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A rare case of anal carcinosarcoma with human papilloma virus infection in both biphasic tumor elements: An immunohistochemical, molecular and ultrastructural study

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ABSTRACT

Carcinosarcoma of the anus is rare and has yet to be reportedly associated with the keratinocyte-specific Human Papilloma Virus (HPV). We describe a case of anal carcinosarcoma with HPV infection in both the epithelial and mesenchymal components of the tumor by immunohistochemistry, chromogenic in-situ hybridization (CISH) and further supported by electron microscopy (EM). Microscopic examination of the tumor showed nests of poorly-differentiated invasive squamous cell carcinoma with basaloid features intermixed with a hypercellular, atypical spindle cell proliferation. Immunohistochemistry demonstrated that the epithelial component was positive for AE1/AE3, p63, CK5/6 and p16, whilst the mesenchymal component was positive for smooth muscle actin, vimentin, and focally positive for desmin and p16, consistent with carcinosarcoma. The tumor was negative for GATA-3, CK7 and CK20. CISH demonstrated that the tumor was positive for high risk HPV (subtype 16/18) in both tumor components. EM further supported the presence of intracellular virus particles (~50 nm) that is compatible with HPV infection. Infection of both epithelial and mesenchymal tumor components by HPV has not been previously observed in the gastrointestinal tract. This finding may represent initial epithelial HPV infection with subsequent divergent tumoral differentiation and suggests the presence of viral replication in both biphasic tumor components.

1. Introduction

Over 200 variants of the Human Papillomavirus (HPV) are now recognized and most strains are considered to have a low or negligible carcinogenic risk to humans [1,2]. HPV is epitheliotropic and common sites of infection by the oncogenic forms of HPV include the transformation zone of the uterine cervix, vulva, oropharynx, anal canal and the glans penis [3]. In the anal canal, HPV serotypes 6 and 11 are typically associated with anal condylomas/ low grade anal intraepithelial neoplasia (AIN) whereas high grade AIN and anal squamous cell carcinoma (ASCC) has been linked to high risk HPV serotypes, such as HPV 16 and 18 [4]. The most prevalent serotype in ASCC is HPV 16, being found in up to 90% of cases [5,6].

In many of the areas that HPV can affect, such as the uterine cervix, glans penis, there have been documented cases of tumors that comprise both epithelial and mesenchymal tumor elements (carcinosarcomas). Several reports have described the presence of HPV infection in carcinosarcomas of the uterine cervix and penis within both the epithelial and mesenchymal components of these tumors [7–9]. There are rare instances of carcinosarcoma within the anal canal, however to our knowledge, HPV infection in both epithelial and mesenchymal components has yet to be reported [10]. We describe the first case of carcinosarcoma within the GI tract that has HPV infection in both squamous and mesenchymal components. The implications of this finding are discussed.

2. Case presentation

An 87-year-old man with a past medical history of hypertension, hyperlipidemia and benign prostatic hypertrophy presented to his physician with proctalgia. Examination under anesthesia revealed a firm mass in the distal rectum. Biopsy tissue was obtained from the rectum/ anus, fixed in neutral-buffered formalin and prepared for routine light microscopy. Immunoperoxidase and CISH were performed targeting specific antigens and HPV DNA sequences, respectively. Tissue for EM was then deparaffinized in xylene, rehydrated in

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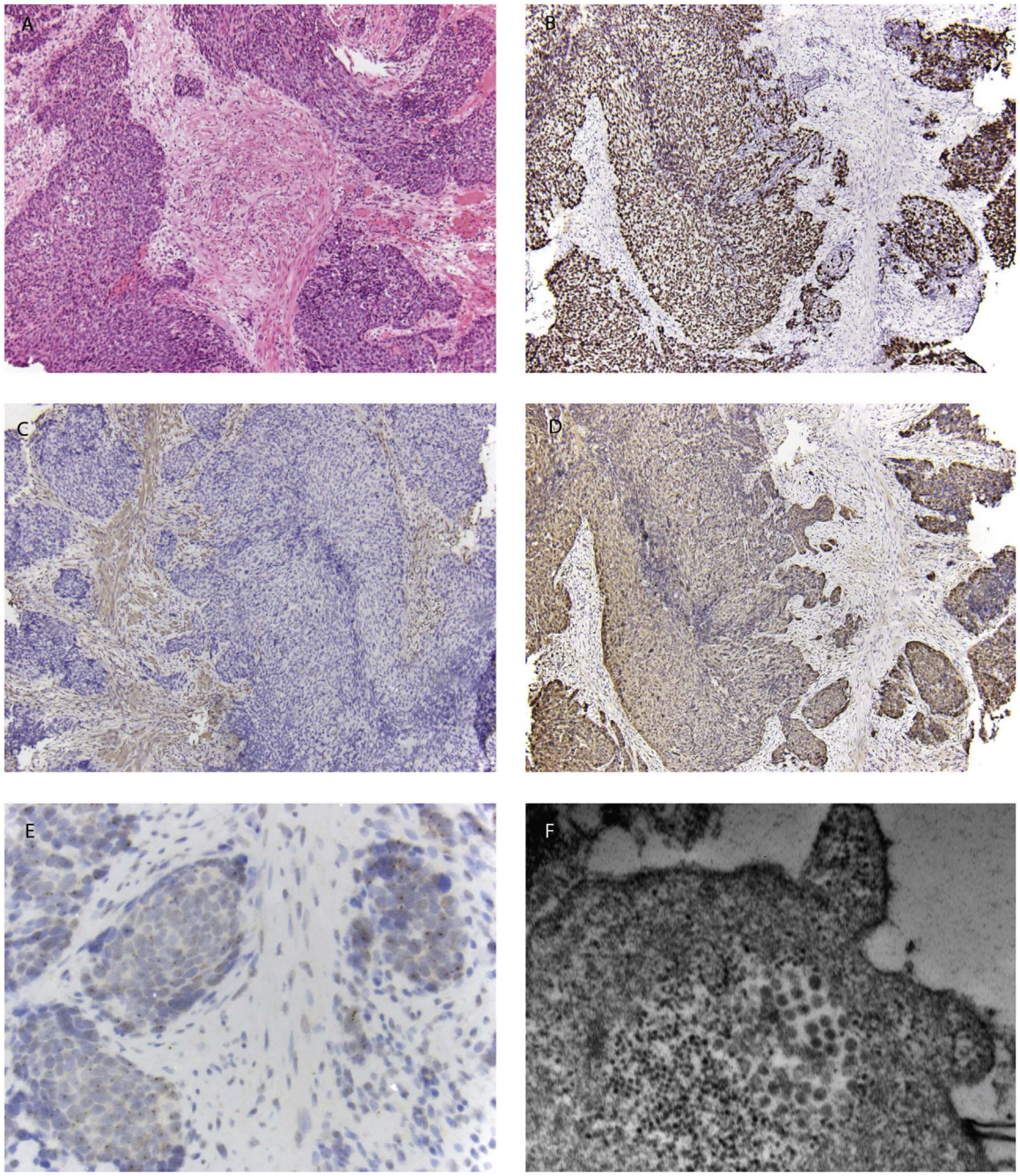

Fig. 1. : A) Hematoxylin and eosin stained sections show nests of neoplastic epithelial cells with intervening atypical spindle cells compatible with carcinosarcoma, x100. B) The epithelial component of the carcinosarcoma was reactive against p63 antibody, x100. C) The mesenchymal component was reactive to smooth muscle actin, x100. D) Both epithelial and mesenchymal tumor components exhibited p16 immunoreactivity, x100. E) HPV 16/18 probes show dot-like signals in both epithelial and mesenchymal components of the tumor, x400. F) Ultrastructural examination of the tumor reveals intracellular particles that are approximately 50 nm in maximal dimension arranged in a lattice formation.

alcohol, post-fixed in Osmium Tetroxide and embedded in Epox A12. Ultrathin sections were cut at 70 nm and stained with uranyl citrate and lead citrate. EM was performed on a JEM 1010 microscope.

2.1. Histology

Microscopic examination of the tumor revealed a biphasic tumor comprising nests of poorly-differentiated invasive squamous cells with intervening atypical spindle cells (Fig. 1A). The squamous component exhibited a basaloid phenotype. Immunohistochemistry using immunoperoxidase stains on paraffin embedded tissue demonstrated that the epithelial component was positive for AE1/AE3, p63 (Fig. 1B), CK5/6, whilst the mesenchymal component was positive for smooth muscle actin (Fig. 1C), vimentin, and focally positive for desmin, consistent with carcinosarcoma (leiomyosarcoma). Of interest, both components exhibited p16 reactivity, suggesting possible HPV involvement in both tumor elements (Fig. 1D). The tumor was negative for GATA-3, CK7 and CK20..

2.2. In-situ hybridization

CISH was performed against HPV subtypes 6, 11, 16, 18, 31 and 33. Both epithelial and mesenchymal tumor cells showed an even, dot-like staining pattern within the tumor nuclei of high risk HPV (subtypes 16/18) (Fig. 1E). This finding confirmed the presence of the same HPV subtype in both tumor components and its integration into the cellular

genome.

2.3. Ultrastructural findings

Ultrastructurally, the tumor exhibited squamous differentiation. Electron-dense particles that measured approximately 50 nm in size were identified and were arranged in a lattice configuration (Fig. 1F). This size and arrangement is compatible with the HPV virus. Although evident microscopically, identification of mesenchymal differentiation was limited by tissue quantity and post-embedding deparaffinization.

3. Discussion

This report demonstrates viral infection by HPV of both epithelial and mesenchymal components of a carcinosarcoma confirmed by immunohistochemistry and in-situ hybridization. Whilst HPV infection of keratinocytes has been well documented in various body sites with squamous epithelia, it has been only rarely described in biphasic tumors, predominantly in the gynecologic tract and in several cases of the genitourinary tract. The presence of HPV 16/18 by ISH demonstrates viral infection within the tumor cells and p16 immunoreactivity in the tumor suggests viral transformation, likely through inactivation of pRB through E7 oncoprotein expression. To our knowledge, this case is the first example of HPV in both biphasic tumor elements of carcinosarcoma within the GI tract.

The following explanations as to the histogenesis of carcinosarcoma have been proposed: 1) that carcinosarcoma is a collision tumor of two distinct epithelial and mesenchymal tumors, 2) that the mesenchymal component is a reactive stromal response to the malignant epithelial component and 3) that it arises from a monoclonal tumor cell that differentiates into epithelial and mesenchymal tissue types [11]. Although it is recognized that bovine papillomavirus has the ability to infect both epithelial and mesenchymal tissues, the infectivity of HPV is thought to be confined to epithelial tissues, which disputes that this case was a collision tumor or a reactive phenomenon [12]. Rather, the finding of high-risk HPV in both epithelial and mesenchymal components, suggests that initial HPV infection of the anal squamous mucosa occurred, which as the tumor grew, a subpopulation of tumor cells differentiated into a mesenchymal phenotype with retention of HPV infection. This has been the prevailing hypothesis for cases of cervical carcinosarcoma with HPV infection [9,13,14].

Although the general prognosis of anal carcinosarcoma is poor, in our case, the patient remains disease-free at 1 year follow-up after chemoradiotherapy which included a daily radiotherapy dose of 180 cGy up to a total dose of 5400 cGy [15]. It is recognized that p16 positive ASCC are more radiosensitive and have improved recurrence free survival and overall survival compared with p16 negative ASCC [5,6]. One could infer from this that immunoreactivity to p16 in at least the squamous component of this leiomyosarcoma may have resulted in better response to radiotherapy and thus improved survival, however, larger studies are needed in p16 positive leiomyosarcomas at this location. Given that this tumor was associated with HPV subtypes 16 and 18, future cases such as this might be avoided by HPV vaccination prior to infection [16].

4. Conclusion

To our knowledge, this is the first case of carcinosarcoma in the GI tract that has HPV infection within both the epithelial and mesenchymal components. Given the epitheliotropic nature of HPV, this finding supports divergent tumoral differentiation as a plausible pathway in the development of this tumor.

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