

The Skin Neurotrophic Network in Health and Disease

R.G. Borroni, F. Truzzi and C. Pincelli

Institute of Dermatology. School of Biosciences and Biotechnologies. University of Modena and Reggio Emilia. Modena. Italy.

Summary. Neurotrophins (NTs) belong to a family of structurally and functionally related proteins that, depending on the tissue context and the receptors involved, promote neuronal cell survival and differentiation or cell death. NTs also exert important functions in other organs besides the nervous system, including the skin. The presence in the skin of diverse cell types which are able to secrete and/or to respond to stimulation by NTs creates a unique network of molecular signaling in the cutaneous microenvironment. This review summarizes currently available data on the expression and function of NTs and their receptors in several cell types in the skin (namely, keratinocytes, melanocytes and fibroblasts). The role of the skin NT network in the development and maintenance of some relevant skin diseases is presented and the potential implications for therapeutic intervention are discussed.

Key words: neurotrophins, Trk, p75NTR, skin, psoriasis, melanoma.

LA RED NEUROTRÓFICA CUTÁNEA EN PIEL SANA Y ENFERMA

Resumen. Las neurotrofinas (NT) pertenecen a una familia de proteínas relacionadas estructural y funcionalmente que, dependiendo del tejido y del receptor implicados, promueven la supervivencia y diferenciación neuronal o la apoptosis. Las NT también ejercen importantes funciones en otros órganos, aparte del sistema nervioso, incluyendo la piel. La presencia de diversos tipos celulares en la piel que son capaces de segregar o responder al estímulo de las NT, crea una red única de señalización molecular en el microambiente cutáneo. Esta revisión resume los datos disponibles actualmente sobre la expresión y función de las NT y sus receptores en diversos tipos celulares de la piel (a saber: queratinocitos, melanocitos, fibroblastos). Se discute el papel de la red de NT cutáneas en el desarrollo y mantenimiento de algunas enfermedades cutáneas relevantes, así como las implicaciones potenciales para una intervención terapéutica.

Palabras clave: neurotrofinas, Trk, p75NTR, piel, psoriasis, melanoma.

Introduction

The nervous system and the epidermis share a common ectodermal origin. Indeed, neurotrophins (NTs) that regulate the development and function of neurons are also involved in the control of epithelial homeostasis and skin remodeling¹⁻⁴. In this respect, NTs fulfill multiple functions and regulate cell proliferation, differentiation, apoptosis, and tissue remodeling^{5,6}. During the last decade, substantial progress has been achieved in defining the roles of NTs and their receptors in the control of skin homeostasis. Here, we review the molecular mechanisms underlying the involvement of NTs in the control of

non-neuronal functions in normal skin, as well as the role of NTs in a number of pathological skin conditions, focusing on psoriasis, melanoma and wound healing. We envision that interfering with these complex interactions might provide novel strategies for treatment of several skin diseases.

Neurotrophins and Their Receptors

NTs belong to a family of structurally and functionally related proteins that, depending on the tissue context and the receptors involved, promote neuronal cell survival and differentiation or cell death⁷. The NT family includes four members: nerve growth factor (NGF), brain-derived neurotrophic growth factor (BDNF); NT-3, and NT-4. NTs exert their effects by binding two classes of transmembrane receptors, the tyrosine kinase high-affinity receptor Trks (TrkA, TrkB, and TrkC) and the low-affinity neuro-

Correspondence:
Carlo Pincelli.
University of Modena and Reggio Emilia.
Department of Medicine. Dermatology Division.
Via del Pozzo 71.
41100 Modena. Italy
carlo.pincelli@unimore.it

trophin receptor p75NTR, the latter belonging to the tumor necrosis factor receptor (TNFR) family. NGF selectively binds TrkA, BDNF and NT4 bind TrkB, and NT3 binds TrkC and, with low affinity, TrkA⁸. All NTs bind p75NTR with equal low affinity⁹. NTs perform different biological functions according to the interaction with Trk or p75NTR¹⁰. When NTs bind to Trk, dimerization and autophosphorylation of the receptor occur, promoting survival and differentiation¹¹. On the other hand, the role of p75NTR is still controversial: in the presence of Trk, p75NTR increases high-affinity NT binding, thereby enhancing Trk ability to promote survival¹². By contrast, in the absence of Trk, p75NTR can induce apoptosis, via its own signal transduction, by interacting with a growing number of downstream molecules¹³. Recently, it has been shown that the proform of NT, proNGF, binds p75NTR in association with its co-receptor sortilin, but does not bind Trk¹⁴. More specifically, sortilin, a member of the vps-10 protein family, binds the “pro” region of NGF, whereas p75NTR binds mature NGF. The p75NTR-sortilin complex couples with proNGF to induce apoptosis¹⁵.

NTs also operate in a number of non-neuronal cells^{16,17}. In the skin, a complex NT network exists in which various cells are either the source or the target of NTs, thus exerting autocrine and paracrine functions^{18,19}. NTs are active on several cell types in the skin. Besides keratinocytes, melanocytes and fibroblasts (discussed below), NGF activates also mast cells²⁰ and prevents their apoptosis²¹. Moreover, NGF stimulates the proliferation of human microvascular endothelial cells²² and is an autocrine survival factor for lymphocytes²³.

Neurotrophins and Keratinocytes

Normal human keratinocytes synthesize and secrete biologically active NGF^{24,25}. In addition they express both the low-affinity (p75NTR) and the high-affinity (TrkA) NGF receptors. Therefore NGF appears as a key player of an autocrine loop through its binding with TrkA, acting as a mitogen and as a survival factor for human keratinocytes^{26,27}. Inhibition of TrkA phosphorylation with the natural alkaloid K252a blocks keratinocyte proliferation²⁶. Correspondingly, the proliferation rate of keratinocytes transfected with NGF is significantly higher than that of mock-transfected cells²⁸ as well as that of TrkA-overexpressing keratinocytes compared to controls²⁹.

The autocrine NGF-TrkA loop in the epidermis is involved in the maintenance of keratinocyte “stemness”. Remarkably, NGF appears to be expressed and secreted in higher levels by epidermal stem cells (ESC) as compared with more differentiated cells *in vitro*^{30,31}. In addition, TA cells, more differentiated than ESC, express p75NTR at higher levels compared to ESC (Truzzi et al, manuscript

in preparation). This is intriguing in view of the fact that ESC are protected from cell death³² and one could speculate that autocrine NGF exerts its anti-apoptotic activity mostly on this keratinocyte subpopulation. On the other hand, it is interesting to notice that the other NTs, which are not elevated in ESC, do not exert anti-apoptotic activity.

Apoptosis plays a fundamental role in epidermal homeostasis by counter-balancing cell proliferation, and apoptotic cells are consistently present in normal human epidermis. In addition to promoting proliferation, NTs and their receptors modulate susceptibility to apoptosis in the epidermis. Although ultraviolet (UV) B downregulates both NGF and TrkA in human keratinocytes²⁸, autocrine NGF also protects human keratinocytes from UVB-induced apoptosis²⁸. Because normal human keratinocytes typically lack TrkB expression, NTs may exert different functions in this system, by binding p75NTR alone. In this context, p75NTR acts as a proapoptotic receptor. This is exemplified by BDNF and NT4, which induce a higher rate of apoptosis in normal human keratinocytes overexpressing p75NTR, as compared to mock-transfected cells. On the other hand, p75NTRsiRNA-transfected keratinocytes fail to undergo cell death after administration of NT4.

Neurotrophins and Melanocytes

Melanocytes are neural crest-derived cells responsible for pigmentation. In human skin they reside at the dermo-epidermal junction and in the hair matrix. The overall low amounts of NTs synthesized by melanocytes do not support their role, under physiological conditions, as an active source of NTs¹⁹. Conversely, melanocytes rather seem to be targets of the NT skin network in a paracrine fashion because they express all the NT receptors both *in vitro* and *in vivo*¹⁹.

One of the effects of NTs on melanocytes is the protection from UV-induced oxidative stress and apoptosis. Not only the secretion of NTs by melanocytes is modulated by UVB, but the secretion of NT3, induced by UVB, promotes the synthesis of tyrosinase and tyrosinase-related peptide (TRP)-1, critical enzymes of melanin biosynthesis¹⁹. When melanocytes are irradiated with UV, NGF markedly reduces apoptosis through upregulation of the antiapoptotic Bcl-2 protein³³. When melanocytes are maintained in growth factor-depleted medium, NGF and NT-3, the latter expressed by dermal fibroblast, promote melanocyte survival³⁴. NGF is also chemotactic for melanocytes and induces at least in part melanocyte dendricity *in vitro*²⁶.

Taken together these data suggest that NGF, constitutively secreted by neighboring epidermal keratinocytes, may preserve epidermal melanocytes from environmental stress.

The role of NT3 on melanocytes is different: this growth factor, strongly expressed by non-proliferating fibroblasts like those in the dermal compartment of non-damaged human skin, could help in melanocyte maintenance under steady-state conditions.

When melanocytes are induced to proliferate *in vitro* with the administration of 12-O-tetradecanoylphorbol13-acetate (TPA), a tumor promoter and a strong activator of protein kinase C, the expression of p75NTR and of TrkA is upregulated. On the other hand, melanocytes constitutively express TrkC but, in contrast with TrkA expression, TrkC expression is decreased after stimulation with TPA, suggesting that although melanocytes can bind both NGF and NT-3, different signals that preferentially induce a specific high-affinity receptor determine which NT would exert its effect.

Neurotrophins and Fibroblasts

Dermal fibroblasts and myofibroblasts, obtained by stimulation with TGF- β , are sensitive to NT cues, as they express all Trks and p75NTR. In addition, they secrete all NTs. In particular, myofibroblasts present higher levels of p75NTR and TrkB than fibroblasts. On the other hand, they express TrkA at lower levels, whereas TrkC does not seem to be expressed by either cell type. Dermal fibroblasts and myofibroblasts also secrete NGF and NT3 at higher levels than NT4 and BDNF.

Thus, both paracrine and autocrine NTs stimulation may be involved in tissue remodeling and wound healing by inducing fibroblast differentiation and migration. All NTs promote fibroblast differentiation into myofibroblasts, as shown by the induction of α -smooth muscle actin expression, with an effect similar to that produced by TGF- β . Moreover, NGF and BDNF increase the tensile strength in a dose-dependent manner, as measured through the Glass Box Device. In addition, NGF, BDNF and NT3 also induce fibroblasts migration.

Neurotrophins in Skin Diseases

Psoriasis is a chronic-relapsing inflammatory disease characterized by acanthosis, hyperkeratosis, increased epidermal turnover. The skin NT network is implicated also in the abnormal epidermal homeostasis of this condition. The altered differentiation is reflected by abnormal expression pattern of NT receptors in psoriatic epidermis. On one hand, both NGF and TrkA are increased in psoriatic epidermis, compared to normal epidermis²⁹. On the other hand, p75NTR levels are significantly lower in psoriatic than in normal keratinocytes, while apoptosis is reduced in psoriatic keratinocytes. The rate of apoptosis in

psoriatic TA cells is significantly lower as compared to TA cells from normal epidermis. Interestingly, TA cells from psoriatic skin express lower levels of p75NTR compared to TA from normal skin. Thus, absence of p75NTR in TA cells could account for the resistance of psoriatic keratinocytes to apoptosis.

Melanoma, one of the most aggressive types of skin cancer, originates from melanocytes. Whereas melanocytes seem to benefit from the cutaneous neurotrophic network mainly as paracrine targets, once malignant transformation occurs melanoma cells acquire self-renewal capabilities mediated also by autocrine NT loops. Indeed all NTs, particularly NT-3 and NT-4, have been detected in conditioned medium of different melanoma cell lines³⁵. In addition, both the low- and the high-affinity NT receptors are expressed by primary and metastatic melanoma. Recent results show that melanoma cells proliferate through autocrine NT stimulation. K252a significantly reduces melanoma cell proliferation by inhibiting Trk phosphorylation. When autocrine NTs are removed from the culture medium by soluble Trk/Fc receptors, proliferation is significantly reduced. NGF, NT-3, and NT-4 induce cell migration in melanoma cell lines in a Boyden/Matrigel *in vitro* assay. Metastatic cell lines seem to be more susceptible to NT-induced migration as shown by independent chemokinesis assays. The migratory phenotype is necessarily dependent on the presence of both the high- and low-affinity NT receptors. Cells treated with p75NTR small interfering RNA (p75NTRsiRNA) fail to respond to NT stimulation. Similarly, the administration of K252a blocks melanoma cell migration, confirming that NTs stimulate melanoma cell migration and invasion via the cooperation of the low- and high-affinity receptors³⁵.

The relevance of the aforementioned data to skin pathology is particularly evident with respect to melanocytic nevi and melanoma. Several studies confirmed the expression of NTs and their receptors in surgical specimens of benign and malignant melanocytic tumors, which increases during progression from radial to vertical growth phase, to metastatic lesions. This is particularly true for TrkB, BDNF and NT-3³⁶. Interestingly TrkC, the specific receptor for NT-3, is significantly more expressed in thin melanomas compared to all other melanocytic lesions, indicating an autocrine stimulation during early melanoma progression³⁷. It is remarkable that whereas epidermal and hair follicle melanocytes do not express p75NTR, some nevi express p75NTR in a proportion of cells. The percentage of p75NTR-positive cells is even higher in primary cutaneous melanomas compared to melanocytic nevi, and melanoma metastases show the highest percentage of p75NTR-positive tumor cells³⁸. It is also noteworthy the correlation of p75NTR expression with spindle cell morphology. The strong p75NTR expression in desmoplastic and neurotropic melanomas^{39,40} supports its use a marker

for the diagnosis of these rare subtypes of melanoma that typically lack expression of melanocytic markers such as HMB45, melanA and tyrosinase⁴¹.

One of the first examples of translation of the results achieved by basic research on NTs into clinical practice is the use of NGF for the treatment of chronic cutaneous ulcers refractory to traditional therapies⁴². In conclusion, direct translation of the relevant data from the field of cutaneous neurobiology into clinically-oriented research and development will unravel novel therapeutic strategies for the treatment of skin disease targeting the skin NT network.

Conflict of interest

Authors have no conflict of interest to declare.

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