WHIM syndrome and oral squamous cell carcinoma

Nicole A. Cipriani, MD,[a] Elizabeth Blair, MD,[b] and Jerome B. Taxy, MD,[a] Chicago, Illinois
UNIVERSITY OF CHICAGO MEDICAL CENTER

WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome is an autosomal dominant disease related to a mutation in the chemokine receptor CXCR4 resulting in altered immune function. An increased susceptibility in these patients to human papillomavirus (HPV) manifests as cutaneous warts and, in women, cervical dysplasia and squamous carcinoma. HPV-related squamous carcinoma in other sites has not been documented. We report the occurrence of HPV-related squamous cell carcinoma of the oral cavity in 2 siblings with WHIM syndrome, whose pedigree has previously been described. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:105-108)

WHIM is the acronymic designation for a rare autosomal dominant syndrome characterized by warts, hypogammaglobulinemia, infections, and retention of mature neutrophils in the bone marrow (myelokathexis). The first case was described, in 1964, in a 10-year-old girl with granulocytopenia and infections.[1,2] Since then, <40 cases have been reported.[3] An increased susceptibility to human papillomavirus (HPV) results in multiple, often disfiguring, cutaneous warts and, in women, susceptibility to HPV-related cervical dysplasia or carcinoma.[4-6] Leukopenia with hypogammaglobulinemia renders patients susceptible to infections, especially bacterial infections of the respiratory tract, e.g., otitis, sinusitis, pneumonia, and cellulitis. The risk for bacterial infections is thought to result from retention of mature neutrophils in the bone marrow (myelokathexis) and consequent unavailability of these cells, manifested as peripheral neutropenia. Not only are peripheral granulocyte counts low, but some neutrophils and eosinophils show abnormal morphology, including hypersegmented nuclei and vacuolated cytoplasm. Long-term antibiotic prophylaxis is often used.[4-6]

Although cervical dysplasia and squamous carcinoma have been noted in female WHIM patients,[3] it is unclear why these patients are susceptible to HPV. Furthermore, squamous carcinomas in other locations are not documented. In the general population, there is a recently and increasingly recognized association of HPV with squamous carcinomas in the oral cavity.[7-9] We report the occurrence of HPV-related oral squamous carcinoma in 2 siblings with documented WHIM syndrome.[5]

MATERIALS AND METHODS

Tissue samples for histopathologic study were formalin fixed, decalcified as required, and embedded in paraffin in accordance with generally practiced laboratory procedures. Immunostaining for p16^{INK4A} (Biocare Medical, Concord, CA) was also done in a routine fashion using a Ventana automatic stainer. In situ hybridization for HPV used 2 cocktails (Ventana Medical Systems, Tucson, AZ) for low-risk (HPV 6 and 11) and high-risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66) viruses.

Case histories

The index patient is a 46-year-old woman with a history of WHIM syndrome clinically recognized since the age of 7 years, manifesting as cutaneous warts, granulocytopenia, hypogammaglobulinemia, and recurrent infections including otitis, sinusitis, pneumonia, and cellulitis.[5] She has had several cutaneous basal cell carcinomas, as well as vulvar carcinoma in situ for which she underwent skinning vulvectomy at an outside institution. She presented to her periodontist in June 2008 with 2 discolored gingival lesions adjacent to her maxillary incisors. Of note, she reported chronic tooth discoloration from use of antibiotics in her youth, which required capping. She is a nonsmoker and reports only occasional use of alcoholic beverages. A biopsy of one of the gingival lesions showed invasive squamous cell carcinoma and was followed by resection of the upper alveolar ridge, consisting of the 4 upper incisors and adjacent maxilla (Fig. 1). The anterior gingival surface demonstrated 2 ulcerated lesions: a 0.4 cm lesion between teeth 9 and 10, and a 0.3 cm lesion above tooth 8. The specimen was decalcified, serially sectioned sagittally, and entirely submitted for histologic examination. Both lesions demonstrated in situ and invasive nonkeratinizing squamous cell carcinoma, extending into the respective gingival sulci (Fig. 2, A). The surface epithelium demonstrated koilocyto-

Fig. 1. Resected upper maxilla (teeth are capped). White arrows indicate separate squamous carcinomas adjacent to teeth 8 and 9-10, extending into the gingival sulci.

1) Hematopoiesis of myeloid and lymphopoiesis of B cells.
2) Organogenesis of cardiac and neural systems.
3) Gastrointestinal angiogenesis.
4) Oncogenesis and metastasis of a variety of human neoplasms, including breast and prostate carcinomas, small cell lung cancers, myeloid neoplasms, and pediatric sarcomas.
5) Human immunodeficiency virus infection, as a co-receptor for the virus.
6) Chemotaxis of hemato/lymphopoietic cells to lymphoid organs (bone marrow, spleen, and lymph nodes).[12]

The role of CXCR4-CXCL12 in bone marrow chemotaxis has been studied in vitro and in mouse models. Normally, osteoblasts and reticular cells in the bone marrow express CXCL12 at high levels.[14] Ligand-receptor binding induces tyrosine phosphorylation of the intracellular domain, resulting in downstream signaling, expression of integrins, and chemotaxis toward the ligand. The CXCR4 receptor can be turned off permanently or temporarily by internalization into vesicles, whence it can be either degraded or recycled back to the surface.[12,15] Cells with a gain of function mutation of CXCR4 demonstrate decreased internalization in response to CXCL12, which results in increased intracellular signaling, increased chemotaxis toward CXCL12 in bone marrow, a mechanical inability to exit the bone marrow, and consequent peripheral neutropenia or leukopenia.[16-18]

Unfortunately, CXCR4's role in HPV infection is not well characterized. HPV is associated with 80%-90% of cervical and anogenital carcinomas,[19] and the female patient reported here did have a history of in situ squamous carcinoma of the vulva. HPV in head and neck squamous cell carcinoma may have an overall association of 25%-50%, depending on the method of viral identification (Southern blot vs. in situ hybridization vs. polymerase chain reaction [PCR]).[20] High-risk virotypes 16 and 18 predominate.[20,21] The male patient had a maxillary sinus squamous carcinoma. In neither patient were we able to examine those tumors to ascertain the presence of HPV changes.

In both of the patients described here, microscopic examination of new oral lesions demonstrated surface viral changes associated with squamous carcinoma. One was positive for p16 oncoprotein, and the other demonstrated HPV by in situ hybridization. The lack of viral reactivity in the sister's lesion may be due to the acid involved in decalcifying the resection specimen. Nonetheless, the cyclin-dependent kinase inhibitor p16 is regarded by some as a surrogate marker for HPV infection in oral squamous epithelium.[22,23] In a recent

sis with nuclear pyknosis and focal binucleation, indicative of HPV infection (Fig. 2, *B*). In situ hybridization for HPV was negative. The p16 immunohistochemical stain was strongly positive (Fig. 3, *A* and *B*). A subsequent biopsy 8 months after resection demonstrated dysplastic squamous epithelium.

The patient's 40-year-old brother also suffered from WHIM syndrome. He had a history of Epstein-Barr virus–related B-cell lymphoma[10] as well as squamous cell carcinoma of the maxillary sinus, diagnosed and treated by maxillectomy at an outside institution. He did not have a history of tobacco or alcohol use. New oral lesions were noted, and biopsies showed in situ and invasive squamous cell carcinoma with evidence of surface viral change, similar to that seen in his sister (Fig. 2, *C* and *D*). In situ hybridization for high-risk HPV was focally present (Fig. 3, *C* and *D*).

DISCUSSION

The diagnosis and manifestations of WHIM syndrome in the 2 patients reported here have been described in detail previously.[5] The immune basis for WHIM syndrome may be related to a mutation in the chemokine receptor CXCR4, a 7-transmembrane protein expressed in a variety of stem and progenitor cells, including hematopoietic, neural and liver stem cells, primordial germ cells, and skeletal muscle and retinal progenitor cells.[11,12] The gene is located on chromosome 2q21, and a number of mutations causing truncation of the intracytoplasmic tail domain have been identified.[13] The receptor's only ligand is CXCL12, and the CXCR4-CXCL12 complex affects:
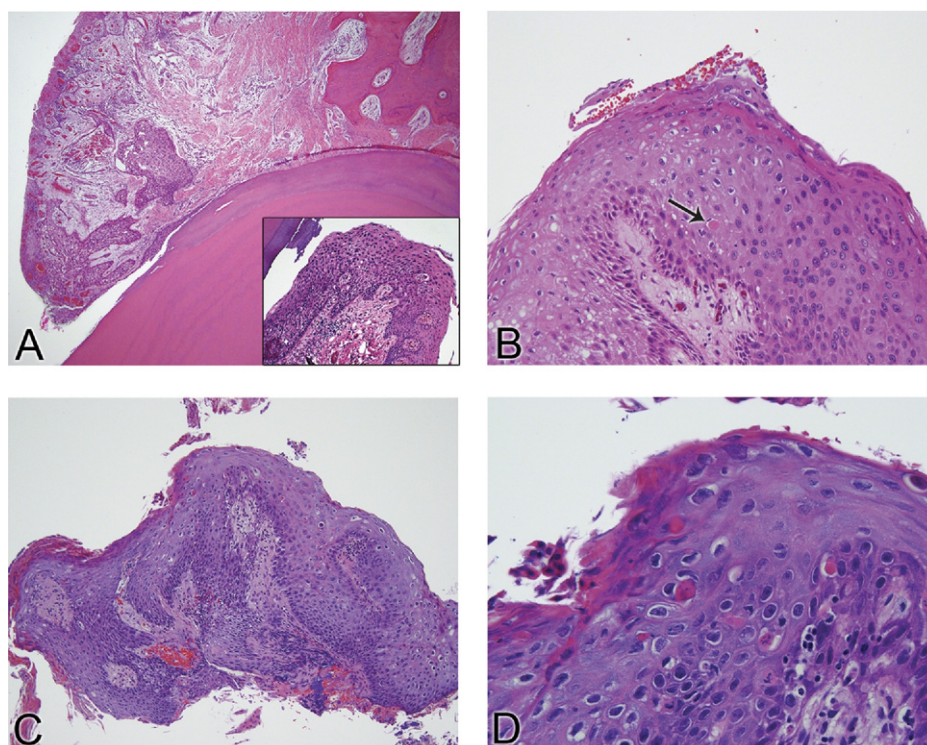
Fig. 2. Female sibling: **A,** in situ (*inset*) and invasive squamous carcinoma adjacent to maxillary bone and tooth; **B,** surface viral change with koilocytosis and dyskeratosis (*arrow*). Male sibling: **C,** in situ squamous carcinoma; **D,** surface viral change.
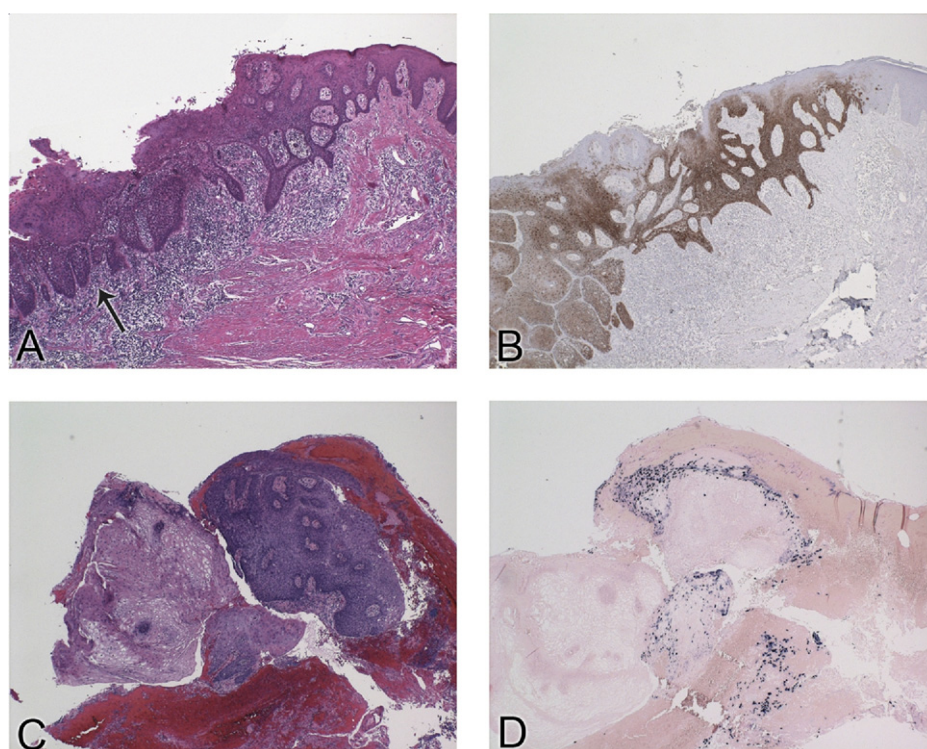

Fig. 3. Female sibling: **A,** Oral mucosa with in situ and invasive squamous carcinoma (*arrow*); **B,** both the dysplastic and the invasive squamous components are diffusely p16 positive. Male sibling: **C,** oral mucosal biopsy with in situ squamous carcinoma; **D,** in situ hybridization demonstrates focal positivity for high-risk human papillomavirus.

evaluation of 41 cases of high-grade squamous dysplasia, 100% of cases (6 out of 6) with p16 positivity demonstrated HPV by PCR. Furthermore, PCR was negative in the p16-negative cases.[22] In a second study comparing p16 expression to HPV in situ hybridization, 9 out of 10 cases with diffuse p16 staining demonstrated positive HPV in situ hybridization.[23]

The identification of high-risk HPV and p16 oncoprotein in these cases suggests but cannot prove an etiologic role for the virus in carcinogenesis, because not all lesions with HPV progress to carcinoma.[24] Some studies suggest that concurrent use of tobacco or alcohol might increase the risk for carcinoma in patients who are already HPV positive.[25] In light of such a "2-hit" hypothesis, the immune dysfunction precipitated by WHIM syndrome might serve as the second hit in patients with oral HPV, further increasing their risk for squamous carcinoma. The development of oral squamous carcinoma in the setting of mucosal HPV infection in these 2 patients with a defective immune response to HPV reinforces a causative role, if somewhat selective, for HPV.

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Reprint requests:

Nicole Cipriani, MD
University of Chicago Medical Center
5841 S. Maryland Ave.
MC 6101
Chicago, IL 60637
nicole.cipriani@uchospitals.edu