Sporadic Trichoepithelioma Demonstrates Deletions at 9q22.3

David Matt, MD; Hong Xin, MD; Alexander O. Vortmeyer, MD; Zhengping Zhuang, MD, PhD; Günter Burg, MD; Roland Böni, MD

Background: Trichoepithelioma (TE) is a benign cutaneous tumor that originates from hair follicles and occurs either in multiple or solitary lesions. Multiple TE is transmitted as an autosomal dominant trait, and a region at 9p21 is thought to be involved in the tumorigenesis. Solitary TE occurs more commonly than multiple TE and is not inherited. Histologically, TE tumors contain horn cysts and abortive hair papillae. A basal cell carcinoma appearance in some or all regions of a TE tumor can happen. In sporadic basal cell carcinoma, frequent deletions at 9q22.3 (Drosophila patched gene) have occurred. The objective of this study is to test whether loss of heterozygosity (LOH) on either 9p21 or on chromosome 9q22.3 could be detected in archival sporadic TE.

Observations: We studied 29 randomly selected cases of sporadic TE by microdissection and polymerase chain reaction using paraffin-embedded, formalin-fixed tissue specimens on glass slides. Analysis was performed with the polymorphic markers IFNA and D9S171 (9p21) as well as D9S15, D9S303, D9S287, and D9S252 (9q22.3).

Results: The LOH at 9q22.3 was identified in 14 (48%) of 29 cases with at least 1 marker, while LOH could not be demonstrated using the markers IFNA and D9S171 (9p21).

Conclusions: The results show that the Drosophila patched gene LOH can be frequently identified in paraffin-embedded sporadic TE after routine processing and indicates a common gatekeeper mechanism for both TE and basal cell carcinoma.


T MICHOEPITHELIOMA (TE) is a benign skin tumor originating from hair follicles. It may occur as a solitary non-familial and a multiple-familial type. Multiple-familial TE (McKusick No. 132700) is an autosomal dominant disease characterized by the presence of many skin-colored small tumors located predominantly in the nasolabial folds, but also on the nose, forehead, upper lip, and occasionally on the scalp, neck, and upper trunk. The tumor suppressor gene believed to be involved in the tumorigenesis of multiple-familial TE has been located in a region at 9p21. Several known tumor suppressor genes, including p15, p16, and p19 have been assigned to this region.

Histologically, TE tumors contain horn cysts, abortive hair papillae, and, uncommonly, areas with the appearance of basal cell carcinoma (BCC) from which it is sometimes almost or completely indistinguishable. The gene for hereditary BCC has been identified and located at 9q22.3-q31, the human homologue of Drosophila patched gene (PTCH), and allelic deletions of the PTCH gene are found in hereditary BCCs. In addition, a substantial subset of sporadic BCC also shows PTCH loss of heterozygosity (LOH). Furthermore, recent studies indicate potential involvement of the PTCH locus in the pathogenesis of TE. We therefore analyzed 29 tumors for LOH at both 9q22.3 and 9p21.

RESULTS

The LOH on 9q22.3 was identified with at least 1 marker in 14 (48%) of 29 cases. D9S252 and D9S303 showed LOH in 7 (35%) of 20 cases; D9S15 in 4 (33%) of 12; and D9S287 in 2 (11%) of 18 (Figure 2 and Table). Results proved homozygous or noninformative for D9S15 in 17 of 29 cases, for D9S303 in 9 of 29, for D9S287 in 11 of 29, and for D9S252 in 9 of 29; these markers were therefore excluded from the calculation. There was consistently no LOH for IFNA or D9S171.
COMMENT

A region at 9p21 has been suspected to be involved in tumorigenesis in multiple familial TE, and deletions within this area harbor known tumor suppressor genes. With this study we tried to elucidate whether genetic changes at 9p21 play a role in the tumorigenesis of sporadic TE. In 29 cases of sporadic TE, tumors have been microdissected and examined using microsatellite markers at 9p21 (IFNA and D9S171), and there was no LOH for either marker. These data suggest that sporadic TE (or at least most cases of sporadic TE) has a different pathogenesis from hereditary counterparts.

Although TE and BCC represent 2 different clinical entities, several lines of evidence suggest common pathogenetic features: (1) Trichoepithelioma and BCC share histopathologic features and may be difficult to differentiate. They occur together. (2) They often occur together. (3) Several genetic studies suggest a common origin of TE and BCC: in a subset of TE, somatic mutations in the over-expressed PTCH gene have occurred, and in a solitary TE, LOH was only demonstrated at the BCC locus, Figure 1.

MATERIALS AND METHODS

HISTOLOGIC SPECIMEN

Formalin-fixed, paraffin-embedded histologic specimens of 29 sporadic TE tumors from 26 different patients (18 men and 8 women) were investigated. None of the patients had a known family history of TE. All TE tumors were nonulcerating, nonbleeding lesions stable for many years.

The histologic specimens were obtained from the archives of the Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland. From each specimen, 1 section was used for hematoxylin-eosin staining and microdissection. To assure correct diagnosis, all histologic slides were reviewed.

MICRODISSECTION TECHNIQUE

In each case, a 5-µm tissue section was obtained for hematoxylin-eosin staining and microdissection. From each section (N = 29) we microdissected between 50 and 100 cells of dermal aggregates of basaloid cells with connection to or differentiation toward hair follicles (Figure 1).

Microdissection was performed under light microscope visualization (magnification ×200) using a 30-gauge needle. Samples from normal cells apart from TE cell structures (eg, inflammatory cells, sebaceous glands) were also obtained in all cases from the same slide for comparison with the tumor samples.

DNA EXTRACTION

Procured cells (dermal aggregates of basaloid cells as well as samples obtained from normal cell structures apart from TE tumors) were immediately suspended in 30 µL of buffer containing 0.05-mol/L Tris-hydrochloride, 1-mmol/L EDTA, 1% Tween 20, 1-g/L proteinase K (pH 8.0), and incubated 2 days at 37°C. The mixture was boiled for 10 minutes at

Figure 1. Histologic study of a solitary trichoepithelioma showing tumor islands composed of basophilic cells surrounded by a stroma before (left) and after (right) microdissection (hematoxylin-eosin, original magnification ×200).
94°C to inactivate proteinase K, and 1.5 µL of this solution was used for polymerase chain reaction (PCR). Analysis of LOH was carried out by PCR amplification of microsatellite polymorphisms.

**PRIMERS AND PCR CONDITIONS**

Two polymorphic DNA markers at the short arm of chromosome 9 (IFNA [9p21] and D9S171 [9p21]) and 4 markers at chromosome 9q22.3 (D9S303 [9q 13-9q22.3], D9S15 [9q 13-9q21.1], D9S252 [9q 13-9q22.1], and D9S287 [9q22.3-9q31]) (Research Genetics, Huntsville, Ala) were used in this study. The PCR was performed in 10 µL units that contained 1 µL of 10 × PCR buffer (Boehringer Mannheim GmbH, Mannheim, Germany), 50 pmol of each primer per liter; 20 nmol/L each of dCTP, dGTP, dTTP, dATP; 0.2 µL [32P] of dCTP [22200 Bq/mmol]; and 0.1 U of Taq DNA polymerase. Reactions were cycled in a thermal cycler (Gene Amp PCR System 9600; Perkin Elmer, Zurich), and amplification consisted of 35 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1 minute at 72°C with a final 10-minute extension at 72°C.

Labeled amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide; 20 mmol/L of EDTA; 0.05% bromophenol blue; and 0.05% xylene cyanol). The samples were denatured for 5 minutes at 94°C and loaded onto a gel consisting of 6% acrylamide (49:1 acrylamide-bis). Samples were electrophoresed at 1600 V for 2 hours. Gels were transferred to 3-mm Whatman paper (Merck & Co Inc, Zurich), dried, and subjected to autoradiography with Typon X-Ray DX 41 film (Typon, Burgdorf, Switzerland). A case was considered informative for a polymorphic marker if normal tissue DNA showed 2 different alleles (heterozygosity). Loss of heterozygosity was defined as absence or substantial reduction of one allele in the tumor DNA evaluated by autoradiography.

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