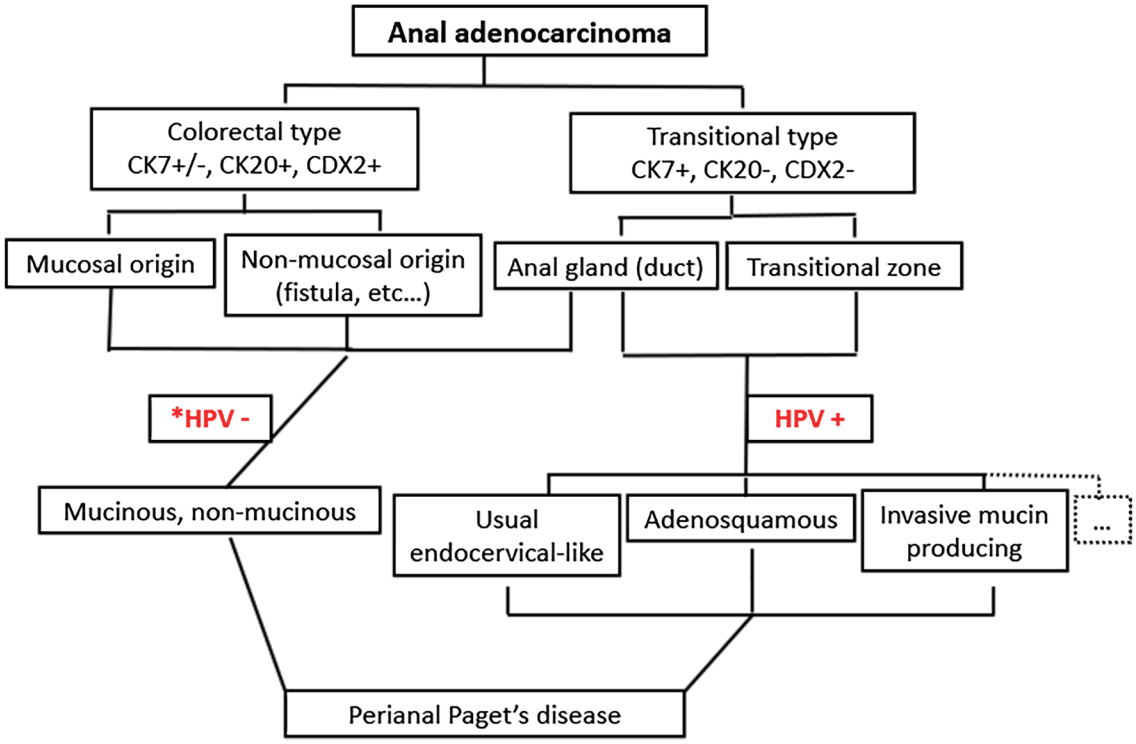

Fig. 6. A proposed classification scheme for anal adenocarcinoma based on cell origin, HPV status, location, and immune profiles. The colorectal type can be of the mucosal origin or non-mucosal origin such as a fistula. The non-colorectal type may originate from either the anal gland (duct) or transitional zone. The HPV+ adenocarcinoma may be further subtyped based on morphology, including the usual endocervical-like adenocarcinoma, adenosquamous carcinoma, or invasive mucin-producing carcinoma. All anal adenocarcinomas can have variable mucin production and can be associated with perianal Paget's disease. *Note: it is believed that most colorectal type adenocarcinomas are HPV independent, but some may be HPV driven as evidenced in Fig. 5.

or even a non-invasive tubular adenoma with HGD,[14] so it is important to recognize PPD when dealing with neoplasms in the anal canal.

Conclusions

Neoplasms of the anal canal are rare, which makes accurate diagnosis a challenge in clinical practice. As a pathologist, knowing the basic anatomy and histology is important to understand the pathogenesis and refine more accurate tumor classification. In the past decades, it has been increasingly understood that HPV plays a key role in carcinogenesis not only for tumors of the head&neck and uterine cervix but also for the anal canal. The malignant tumor types associated with HPV are not limited to SqCC, but also NEC and adenocarcinoma. Therefore, a unified classification scheme based on HPV infection appears to be justified. As reinforced by studies published by other people and the author's personal experience, a tumor classification system based on HPV infection and molecular characterization such as *TP53* mutation status does have clinical and therapeutic significance, which will ultimately improve risk stratification and optimal patient management. After all, the key to achieving an accurate and meaningful diagnosis is the integration of morphologic, immunohistochemical, and molecular findings that can translate into meaningful clinical management.

Acknowledgments

The author thanks Ms. Rena X. Li (Mendon High School) for providing artistic assistance for Figures 1 and 3.

Funding

None.

Conflict of interest

The author has no conflicts of interest related to this publication.

Author contributions

XL is the sole author of this article.

References

[1] Shia J. An update on tumors of the anal canal. Arch Pathol Lab Med 2010; 134(11):1601–1611. doi:10.5858/2009-0668-RAR.1, PMID:21043813.
[2] Nagtegaal ID, Washington MK. World Health Organization Classification of Tumours. Digestive System Tumours, 5 ed. Lyon, France: IARC Press; 2019.
[3] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022;72(1):7–33. doi:10.3322/caac.21708, PMID:35020204.
[4] Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to sex and HIV status: a systematic review and meta-analysis. Lancet Infect Dis 2018;18(2):198–206. doi:10.1016/S1473-3099(17)30653-9, PMID:29158102.
[5] Lisovsky M, Patel K, Cymes K, Chase D, Bhuiya T, Morgenstern N. Immunophenotypic characterization of anal gland carcinoma: loss of p63 and cytokeratin 5/6. Arch Pathol Lab Med 2007;131(8):1304–1311. doi:10.5858/2007-131-1304-ICOAGC, PMID:17683193.
[6] Yang EJ, Quick MC, Hanamornroongruang S, Lai K, Doyle LA, McKeon FD, *et al*. Microanatomy of the cervical and anorectal squamocolumnar junctions: a proposed model for anatomical differences in HPV-related cancer risk. Mod Pathol 2015;28(7):994–1000. doi:10.1038/modpathol.2015.54, PMID:25975286.
[7] Foust RL, Dean PJ, Stoler MH, Moinuddin SM. Intraepithelial neoplasia of the anal canal in hemorrhoidal tissue: a study of 19 cases. Hum Pathol

1991;22(6):528–534. doi:10.1016/0046-8177(91)90228-h, PMID:1650751.
[8] Bell PD, Israel AK, Dunn AL, Liao X. Primary Dedifferentiated Amelanotic Anorectal Melanoma: Report of a Rare Case. Int J Surg Pathol 2019;27(8):923–928. doi:10.1177/1066896919857148, PMID:31232138.
[9] Lobert PF, Appelman HD. Inflammatory cloacogenic polyp. A unique inflammatory lesion of the anal transitional zone. Am J Surg Pathol 1981; 5(8):761–766. PMID:7337162.
[10] Washington K, Rourk MH Jr, McDonagh D, Oldham KT. Inflammatory cloacogenic polyp in a child: part of the spectrum of solitary rectal ulcer syndrome. Pediatr Pathol 1993;13(4):409–414. doi:10.3109/15513819309048229, PMID:8372025.
[11] Sturegård E, Johansson H, Ekström J, Hansson BG, Johnsson A, Gustafsson E, *et al*. Human papillomavirus typing in reporting of condyloma. Sex Transm Dis 2013;40(2):123–129. doi:10.1097/OLQ.0b013e31827aa9b3, PMID:23324975.
[12] Wikström A, Vassilaki I, Hedblad MA, Syrjänen S. The spectrum of genital human papillomavirus infection among men attending a Swedish sexually-transmitted infections clinic: human papillomavirus typing and clinical presentation of histopathologically benign lesions. Acta Derm Venereol 2013;93(2):223–227. doi:10.2340/00015555-1375, PMID:22688658.
[13] Srodon M, Stoler MH, Baber GB, Kurman RJ. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). Am J Surg Pathol 2006;30(12):1513–1518. doi:10.1097/01.pas.0000213291.96401.48, PMID:17122506.
[14] Liao X, Liu X, Fan X, Lai J, Zhang D. Perianal Paget's disease: a clinicopathological and immunohistochemical study of 13 cases. Diagn Pathol 2020;15(1):29. doi:10.1186/s13000-020-00952-w, PMID:32209119.
[15] del Pino M, Bleeker MC, Quint WG, Snijders PJ, Meijer CJ, Steenbergen RD. Comprehensive analysis of human papillomavirus prevalence and the potential role of low-risk types in verrucous carcinoma. Mod Pathol 2012;25(10):1354–1363. doi:10.1038/modpathol.2012.91, PMID:22684225.
[16] Zidar N, Langner C, Odar K, Hošnjak L, Kamarádová K, Daum O, *et al*. Anal verrucous carcinoma is not related to infection with human papillomaviruses and should be distinguished from giant condyloma (Buschke-Löwenstein tumour). Histopathology 2017;70(6):938–945. doi:10.1111/his.13158, PMID:28012208.
[17] Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, *et al*. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol 2013;32(1):76–115. doi:10.1097/PGP.0b013e31826916c7, PMID:23202792.
[18] Hernandez BY, McDuffie K, Zhu X, Wilkens LR, Killeen J, Kessel B, *et al*. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomarkers Prev 2005;14(11 Pt 1):2550–2556. doi:10.1158/1055-9965.EPI-05-0460, PMID:16284377.
[19] Wai KC, Strohl MP, van Zante A, Ha PK. Molecular Diagnostics in Human Papillomavirus-Related Head and Neck Squamous Cell Carcinoma. Cells 2020;9(2):500. doi:10.3390/cells9020500, PMID:32098320.
[20] Liu Y, McCluggage WG, Darragh TM, Farhat N, Blakely M, Sigel K, *et al*. p16 Immunoreactivity Correlates With Morphologic Diagnosis of HPV-associated Anal Intraepithelial Neoplasia: A Study of 1000 Biopsies. Am J Surg Pathol 2021;45(11):1573–1578. doi:10.1097/PAS.0000000000001769, PMID:34231547.
[21] Abu-Farsakh S, Drage MG, Huber AR, Turner BM, Varghese S, Wang X, *et al*. Interobserver agreement in the diagnosis of anal dysplasia: comparison between gastrointestinal and gynaecological pathologists and utility of consensus conferences. Histopathology 2022;80(4):648–655. doi:10.1111/his.14578, PMID:34601750.
[22] Koulos J, Symmans F, Chumas J, Nuovo G. Human papillomavirus detection in adenocarcinoma of the anus. Mod Pathol 1991;4(1):58–61. PMID:1850518.
[23] Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, *et al*. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. Cancer 2004;101(2):270–280. doi:10.1002/cncr.20365, PMID:15241823.
[24] Grulich AE, Poynten IM, Machalek DA, Jin F, Templeton DJ, Hillman RJ. The epidemiology of anal cancer. Sex Health 2012;9(6):504–508. doi:10.1071/SH12070, PMID:22958581.
[25] Zhu X, Jamshed S, Zou J, Azar A, Meng X, Bathini V, *et al*. Molecular and immunophenotypic characterization of anal squamous cell carcinoma reveals distinct clinicopathologic groups associated with HPV and TP53 mutation status. Mod Pathol 2021;34(5):1017–1030. doi:10.1038/s41379-020-00729-y, PMID:33483624.
[26] Graham RP, Arnold CA, Naini BV, Lam-Himlin DM. Basaloid Squamous Cell Carcinoma of the Anus Revisited. Am J Surg Pathol 2016;40(3):354–360. doi:10.1097/PAS.0000000000000594, PMID:26866355.
[27] Bruyere D, Monnien F, Colpart P, Roncarati P, Vuitton L, Hendrick E, *et al*. Treatment algorithm and prognostic factors for patients with stage I-III carcinoma of the anal canal: a 20-year multicenter study. Mod Pathol 2021;34(1):116–130. doi:10.1038/s41379-020-0637-6, PMID:32728225.
[28] Modlin IM, Sandor A. An analysis of 8305 cases of carcinoid tumors. Cancer 1997;79(4):813–829. doi:10.1002/(sici)1097-0142(19970215)79:4<813::aid-cncr19>3.0.co;2-2, PMID:9024720.
[29] Osagiede O, Habermann E, Day C, Gabriel E, Merchea A, Lemini R, *et al*. Factors associated with worse outcomes for colorectal neuroendocrine tumors in radical versus local resections. J Gastrointest Oncol 2020;11(5):836–846. doi:10.21037/jgo-20-193, PMID:33209480.
[30] Maione F, Chini A, Milone M, Gennarelli N, Manigrasso M, Maione R, *et al*. Diagnosis and Management of Rectal Neuroendocrine Tumors (NETs). Diagnostics (Basel) 2021;11(5):771. doi:10.3390/diagnostics11050771, PMID:33923121.
[31] Gut P, Waligórska-Stachura J, Czarnywojtek A, Sawicka-Gutaj N, Bączyk M, Ziemnicka K, *et al*. Hindgut neuroendocrine neoplasms - characteristics and prognosis. Arch Med Sci 2017;13(6):1427–1432. doi:10.5114/aoms.2017.64979, PMID:29181074.
[32] Cimino-Mathews A, Sharma R, Illei PB. Detection of human papillomavirus in small cell carcinomas of the anus and rectum. Am J Surg Pathol 2012;36(7):1087–1092. doi:10.1097/PAS.0b013e3182549b6d, PMID:22531171.
[33] Bernick PE, Klimstra DS, Shia J, Minsky B, Saltz L, Shi W, *et al*. Neuroendocrine carcinomas of the colon and rectum. Dis Colon Rectum 2004;47(2):163–169. doi:10.1007/s10350-003-0038-1, PMID:15043285.
[34] Chen I, Zhang D, Velez M, Kovar S, Liao X. Poorly differentiated neuroendocrine carcinomas of the gastrointestinal tract: A single-institute study of 43 cases. Pathol Res Pract 2021;226:153614. doi:10.1016/j.prp.2021.153614, PMID:34555651.
[35] Voong KR, Rashid A, Crane CH, Minsky BD, Krishnan S, Yao JC, *et al*. Chemoradiation for High-grade Neuroendocrine Carcinoma of the Rectum and Anal Canal. Am J Clin Oncol 2017;40(6):555–560. doi:10.1097/COC.0000000000000211, PMID:26237193.
[36] Shiels MS, Kreimer AR, Coghill AE, Darragh TM, Devesa SS. Anal Cancer Incidence in the United States, 1977-2011: Distinct Patterns by Histology and Behavior. Cancer Epidemiol Biomarkers Prev 2015;24(10):1548–1556. doi:10.1158/1055-9965.EPI-15-0044, PMID:26224796.
[37] Meriden Z, Montgomery EA. Anal duct carcinoma: a report of 5 cases. Hum Pathol 2012;43(2):216–220. doi:10.1016/j.humpath.2011.04.019, PMID:21820151.
[38] Herfs M, Roncarati P, Koopmansch B, Peulen O, Bruyere D, Lebeau A, *et al*. A dualistic model of primary anal canal adenocarcinoma with distinct cellular origins, etiologies, inflammatory microenvironments and mutational signatures: implications for personalised medicine. Br J Cancer 2018;118(10):1302–1312. doi:10.1038/s41416-018-0049-2, PMID:29700411.
[39] Voltaggio L, McCluggage WG, Iding JS, Martin B, Longacre TA, Ronnett BM. A novel group of HPV-related adenocarcinomas of the lower anogenital tract (vagina, vulva, and anorectum) in women and men resembling HPV-related endocervical adenocarcinomas. Mod Pathol 2020;33(5):944–952. doi:10.1038/s41379-019-0437-z, PMID:31857682.
[40] Katerji R, Liao X, Huber A, Zhang D. An Unusual Case of Human Papillomavirus-Related Anorectal Adenocarcinoma With Progression to Perianal Paget's Disease. Int J Surg Pathol 2021;29(6):672–676. doi:10.1177/1066896920982367, PMID:33356675.
[41] Damin DC, Ziegelmann PK, Damin AP. Human papillomavirus infection and colorectal cancer risk: a meta-analysis. Colorectal Dis 2013;15(8):e420–e428. doi:10.1111/codi.12257, PMID:23895733.
[42] Bodaghi S, Yamanegi K, Xiao SY, Da Costa M, Palefsky JM, Zheng ZM. Colorectal papillomavirus infection in patients with colorectal cancer. Clin Cancer Res 2005;11(8):2862–2867. doi:10.1158/1078-0432.CCR-04-1680, PMID:15837733.
[43] Cheng JY, Sheu LF, Meng CL, Lee WH, Lin JC. Detection of human papillomavirus DNA in colorectal carcinomas by polymerase chain reaction. Gut 1995;37(1):87–90. doi:10.1136/gut.37.1.87, PMID:7672688.
[44] Gornick MC, Castellsague X, Sanchez G, Giordano TJ, Vinco M, Greenson JK, *et al*. Human papillomavirus is not associated with colorectal cancer in a large international study. Cancer Causes Control 2010;21(5):737–743. doi:10.1007/s10552-010-9502-0, PMID:20087645.
[45] Park JJ, Sun D, Quade BJ, Flynn C, Sheets EE, Yang A, *et al*. Stratified mucin-producing intraepithelial lesions of the cervix: adenosquamous or columnar cell neoplasia? Am J Surg Pathol 2000;24(10):1414–1419. doi:10.1097/00000478-200010000-00012, PMID:11023104.
[46] Assarzadegan N, Brooks E, Voltaggio L. HPV-driven anal neoplasia: review and recent developments. Pathology 2022;54(2):184–194. doi:10.1016/j.pathol.2021.07.003, PMID:34645567.
[47] Sekar A, Rana SS, Bardia A. SMILE-like lesion in the anal canal. Autops Case Rep 2021;11:e2021289. doi:10.4322/acr.2021.289, PMID:34249793.
[48] Guo L, Kuroda N, Miyazaki E, Jin Y, Toi M, Hamauzu T, *et al*. Anal canal neuroendocrine carcinoma with Pagetoid extension. Pathol Int 2004;54(8):630–635. doi:10.1111/j.1440-1827.2004.01673.x, PMID:15260855.