Archives of Biological and Biomedical Research
An International Journal

http://dx.doi.org/10.18576/abbr/010203

Immunohisto Chemical Expression Patterns of Glial Cell Line-Derived Neurotrophic Factor and its Cognate Receptor GFRα-1 in Lichen Planus

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Received: 25 Aug. 2017, Revised: 28 Sep. 2017, Accepted: 29 Sep. 2017.
Published online: 31 Oct. 2017.

Abstract:

Background: Glial cell line-derived neurotrophic factor (GDNF), produced by basal cell keratinocytes, is a neurotrophic factor that plays diverse developmental roles and together with its cognate receptor (GFR Rα-1) are distal members of the transforming growth factor- β super family. To date, their expression pattern in lichen planus is still unknown. **Objectives:** To investigate the immunohis to chemical expression patterns of GDNF and its cognate receptor (GFRα-1) in lichen planus and compare the results with the normal skin from healthy controls. **Patients and Methods:**Twenty patients with lichen planus were examined in addition to twenty age and sex matched healthy subjects (the control group). Punch biopsies from lesional and normal healthy skin were performed. Sections were examined using immunohis to chemical staining methods for GDNF and GFRα-1proteins expression patterns expression analysis. **Results:** There was strong GDNF protein expression in the basal cell keratinocytes and moderate gene expression in the spinous layer and weak gene expression in the granular layer of the healthy skin. In contrast, in lichen planus, GDNF protein expression was absent in the basal cell keratinocytes and lower reaches of the spinous layer. Weak gene expression was noted in the upper reaches of the epidermis. In both healthy and lichen planus skins, the expression of GFRα-1in the adnesal structures showed no differences.**Conclusion:** The altered GDNF protein expression may contribute to the pathogenesis of lichen planus.

Keywords: GDNF; GFRα-1; Immunohis to chemistry; Lichen planus

1 Introduction:

Lichen planus is an inflammatory, severely pruritic disease characterized by distinictive papulosquamous lesions with violaceous color, polygonal shape, commonly found on the flexor surfaces of the upper extrimities. It may be accompanied by oral and genital mucous membrane affection. The disease affects less than 1% of the adult population [1].Germ cell

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line-derived neurotrophic factor (GDNF) belongs to a family of neurotrophic factor, the GDNF family, which is closely related to the transforming growth factor-beta (TGF- β) superfamily [2-4]. GDNF family ligands were originally discovered in the nervous system as potent survival factor for embryonic and adult central dopaminergic, noradrenergic and motor neurons as well as a variety of peripheral neurons [5].In the skin, GDNF has important roles in the development and maintenance of skin, nerve fibers, hair growth and hair follicle cycling [6, 7]. GDNFβ-1 is produced by many cell types including epidermal keratinocytes and is considered as one of the keratinocytes-derived cytokines[8].There is an abnormal TGF-β signal transduction in CD8+Tcells of oral lichen planus, which may contribute to the persistence of the chronic inflammation in the disease [9].So, neurotrophic factors such as GDNF seem to have an important role in the homeostasis of the skin.

To date the expression patterns of GDNF and its cognate receptor (GFRα-1) in lichen planus are unknown. To address this issue, we investigated the expression of GDNF protein in LP using immunohistochemical methods.

2 Methods:

2.1 Study Design and Patients Selection

A cross sectional case-control study was carried out on20 patients with lichen planus, including 13 males and 7 females. They were recruited from the Dermatology, Venereology and Andrology Outpatient Clinic at Sohag and Aswan University Hospitals, Egypt, after approval of university hospital ethical committee and in accordance to the guidelines of the Helsiniki declaration. In addition, 20 healthy age and sex matched subjects selected as the control group. Prior to initiation of the study, every subject was informed about the aim of the study and gave a written consent. Patients with lichen planus (no mucosal lesions) who did not receive any treatment for at least three months were included in the study.with skin lesions only and those receiving immunosuppressive treatments were excluded from the study.

2.2 Immunohistochemical Assays of GNDF and GFRα-1 Protein Expression Patterns

Punch skin biopsies were obtained from the lichen planus lesions of the patients and healthy controls (under local anesthesia, 0.5%lidocaine). The samples were fixed in formalin and processed for paraffin sectioning and immunohistochemical studies at the laboratory of Biology and Zoology Department, Faculty of Science, Sohag University, Egypt. Paraffin sections were deparaffinized and hydrated then they were washed in TBS buffer (Tris buffer solution composed of Tris HCL: 6.19 g, NaCL: 8.8 g, Aq. Dest.: 1000 ml at pH 7.6) for further hydration. Subsequently, the slides were washed in TBS + 3.5 % H2O2, and then they were washed again in TBS buffer. Antigen retrieval was done using trypsin (dilution: 0.1% and duration of exposure, for 15 min) [10, 11].

Then, sections were incubated in protein blocking agent. Primary antibodies (Rabbit polyclonal IgG anti-human GDNF antibody and Rabbit polyclonal IgG anti-human GFRα-1 antibody, Sigma-Aldrich) were diluted (1:500) in TBS containing 2% normal serum (gout for both GDNF and GFRα-1, Sigma-Aldrich). The slides were incubated with 50µl of the primary antibody solution for 60 min at room temperature.

The sections were then washed three times in TBS buffer for 5 minutes at room temperature and then incubated with 50µl of goat anti-rabbit secondary antibody (Secondary antibodies included biotinylated goat anti-rabbit antibody and biotinylated rabbit anti-goat antibody, Sigma-Aldrich) diluted 1:200 in TBS blocking buffer containing normal serum (gout serum for both GDNF and GFRα-1). After washing in TBS buffer (three times for 5min at room temperature), the detection system (peroxidase from horse raddish (Sigma-Aldrich) diluted at 1:1000 in PBS (Phosphate buffer solution, pH 6) buffer was added for 30 minutes at room

temperature. The slides were examined, by two observers (Dr. Adly and Dr. Hussein), in agreement. GDNF and GFRα-1 protein expression appeared as diffuse cytoplasmic staining. The staining results were scored as negative, weak, moderate and strong staining, following previous studies [12-16].

3 Results:

The mean age of the patients was 43.50±8 years and that control group was 40.60±10.11. In the healthy skin (control group), GDNF gene expression was strong in the basal cell keratinocytes and decreased gradually in the upper reaches of the epidermis (moderate expression in the spinous layer and weak reactivity in the granular cell layers). In contrast, in lichen planus skin GDNF gene expression was absent in the basal cell keratinocytes and lower reaches of the spinous layer. Weak gene expression was noted in the upper reaches of the epidermis. In both healthy and lichen planus skins, the gene expression of GFRα-1 was strong in the basal cell layer and moderate in the spinous layer and weak in the granular cell layer. The expression of GDNF and GFRα-1 genes was strong in the adnexa of both lesional and healthy skins (hair follicles, sweat and sebaceous glands) (Table 1 and Fig.1A, B, C, D, E &F).

Table 1. Expression patterns of GDNF and GFR-alpha 1 proteins in the healthy and lichen planus skins.

Aspects	GFRα-1 proteins		GDNF protein	
	Healthy skin	lichen planus skin	Healthy skin	lichen planus skin
Basal cell	Strong	Strong	Strong	Negative
Spinous cell	Moderate	Moderate	Moderate	Negative
Granular cell	Weak	Weak	Weak	Weak
Cornified layer	Weak	Weak	Weak	Weak
Adnexal structures	Strong	Strong	Strong	Strong

GDNF: Glial cell line-derived neurotrophic factor, GFR Rα-1: its cognate receptor

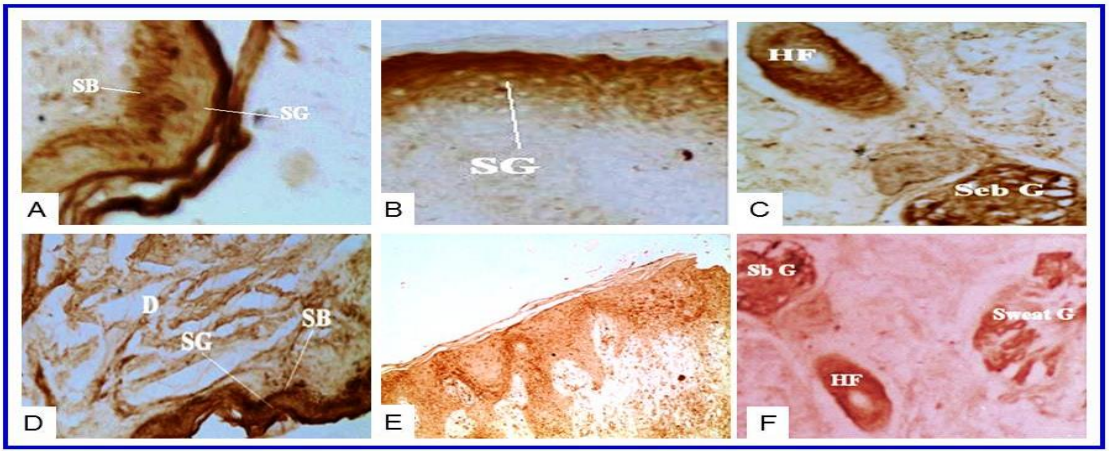

Fig.1. Immunohistochemical expression of GDNF and GFRα-1 proteins in the healthy and lichen planus skins. In healthy skin, the expression of GDNF protein was strong (basal cell keratinocytes), moderate (spinous layer) and weak (granular cell layer) **(A).** In lichen planus skin, GDNF expression was absent in the basal and spinous layer and weak in the upper reaches of the epidermis **(B).** Strong GDNF expression in the adnexal structures in lichen planus skin **(C).** In both healthy and lichen planus skins, GFRα-1 protein expression was strong (basal cell keratinocytes and melanocytes), moderate (spinous and granular cell layers). In both healthy and lichen planus skins, the expression of GDNF and GFRα-1 proteins was strong in the adnexal structures **(D-F).** (D: dermis, HF: hair follicle, SB:stratum basal, Sb G or Seb G: sebaceous gland, SG=stratum granular)

4 Discussion:

Here we report, for the first time, the expression pattern of GDNF and its congnate receptor GFRα-1 in lichen planus.In the healthy skin, the expression of GDNF and GFRα-1 by the epidermal keratinocytes and adnexal structuressuggests its possible roles in the keratinocytehomeostasis [13, 14, 17]. We found strong GDNF and GFRα-1 protein expression in the basal cell keratinocytes with gradual decrease towards the granular cell layer. These results are in accordance with those of Adly et al [13-15].This differential expression of GDNF suggests that its expression correlates with the degree of differentiation and keratinization of the keratinocytes [18].GDNF proteins represent distant members of the TGF-β1 superfamily. The strong expression of GDNF and GFRα-1 in our study is in agreement with several studies indicating that the members of TGF-β1 superfamily are not only expressed but also involved in the control of keratinocyte proliferation and differentiation [19]. It is possible that GDNF and their receptors may operate as an additional neuronal signaling molecule regulating skin functions [20].

In lichen planus, we found loss of GDNF protein expression in the basal cell keratinocytes and moderate expression in the upper reaches of the epidermis. It is possible that the loss of GDNF protein expression in the basal cell keratinocytes may contribute to the mechanisms of apoptotic cell death (Civatte bodies) in these lesions. GDNF and other neurotrophic factors such as nerve growth factors promote cell survival by suppressing the intrinsic cellular apoptotic machinery [21, 22]. This occurs through binding of these neurotrophic factors to membrane receptors and by regulation of intracellular signaling molecules such as Bcl-2, TNF-a and sFas [6, 23].

GDNF and its cognate receptor GFRα-1 are distal members of TGF-β superfamily. The down-regulation of GDNF in lichen planus as revealed by our study are in agreement with expression pattern of TGF-β1 in the lichen planus [24].El-Rebery et al [25] evaluated the possible role of TGF-β1 in the pathogenesis of lichen planus. TGF-β1 was analyzed in skin biopsies of patients with lichen planus and healthy skin (control). TGF-β1was expressed in keratinocytes of 70% of the control group and 48% of lichen planus cases with a significant difference in intensities of TGF-β1 protein expression. The authors concluded that the down regulation of TGF-β1expression in lichen planus may participate in immune dysregulation of the disease. Taghavi et al [24] examined the serum levels of TNF α-1and TGF-β in patients with oral lichen planus. There was a significant decrease in the serum levels of TGF-β1and a significant increase in the serum levels of TNF- α-1 in patients with oral lichen planus [26].

GDNF is a neurotrophic factor functioning mainly in the nervous system [27].It has important roles in the development and maintenance of skin nerve fibers [28, 29]. GDNF binds to a specific co-receptor GFRα-1, leading to the formation of a heterotetramer complex, which then interacts with receptor tyrosine kinase RET (the signaling receptor). Ongoing studies continue to uncover potential new roles for the components of the neurosensory system in skin homeostasis and disease states. As our understanding of the interactions among the cutaneous neurosensory system and the various components of the skin and the immune system in times of health and disease increases, specific treatments modulating the neurocutaneous system will find their way into the armamentarium of daily dermatologic therapy [30].

In conclusion, herein we report the expression pattern of GDNF and GFRα-1 in lichen planus skin. As the pathogenesis of lichen planus is related to apoptosis of the basal cell keratinocytes, it is possible that the down regualtion of GDNF in lichen planus skin contributes to the development of these lesions. Our findings may have some therapeutic ramifications. Our results suggest important roles for these proteins in the pathogenesis of lichen planus. Further studies are recommended to investigate the expression of these molecules

at the gene and mRNA levels. This will help the understanding of the pathogenesis of the disease and finding new lines for its treatment.

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