Ann Ist Super Sanità 2015 | Vol. 51, No. 1: 5-10
DOI: 10.4415/ANN_15_01_03

NGF and therapeutic prospective: what have we learned from the NGF transgenic models?

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Abstract

It has been shown that topical nerve growth factor (NGF) administration induces healing action on human cutaneous, corneal and pressure ulcers, glaucoma, maculopathy and retinitis pigmentosa suggesting a therapeutic potential of NGF in human ophthalmology and cutaneous ulcers. A similar therapeutic suggestion has emerged for the NGF gene therapy of Alzheimer's disease and ischemic heart injury. Moreover, over the last few years, the role and biological properties of NGF have also been investigated with transgenic mice over-expressing and down-expressing NGF. However, the results obtained with these transgenic mice seem suitable to confirm and/or support the evidence obtained with exogenous administration of NGF regarding the suggested clinical potentiality of NGF. The aim of the present brief review is to report and comment on these two different findings of NGF's healing properties.

Key words

- NGF receptor
- transgenic mice
- knockout mice
- NGF and therapy

NGF: DISCOVERY AND ONGOING FINDINGS

Since its discovery, nerve growth factor (NGF) has long occupied a critical role in developmental neurobiology because of the many important neuronal functions it has been shown to have [1]. NGF is the first discovered and best-characterised member of a family of neurotrophic factors, collectively indicated as neurotrophins, which include brain-derived neurotrophic factors and neurotrophin-3 (NT-3). These neurotrophic factors share significant structural homologies and overlapping actions [2] exerting its action on growth and survival of peripheral sensory and sympathetic neurons [3] (see *Figure 1A, B*) and on a number of brain neurons, particularly forebrain cholinergic neurons (FBCN) that are the major NGF-target cells within the central nervous system [4-6]. The molecule, initially de-

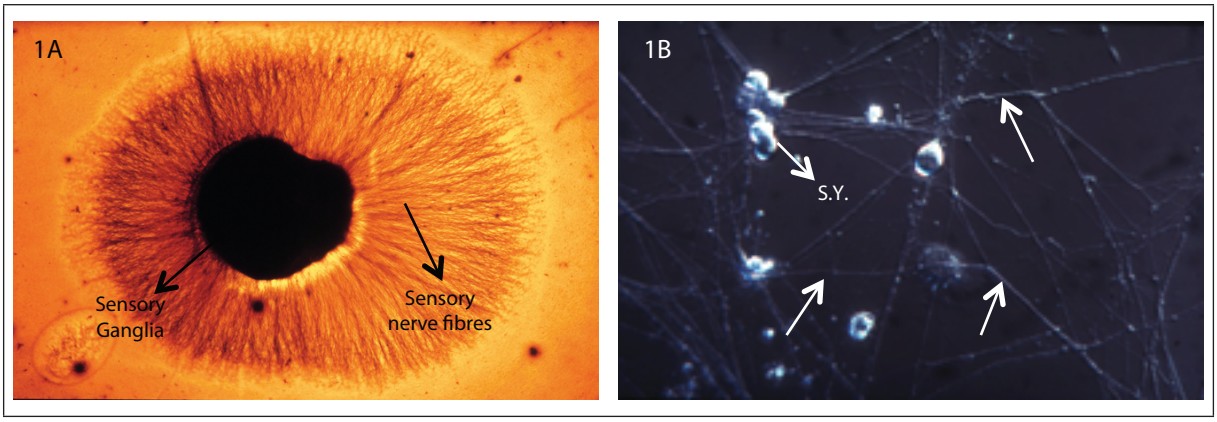

Figure 1

A) Photomicrographs of sensory ganglia (SG) removed from an eight-day-old chick embryo and cultured for 24h at 37.8 °C in the presence of nerve growth factor (NGF) showing a dense halo of sensory nerve fibres stimulated by NGF (arrows). **B)** Sympathetic nerve cells (SY) isolated from the superior cervical ganglia of newborn rats and cultured *in vitro* in the presence of 10 µg of purified NGF for four days. Note the presence of a large network of neuritis stimulated by NGF from single neurons (arrows).

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scribed as a neurotrophic factor acting only or mainly on growth and differentiation of peripheral sympathetic and sensory neurons (*Figure 1A, B*), resulted to possess a number of other target cells within the nervous system as well as extra-neuronal targets including cells in cutaneous, immune, endocrine [3, 6, 7] and adipose tissue [8]. The biological activity of NGF is mediated by two distinct receptors: TrkA (a tyrosine kinase receptor) and p75 (a member of the tumour necrosis factor receptor superfamily) [2, 9]. NGF's functional roles are supported by findings demonstrating that administration of anti-NGF antibodies in developing rodents down-regulates the circulating level of NGF, induces damages of peripheral and sympathetic NGF-target cells and ultimately leads to immunosympathectomy [10]. Because NGF is a rather high molecular-weight protein, it is unable to cross the blood-brain-barrier, and intracerebral administration produces undesired side effects. The role of NGF on brain target neurons has been studied using of NGF conjugated with small molecules [11] or gene therapy and transgenic animal models. These findings paved the way for further investigations on the role of NGF in learning and memory that undergo degeneration during age-related disorders including the role of NGF in learning, memory, brain neuronal degenerative diseases and Alzheimer's disease (AD) [4, 5, 12].

We have recently demonstrated that topical NGF administration promotes, in human cutaneous ulcers induced by pressure, diabetes, rheumatoid arthritis and corneal ulcers [13-16], and safety protects damaged retinal cell's degeneration in patients affected by glaucoma [5], maculopathy [17] and retinitis pigmentosa [18]. More recently, findings published by others indicated that NGF administered through gene therapy protected FBCN that degenerate in patients with AD, and reduced cell damages in myocardial infarction [19] and spinal cord injury [20]. These findings are summarised in *Table 1*.

During the last two decades, the biological properties of NGF have also been investigated using NGF transgenic mice models, over-expressing NGF (*Figure 2A*) or lacking NGF, knockout (KO) mice (*Figure 2B*). These transgenic animal models display a number of neuronal and non-neuronal deficits similar to those observed after exogenous administration of purified NGF or anti-NGF-antibody (ANA), but also revealed some contradictory effects, not only among different strains of NGF transgenic mice models, but also between NGF transgenic mice and mice treated with exogenous NGF administration. The aim of this brief review is, therefore, to compare and critically assess these differences and to discuss the NGF transgenic mouse model in order to support the hypothesis.

THE GENERATION OF TRANSGENIC MICE

In 1953, Watson and Crick published the structure of the double-strand helix model for DNA [21]. This discovery, and subsequent molecular related studies, provided a powerful tool for understanding biological, molecular and genetic mechanisms for a number of pathologies and human therapeutic applications [22-24]. Thus, the knowledge of the DNA structure was the first step to understand and interfere at the genomic

Table 1
NGF therapeutic potentiality based on human diseases

Disease	Dose	Treatment	Side effects	Result	Ref.
Neurotrophic keratite	10 µg*	4 weeks	None	Healed	[1] Lambiase A *et al.*
Cornea ulcer	10 µg*	6-8 weeks	None	Healed	[2] Lambiase A *et al.*
Glaucoma	10 µg*	12-15 weeks	None	Protective	[3] Lambiase A *et al.*
Maculopathy	10 µg*	15-20 weeks	None	Protective	[3] Lambiase A *et al.*
Vasculitic ulcer	20 µg**	20 weeks	None	Healed	[4] Tuveri M *et al.*
Pressure ulcer	20 µg**	10 weeks	None	Healed	[5] Landi F *et al.*
Diabetic ulcer	0.3 µg/kg***	6 weeks	None	Healed	[6] Generini S *et al.*
AD	Gene deliver	10-12 weeks	None	Improved	[7] Tuszynski MH *et al.*
Miocardial infarction	Gene deliver	?	None	Improved	[8] Meloni M *et al.*

*Eye drop; **Topical application; ***Gene deliver.

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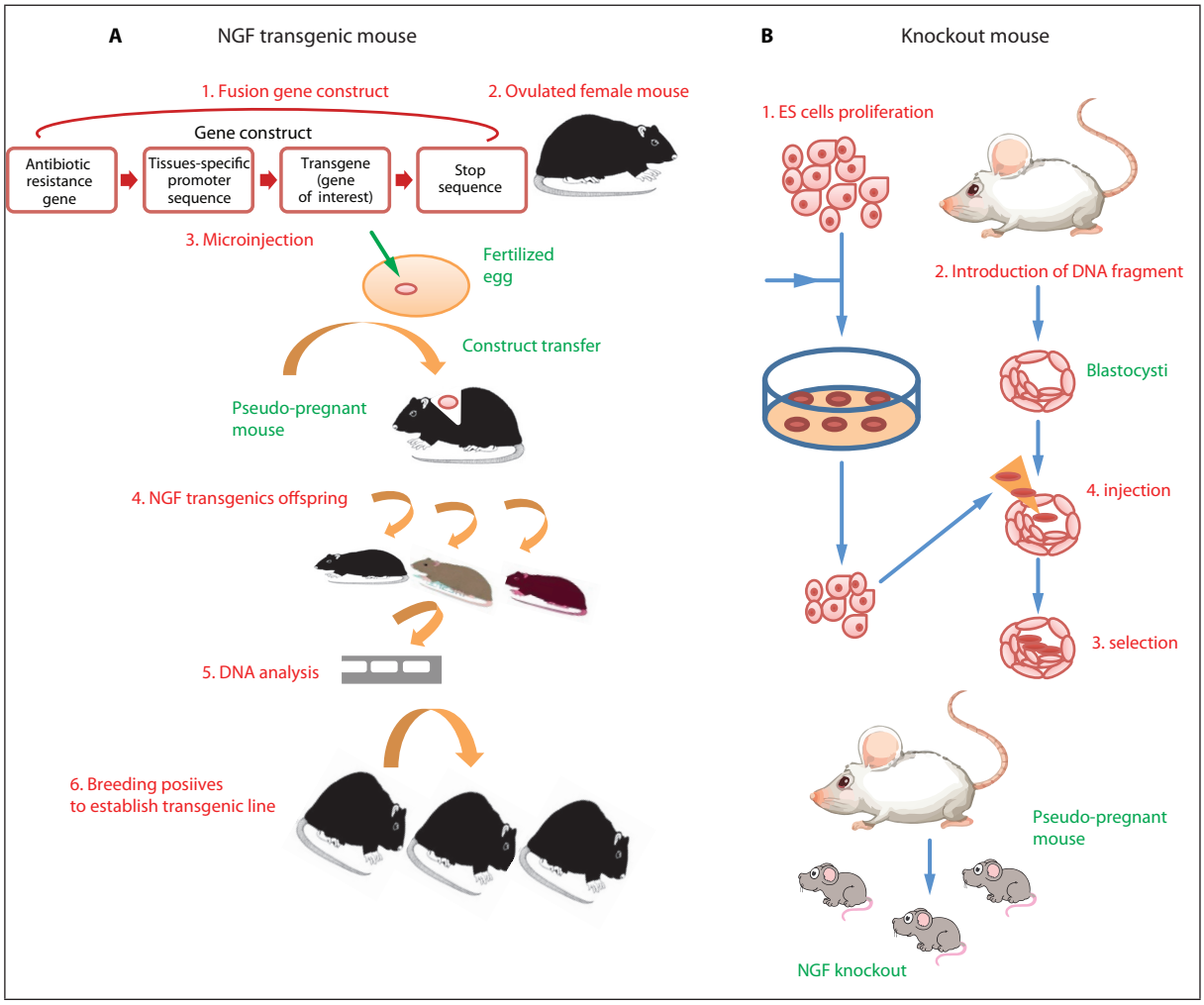

Figure 2
Schematic representation illustrating the critical steps for generating NGF-producing transgenic mice, over-expressing NGF (**A**) or transgenic mice, under-expression or inhibiting constitutive endogenous molecules (**B**), knock-out mice. The key difference between knock-in/out and transgenic mice are that knock-in/out is targeted, meaning the desired gene is inserted/interrupted into a specific locus in the target genome via homologous recombination. This is important because it means the gene will achieve biological (*i.e.*, natural) expression patterns and levels. By contrast, transgenic models use random integration: one or more copies of the desired gene could end up anywhere in the host genome [29].

level and to generate modified organism, transgenic and KO animals. For most of these studies, the mouse has been selected due to the striking similarity of genetic properties (over 95%) between the mouse and human genome. Indeed, transgenic (gene enhancing) and KO (gene suppressing) mice provided a novel important strategy for studying development and mechanisms of a number of genetic and non-genetic pre- and post-natal diseases by introducing specific loss-of-function or gain-of-function mutations into genes and generating a great number of transgenic rodents. These experimental approaches allowed for the possibility to investigate the mechanism(s) through which specific signals were involved in human physiological and/or pathological events and eventually modify them. The generated NGF transgenic mice proved to be useful for studying a number of diseases, not only those afflicting laboratory and wild animals, but, most importantly, humans. Based on the available finding on the NGF spectrum of action on neuronal and non-neuronal cells and brain neuronal cells, it was reasonable to hypothesise that developing NGF transgenic mice would provide further understanding about the clinical potentiality of NGF.

NGF TRANSGENIC MICE

In 1994, Snider [25] and Smeyne *et al.* [26] generated the first TrkA transgenic mice characterised by severe sensory and sympathetic nerve cell deficits. During the same year, Crowley *et al.* [27] published the results of a new generated transgenic NGF KO mouse displaying severe deficits of peripheral sensory and sympathetic neurons and, surprisingly, no deleterious effects were observed in FBCN that received a critical trophic support from the NGF produced and released by the hippocampus and cortex [9]. Why these NGF transgenic mice show peripheral neurons loss and no effect on brain NGF target cells, and why exogenous administration of NGF is unable to compensate for the deficits

induced by the endogenous release of NGF in NGF-targets is not clear. In 2004, Coppola, *et al.* [28] generated a TrkA KO mouse, characterised also by B-cell abnormalities but normal post-natal development and survival, compared to other generated transgenic mice that die during the first two post-natal weeks [29].

In 2000, Cattaneo *et al.* [29] generated one more transgenic mouse, indicated as AD11, expressing recombinant neutralising anti-NGF monoclonal antibodies and characterised by severe deficits of the sympathetic nervous system, loss of FBCN, but also muscle dystrophy affecting the spinal cord and hind limb extensor muscle, and diffuse cell death in the spleen of adult mice [30-32]. Notably, the AD11 mice displayed a normal postnatal life compared to other NGF or TrkA KO mice that died during the first two post-natal weeks [26, 27]. In addition, no evident signs of neuropathological deficits before 60 days of age were evident. However, these rodents did develop clear signs of neuronal degeneration of the peripheral and central nervous systems that became progressively more evident [29]. Surprisingly, the exogenous administration of NGF can promote complete reversion and recovery of the deficits induced in FBCN by the neutralising NGF antibodies released by the AD11 mouse [31].

To summarise, while the findings observed with NGF transgenic models confirmed the functional role of NGF on peripheral and brain neurons observed following exogenous NGF administration, they also revealed effects not previously reported using a different experimental approach. For example, the deficits observed in NGF neutralising AD11 mice in cells of the immune and central nervous systems, as well as the action in brain stem cell response, have not been observed with exogenous anti-NGF-antibody administration either during foetal life or during adult life. Likewise, it is not clear why some NGF transgenic mice will die during the early post-natal life and others will survive normally throughout their post-natal life. On the contrary, short or long-term administration of NGF or anti NGF administrations have no deleterious effect on mouse survival induce [1]. Other differences between the two experimental approves include the mechanism through which exogenous administration of NGF reverses the deficits of FBCN in the brain of AD11 mice, in times of constant presence and/or release of neutralising anti-NGF monoclonal antibody by AD11 KO mice. Why the AD11 mice do not develop cutaneous ulcers, similar to those induced by circulating anti-NGF antibodies in NGF autoimmunisation rodents is unaccounted for [33]. A number of other questions remain unresolved. Thus, while NGF transgenic clearly demonstrated that exogenous NGF induces protective and healing action on a number of human disorders (such as cutaneous ulcers and retinal cell degeneration) and NGF genes protect brain cells and cardiac cells, prospecting as potential therapeutic application of NGF, the available published data with NGF transgenic mice seem, despite the numerous contributions regarding the role of NGF and the molecular mechanisms involved, to not allow for the support of the observations obtained with exogenous NGF administration.

A number of observations obtained with the AD11 transgenic mice support the hypothesis that NGF can play a critical protective role on degenerating FBCN and possibly in the pathogenesis of human AD [34-36]. It should be taken into consideration, however, that AD is characterised not only by the altered presence of NGF and of NGF receptor expression in NGF-target neurons, but also by deregulations of a number of other different molecular signalling and survival factors. It should, therefore, demonstrate that the NGF molecule is the only or a very critical important factor that can prevent the development and/or protect the diverse deleterious events leading to AD. At present, however, no convincing evidence exists supporting the hypothesis of a direct link between NGF and the potential clinical approach in AD. Thus, the initial enthusiastic hope that the generation of NGF would provide mechanisms supporting or denying the potential therapeutic application of NGF needs at the moment is tempered by the different observations obtained with the two experimental approaches.

CONCLUDING REMARKS

We have recently reported that topical NGF administration promotes healing of human cutaneous and corneal ulcers, and protects degenerating retinal cells in patients affected by glaucoma, maculopathy and retinitis pigmentosa [5, 17, 18]. Other studies have shown that the delivery of NGF through NGF gene therapy protects damaged brain neurons [33, 36] and myocardial cells [19]. Though the results obtained with NGF transgenic mice models largely confirms the role of NGF on peripheral sensory and sympathetic neurons and on neurons of the central nervous system, not much has been learned by the published findings with NGF transgenic mice about the therapeutic properties of NGF on cutaneous corneal ulcers and retinal cell protections, as has been demonstrated with exogenous purified and gene therapies. From these two experimental approaches have emerged differences that might generate an erroneous interpretation; including the hypothesis, NGF transgenic models are unable or are insufficient to reproduce the effects obtained by exogenous NGF administration. These differences may temper the original enthusiastic belief that the generation of NGF transgenic mice would provide additional important evidence about the therapeutic properties of NGF. Anyhow, further studies are needed to identify the mechanisms through which NGF acts on damaged cells and to elucidate the role of exogenous NGF and ANA administration versus the endogenous release of NGF and the neutralizing NGF proteins before determine the exact therapeutic properties of NGF within and outside the brain. It is reasonable to hope that the development of other NGF transgenic mouse strains and further basic and clinical experimental approaches with exogenous NGF administration will provide further data, a better understanding and, hopefully, the NGF clinical applications.

Acknowledgements

This study was supported by National Research

Council and NGF Onlus to Luigi Aloe. The Authors thank George N. Chaldakov for discussion and critical comments.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

Received on 11 September 2014.
Accepted on 2 December 2014.

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