Expression patterns of the glial cell line–derived neurotrophic factor, neurturin, their cognate receptors GFRα-1, GFRα-2, and a common signal transduction element c-Ret in the human skin hair follicles

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Background: Glial cell line–derived neurotrophic factor (GDNF) and a related family member, neurturin (NTN), and their cognate receptors (GFRα-1 and GFRα-2, for GDNF and NTN, respectively) are distal members of the transforming growth factor-β superfamily. They are involved in the control of murine hair follicle (HF) cycling. This study tests the hypothesis that GDNF and NTN, and their cognate receptors, are expressed in the human HF and their expression varies in the different stages of the HF cycle.

Methods: The expression pattern of these proteins was examined in human HF by immunofluorescence, immunoalkalinephosphatase staining methods, and reverse transcription-polymerase chain reaction (GDNF). The functional effects (GDNF and NTN) were examined in organ culture of the microdissected HFs.

Results: GDNF, NTN, GFRα-1, GFRα-2, and c-Ret proteins were weakly expressed in catagen and telogen HFs. In contrast, they were strongly expressed in the epithelial and mesenchymal compartments of the anagen HF. GDNF gene was transcribed, both in the human scalp skin and in the isolated anagen HFs (reverse transcription-polymerase chain reaction). In HF organ culture, GDNF (but not NTN) increased the number of the proliferating HF keratinocytes (Ki 67 + cells). GDNF partially protected HFs from transforming growth factor-β2–induced premature catagen transition.

Limitations: None.

Conclusions: GDNF, NTN, GFRα-1, GFRα-2, and c-Ret proteins are differentially expressed in the different stages of HF cycle. GFRα-mediated signaling involves c-Ret and may play a role in human HF biology. (J Am Acad Dermatol 2008;58:238-50.)

Glial cell line–derived neurotrophic factor (GDNF), neurturin (NTN), artemin, and persephin are members of the GDNF family, which belongs to the transforming growth factor (TGF)-β superfamily.[1-3] Ligands of the GDNF family signal through a receptor complex consisting of a transmembrane tyrosine kinase signaling subunit (c-Ret), which is common to all GDNF family ligands, and a ligand binding subunit GFR-α. Four different GFRα family members, GFRα-1, GFRα-2, GFRα-3, and GFRα-4, operate as cognate receptors

Abbreviations used:

CTS:	connective tissue sheath
DP:	dermal papilla
GDNF:	glial cell line–derived neurotrophic factor
HF:	hair follicle
IR:	immunoreactivity
IRS:	inner root sheath
NTN:	neurturin
ORS:	outer root sheath
PCR:	polymerase chain reaction
RT:	reverse transcription
TBS:	Tris-buffered saline
TGF:	transforming growth factor

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for GDNF, NTN, artemin, and persephin, respectively.[4,5] GDNF family ligands were originally discovered in the nervous system as survival factors for embryonic and adult neurons.[1,6-8] GDNF ligands and their receptors are expressed in the developing retina, inner ear, skeletal muscle, chromaffin cells, and mice thymocytes.[1,9]

GDNF ligands and their receptors play important roles in promoting neurite and axon outgrowths from neurons.[10,11] They exert protective functions for degenerating and chemically damaged neurons.[12] They are also essential factors for the development of organs formed on the basis of complex epithelial-mesenchymal interaction such as kidneys and teeth.[1,13,14] The GDNF family of proteins plays a critical role in the development and homeostasis of ectodermally derived structures. Albers et al[15] examined the functional properties of GDNF-dependent nociceptors and the cellular and physiologic properties of sensory neurons of wild-type and transgenic mice that overexpress GDNF in the skin using a skin, nerve, dorsal root ganglion, and spinal cord preparation, immunolabeling, and reverse transcriptase (RT)-polymerase chain reaction (PCR) assays. They reported an increase in peripheral conduction velocity of C-fibers in mice that overexpress GDNF in the skin. They showed that isolectin B4−positive neurons, many of which are responsive to GDNF, exhibited significantly lower thresholds to mechanical stimulation relative to wild-type neurons. Their data raise the notion that enhanced expression of GDNF in the skin can change mechanical sensitivity of isolectin B4−positive nociceptive afferents and that this may occur through enhanced expression of specific types of channel proteins.

GDNF family members are involved in hair cycle control in mice.[7,15,16] GDNF and NTN are detected in a classic neuroectodermal-mesodermal interaction system, the murine hair follicle (HF), where they inhibit HF regression (catagen).[16-18] Botchkareva et al[16] have investigated the expression patterns and potential functions of GDNF, NTN, GFRα-1, and GFRα-2 in murine skin during the cyclic transformation of the HF from its resting state (telogen) to active growth (anagen) and then through regression (catagen) back to telogen. GDNF protein and GFRα-1 messenger RNA were strongly expressed in telogen skin, which lacks NTN and GFRα-2 transcripts. Early anagen development was associated with a marked decline in the skin content of GDNF protein and GFRα-1 transcripts. During the anagen-catagen transition, GDNF, GFRα-1, NTN, and GFRα-2 transcripts reached maximal levels. Compared with wild-type controls, GFRα-1 ($^{+}/^{-}$) and GFRα-2 ($^{-}/^{-}$) knockout mice showed a significantly accelerated catagen development. Interestingly, the administration of either GDNF or NTN markedly retarded HF regression in organ-cultured mouse skin.

As both cutaneous epithelium and the nervous system have a common neuroectodermal origin, it is conceivable that GDNF ligands and their receptors are also involved in HF morphogenesis.[1,9] In support, GDNF messenger RNA transcripts are expressed in developing rat skin[19]; these neurotrophic factors are also expressed in murine HFs.[20] The expression pattern of GDNF ligands and their receptors in the human HF is unknown to date.

This investigation tests the hypothesis that GDNF and NTN, and their cognate receptors, are expressed in the human HF and their expression varies with the different stages of the HF cycle. To test this hypothesis, we examined the expression pattern of these proteins in the healthy human scalp HFs during the different stages of HF cycle, and the functional effects exerted by GDNF and NTN on the human HF in organ culture.

METHODS

Skin samples

A total of 50 normal-appearing human scalp skin specimens were obtained from 50 women (age: 53-57 years; after informed consent) undergoing elective cosmetic plastic surgery. None of these women had any accompanying primary hair disorder. The specimens were obtained from both the frontal and temporal regions of the scalp. After surgery, samples were maintained in Williams E Medium (Biochrom KG Seromed, Berlin, Germany) for transportation at 4°C and cryopreservation or culture within 24 hours. Skin specimens used for cryosections were shock-frozen in liquid nitrogen and stored at −80°C until used. Before immunostaining, samples were embedded in cryogel and processed for longitudinal cryosections (8 μm). Sections were dried, fixed in cold acetone (−20°C), and stored at −20°C until used for immunohistochemistry. Skin samples used for semiquantitative RT-PCR analysis were classified into 4 groups. The first group included intact full-thickness scalp skin samples immediately frozen in liquid nitrogen. The second group consisted of scalp skin samples cut through at the dermosubcutaneous fat interface below the sebaceous gland, from which the HFs were quickly microdissected[21] and frozen in liquid nitrogen. The samples of the third group were frozen in liquid nitrogen after extraction of terminal anagen VI HFs. The fourth group included samples of the frontal skin containing only vellus hairs, which were quickly frozen in liquid nitrogen. All samples were stored at −80°C for further RT-PCR analysis.