The Sebaceous Nevus: A Nevus with Deletions of the PTCH Gene

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Abstract

Sebaceous nevi (SN) are congenital malformations of the skin that appear as slightly raised, yellowish, smooth or velvety, hairless plaques on the head or neck. The growth of the lesion is accelerated at puberty, showing a verrucous and nodular appearance. During adult life, it may be complicated by secondary tumor development, which is regarded as third stage in the natural history of the lesion (1). Quite commonly, a large variety of appendage tumors may develop within SN, e.g., syringocystadenoma papilliferum, primitive follicular germs, trichoblastomas, and, most often, BCCs (1–3). For this reason, SN are usually excised. Despite the fact that substantial data have proven the potential of SN to develop mainly into BCC, the molecular basis for the carcinogenesis of SN remains unknown.

During the past few years, the genetic changes in BCCs have been described in detail. For the nevoid BCC syndrome, an autosomal dominant disorder characterized by multiple BCCs, one particular locus, the human homologue of Drosophila patched gene (PTCH gene) at 9q22.3 has been shown to be involved and to act as a tumor suppressor gene (4–6). The inactivation of a tumor suppressor gene frequently occurs through point mutation of one homologue and loss of the other homologue by nondisjunction, deletion, or mitotic recombination. The deletion of genetic material results in LOH when alleles are excised. Despite the fact that substantial data have proven the potential of SN to develop mainly into BCC, the molecular basis for the carcinogenesis of SN remains unknown.

This study was undertaken to investigate the possible LOH in the 9q22.3 region (PTCH gene) in a set of randomly selected SN. We aimed to compare the pattern of LOH with that found in BCCs and expound the close association between SN and BCC with cytogenetic evidence.

Materials and Methods

Histological Specimens. Twenty-one paraffin-embedded histological specimen of SN (from 17 males and 4 females; mean age, 29 ± 15 years), obtained from the archives of the Department of Dermatology, University Hospital of Zurich, were investigated in this study. For each specimen, one section was used for H&E staining and microdissection (Fig. 1). To assure correct diagnosis, we reviewed all histological slides.

Microdissection. In each case, a 5-μm tissue section was obtained for H&E staining and microdissection. Microdissection was performed under light microscope (magnification, ×200). Basaloid cells were selectively removed with a disposable 30-gauge needle. These basaloid cells were preferentially located in association with areas showing sebaceous glands. In addition, normal epidermal layer apart from SN (control) was procured. At least 50–100 cells were microdissected in each sample.

DNA Extraction. Procured cells were immediately suspended in a 20-μl solution containing 0.05 m Tris-HCl, 1 m M EDTA, 1% Tween 20, and 2.5 mg/ml proteinase K (pH 8.0) and incubated for 2 days at 37°C. The mixture was boiled for 10 min at 94°C to inactivate proteinase K, and 1.5 μl of this solution was used as template DNA in PCR amplification.

Primers and PCR Conditions. Four polymorphic DNA markers (obtained from Research Genetics, Huntsville, AL) were used: D9S252 (9q22), D9S15 (9q21), D9S287 (9q22), and D9S303 (9q21–22).

The PCR was performed in 10-μl volumes by using 1.5 μl of template DNA, as described above; 50 pmol of each primer; 20 ng each dATP, dCTP, dGTP, and dTTP; 0.2 μl of [32P]dCTP (6000 Ci/mmol); 0.1 unit of Taq polymerase; and 1 μl of 10× buffer [100 m M Tris-HCl (pH 8.3), 500 mM KCl, 15 mM MgCl₂, and 0.1% w/v gelatin]. Each sample was overlaid with mineral oil and was amplified in a thermal cycler (Gene Amp PCR System 9600; Perkin-Elmer, Zurich, Switzerland) for 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min at the temperature described above for each primer, and extension for 1 min at 72°C, with a final 10-min extension at 72°C.

LOH Analysis. Labeled amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). Samples were then denatured for 5 minutes at 94°C, followed by rapid cooling, and loaded on to a commercial 8% acrylamide gel (Gel-Mix8; Life Technologies, Inc.). The gel was run at 1600 V for 3 h. After electrophoresis, the gel was transferred to 3-mm Whatman paper and dried. Autoradiography was performed with Typon X-Ray DX-41 film (Typhon, Burgdorf, Switzerland). A case was considered informative for a polymorphic marker if normal tissue DNA showed two different alleles. LOH of the informative polymorphic loci was compared with heterozygosity at the analyzed locus by visually evaluating allele band intensity of normal epidermal layer apart from SN basaloid cells and basaloid cells in conjunction with sebaceous glands from the same patient. Complete loss or >50% reduction in a single band intensity in an informative locus was scored as LOH by two independent observers.

Results

LOH analysis was performed on 21 SN using four polymorphic markers at 9q22.3 (Table 1). Twenty SN were informative at one or more loci. The DNA from one sample (case 13) failed to be amplified by any of the polymorphic markers, probably because of...
the extreme fragmentation of DNA from this sample preserved in paraffin.

Of the 20 informative SN, 8 (40%) exhibited LOH at least at one locus. We found one case (case 4) of LOH occurring simultaneously at all four loci, two cases (cases 7 and 9) with loss at three loci, and two cases (cases 1 and 20) with loss at two loci. The frequencies of LOH at these four loci excluding noninformative cases were 50% (7 of 14) at D9S252, 30.8% (4 of 13) at D9S15, 23.5% (4 of 17) at D9S287, and 11.7% (2 of 17) at D9S303, respectively.

**Discussion**

BCC and SN share 9q22.3 deletions and are, therefore, likely to be pathogenetically related. Deletions at the PTCH locus in basaloid cells of SN suggest the presence of a tumor suppressor gene, which could be responsible for the transformation of SN to BCC and, possibly, into other appendageal tumors.

Tumor suppressor genes play an important role in the carcinogenesis of many tissue types. These genes normally exert a negative control on cell growth and inactivation of both homologues (“two hits”) is required for a growth-promoting effect. One hit is almost invariably a point mutation. The other homologue is frequently lost through a gross chromosomal rearrangement such as deletion or nondisjunction, which is manifested as LOH for polymorphisms surrounding the gene (8).

The frequency of LOH in SN as found in our study is strikingly similar to the frequency found in a series of BCC (9). The possible reasons for why LOH was not detected in 50% of SN include: mutation without deletion; intragenic microdeletion, or deletions that did not expand to adjacent microsatellite markers; methylation of CpG islands; and other undetected genetic factors that might contribute to SN/BCC development (10). The genetic defect at 9q22.3 might possibly also contribute to the development of other appendageal tumors in SN, e.g., syringocystadenoma papilliferum, primitive follicular germs, and trichoblastomas. To date, however, these tumors have not been genetically analyzed.

Here, we provide the first evidence of the involvement of the tumor suppressor gene PTCH in SN. Our study may support, on a molecular basis, the clinical observation of the development of a subset of SN into BCC.

**Table 1** Results of LOH studies using the markers D9S252, D9S15, D9S287, and D9S303 in SN

<table>
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<th>Case no.</th>
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*NL, no loss; --, noninformative/failed amplification.*
References

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