Occult Neurofibroma and Increased S100 Protein in the Skin of Patients With Neurofibromatosis Type 1

New Insight to the Etiopathomechanism of Neurofibromas

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Background: Neurofibromas represent proliferation of the connective tissue cells of peripheral nerves and deposition of collagenous extracellular matrix. There is evidence that the appearance and growth of neurofibromas may be associated with prior or ongoing mechanical trauma in patients with neurofibromatosis type 1 (NF1).

Objective: To study the histologic characteristics of apparently healthy skin of patients with NF1.

Design: The histologic features of healthy-looking skin of patients with NF1 were analyzed.

Setting: University hospital.

Patients: Ten patients who fulfilled the criteria for NF1.

Interventions: Punch biopsy specimens of healthy-looking skin of the forearm from 9 volunteer patients and of the upper eyelid during cosmetic operation from 1 volunteer patient were obtained.

Main Outcome Measures: The main outcomes were not predicted, and the hypothesis was formulated during data collection.

Results: Apparently unaffected skin of 5 patients with NF1 was studied by routine histologic testing with respect to expression of S100 protein. Unexpectedly, analysis of the samples revealed the presence of a small neurofibroma tumor in one of the samples. The tumor was located in deep dermis around a hair follicle. In addition, neurofibromatous tissue not large enough to be called a tumor was found on the same anatomical location in another patient. In further studies, 10 punch biopsy specimens of apparently healthy skin from patients with NF1 were similarly sectioned and analyzed. No tumors were found in these additional samples. In 4 patients, however, abundant S100 protein–positive cells were located within collagenous extracellular matrix surrounding hair follicles.

Conclusions: The skin of patients with NF1 might be more widely affected than previously thought and occult neurofibromas are not rare.

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PATIENTS AND METHODS

For research purposes, 6-mm punch biopsy specimens were taken of healthy-looking skin of the forearm from 9 volunteer patients with NF1 and of the upper eyelid during cosmetic operation from 1 volunteer patient with NF1. The patients fulfilled the criteria for NF1. The study was accepted by the Ethical Committee of Medical Faculty of Oulu University, Oulu, Finland.

The skin biopsy specimens were fixed in 10% formalin solution, embedded in paraffin, and cut into 5-µm sections. Sections were prepared with routine hematoxylin-eosin stains. Dermal Schwann cells were detected by immunohistochemical labeling for the S100 protein using a polyclonal rabbit, anti-bovine S100 antibody and the streptavidin-biotin technique (StreptABComplex/HRP kit; Dako A/S, Glostrup, Denmark) according to the instructions of the manufacturer.

RESULTS

Histologic analysis of 6-mm punch biopsy specimens of apparently healthy-looking skin of 10 patients with NF1 was performed. Seven biopsy specimens showed increased numbers of fibroblasts around hair follicles, and 4 of them also contained S100-positive spindle cells among fibroblasts (Figure 1). The specimens were further analyzed in serial sections, and in one specimen, a small tumor of 3 to 5 mm in diameter was found to encircle a hair follicle. Histopathologic findings were consistent with a neurofibroma: the spindle-shaped tumor cells with regular nuclei were embedded in a stroma of delicate collagen bundles (Figure 2). Furthermore, S100 immunolabeling demonstrated a positive reaction in most of the tumor cells. Since dendritic cells of the epidermis are not known to form tumors with these histopathologic findings, the S100 protein–positive cells in this specimen were identified as Schwann cells (Figure 3). No corresponding findings were found in similar sections from 6 healthy control subjects.

COMMENT

We describe neurofibromas observed by chance in healthy-looking skin of a patient with NF1. This patient reported neither previous operation nor trauma in this area. The typical histopathologic appearance and an abundant number of S100-positive cells identified the tumor as neurofibroma. To our knowledge, this is the first report describing neurofibromas occurring in unaffected or apparently healthy skin of a patient with NF1.

The NF1 gene has been referred to as tumor suppressor gene since cells of malignant schwannomas of patients with neurofibromatosis may display loss of heterozygosity of the NF1 gene. Somatic mutations of the NF1 gene have also been found in malignant tissues of otherwise healthy persons. Furthermore, the levels of NF1 protein and/or messenger RNA have been reported to be altered in certain proliferative diseases, such as transitional cell carcinoma and psoriasis. On the other hand, neurofibromas have been described to develop on locations of previous mechanical trauma. Interestingly, recent results demonstrate up-regulation of NF1 gene expression in response to tissue trauma and suggest that a “second hit” in a form of somatic mutations in lesional neurofibromatosis cells is not necessarily a prerequisite for the development of neurofibromas. Atit et al have shown that, in mice with NF1 mutations, injury promoted specific features of neurofibromas found in human patients.
including fibroblast hyperplasia and collagen accumulation. This study supports the view that trauma and dysfunction of NF1 mutant fibroblasts could contribute to human neurofibroma formation. The present study shows microscopic neurofibroma around hair follicles; in the latter case, there was an adjacent arrector pili muscle. We speculate that continuous mechanical stress or injury caused by the muscle to the hair follicle might induce the formation of neurofibroma.

A recent report showed folliculo-sebaceous stimulation in neurofibromas in 2 unrelated patients with NF1. In one of these cases, there was a typical microcomedo in the center of the neurofibroma, and neurofibromas were regarded as stimulators of these follicular structures. However, we propose an opposite finding in which developing neurofibromas were found in healthy-looking skin around hair follicles. This finding suggests that hair follicles might be stimulators of the fibroblasts of surrounding collagenous tissue and Schwann cells of adjacent nerve endings.

In conclusion, our data suggest that, apart from clinically involved skin, apparently healthy-looking skin of patients with NF1 can be affected. Passive and/or active mechanical stress from arrector pili muscles is speculated to be a factor that induces the development of neurofibromas. Our findings do not, however, exclude the possibility of somatic NF1 mutations in these tumors and contradict the “second hit” hypothesis, which includes the loss of normal function of both NF1 alleles. In fact, genetic and epigenetic factors may both be operative in the pathogenesis of neurofibromas.

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