REVIEW

Uncoupling neurotrophic function from nociception of nerve growth factor: what can be learned from a rare human disease?

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Abstract

Nerve growth factor (NGF) is a powerful trophic factor that provides essential support for the survival and differentiation of sympathetic and sensory neurons during development. However, NGF also activates nociceptors contributing significantly to inflammatory pain and neuropathic pain after tissue injury. As such anti-NGF based therapies represent a promising strategy for pain management. Because of dose-dependent serious side effects such as back pain, injection site hyperalgesia, clinical trials of using NGF to treat various disorders such as diabetic neuropathies, chemotherapy-induced and human immunodeficiency virus-associated peripheral neuropathies were all discontinued. Thus far, worldwide clinical applications of NGF in treating patients are very limited except in China. Hereditary sensory autonomic neuropathy type V (HSAN V) is an extremely rare disease. Genetic analyses have revealed that HSAN V is associated with autosomal recessive mutations in NGF. One of the mutations occurred at the 100th position of mature NGF resulting in a change of residue from arginine to tryptophan (R100W). Although those HSAN V patients associated with the NGF^{R100W} mutation suffer from severe loss of deep pain, bone fractures and joint destruction, interestingly patients with the NGF^{R100W} mutation do not show apparent cognitive deficits, suggesting important trophic support function is preserved. We believe that NGF^{R100W} provides an ideal tool to uncouple the two important functions of NGF: trophic *versus* nociceptive. Studies from investigators including ourselves have indeed confirmed in animal testing that the NGF^{R100W} no longer induced pain. More importantly, the trophic function seemed to be largely preserved in NGF harboring the R100W mutation. On the mechanistic level, we found that the NGF^{R100W} mutation was capable of binding to and signaling through the tyrosine receptor kinase A receptor. But its ability to bind to and activate the 75 kDa neurotrophic factor was significantly diminished. The significance of these findings is at least two folds: 1) the NGF^{R100W} mutation can be used as an alternative to the wildtype NGF to treat human conditions without eliciting pain; and 2) the 75 kDa neurotrophic factor may serve as a novel target for pain management. We will discuss all the details in this mini-review.

Key Words: *hereditary sensory and autonomic neuropathy V; nerve growth factor; NGF^{R100W} mutation; pain; tyrosine receptor kinase A; p75 neurotrophic factor receptor*

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Nerve growth factor (NGF) is the most thoroughly characterized neurotrophic factor being studied intensely over the past 60 years (Levi-Montalcini, 1964; Aloe et al., 2012). NGF provides support for the survival, development, and differentiation of both basal forebrain cholinergic neurons in the brain and peripheral neurons such as peripheral sensory neurons (Aloe et al., 2012). Because of its robust trophic function, NGF has been extensively investigated for its potential to treat neurodegenerative diseases such as Alzheimer's disease, peripheral neuropathies such as diabetic neuropathy and human immunodeficiency virus-induced neuropathy, and more (Anand et al., 1996; Aloe et al., 2012). However, all clinical trials using NGF were discontinued largely due to significant side effects including back pain, injection site hyperalgesia, myalgia, and weight loss (Anand et al., 1996; Aloe et al., 2012). Significant pain induced by efficacious doses of NGF is a major obstacle in clinical application of NGF. We have performed a PubMed literature search of articles published in the period January 2009–November 2018 on the subject of hereditary sensory and autonomic neuropathy V involving mutations in NGF.

Recently, a homozygous mutation in NGF (NGF^{R100W}) has been linked to consanguineous families with hereditary, sensory, and autonomic neuropathies type V (HSAN V) (Einarsdottir et al., 2004; Minde et al., 2004). HSAN V is an autosomal recessive genetic disorder characterized by sensory neuropathies accompanied by loss of pain. Clinical symptoms of HSAN V are similar to HSAN IV, which was associated with mutations in the tyrosine receptor kinase A (TrkA), a receptor for NGF. However, unlike HSAN IV patients, patients with HSAN V do not suffer from mental retardation and

anhidrosis. This suggests the HSAN V NGF mutation retains important trophic functions, but without pain-eliciting effect. Careful studies on NGF^{R100W} will likely yield important clues to uncoupling the two important biological functions of NGF: nociception *versus* trophic function.

The Cattaneo group examined binding/biochemical properties using NGF^{R100E}, a mutation at the same site as NGF^{R100W} (Covaceuszach et al., 2010; Capsoni et al., 2011). Their studies revealed that NGF^{R100E} binding to TrkA is robust. But the binding affinity to $p75^{NTR}$, the 75 kDa neurotrophic factor receptor for NGF, is substantially reduced. We were able to produce and characterize the naturally occurring mutant, NGF^{R100W}, and have demonstrated that NGF^{R100W}, like NGF^{R100E}, failed to bind to and activate $p75^{NTR}$-mediated signaling pathways (Sung et al., 2018). Ultimately, we were able to show that NGF^{R100W} was as active as wildtype NGF (NGF^{WT}) in binding and eliciting TrkA-mediated signaling pathways such as phosphatidylinositol-4,5-bisphosphate 3-kinase/Akt and extracellular regulated protein kinase (Erk)1/2. NGF^{R100W} was transported retrograde along the axon of dorsal root ganglion and supported dorsal root ganglion survival and differentiation (Sung et al., 2018), a function likely attributed to TrkA.

We tested the ability of NGF^{R100W} to elicit painful responses both *in vitro* and *in vivo*, using NGF^{WT} as a comparison. Our studies demonstrated that NGF^{WT} induced acute potentiation of proton-evoked current in cultured dorsal root ganglion neurons by single cell voltage clamp, but NGF^{R100W} failed to induce potentiation (Sung et al., 2018). Since such fast onset potentiation occurred through post translational change on nociceptive ion channels, failure of those local and acute modification of NGF^{R100W} could be ascribed possibly to absence of $p75^{NTR}$ signaling. Indeed, the $p75^{NTR}$ is implicated in hyperalgesia as shown in many different studies: 1) binding of NGF to $p75^{NTR}$ activates sphingomyelin hydrolysis, which then liberates ceramide (Dobrowsky et al., 1994); 2) Ceramide is known to increase the number of action potentials in sensory neurons (Zhang et al., 2002, 2006); 3) Inhibition of c-Jun N-terminal kinase activity, another $p75^{NTR}$-downstream signaling effector, was shown to reduce or block thermal hyperalgesia/allodynia (Doya et al., 2005; Obata et al., 2006; Zhuang et al., 2006); 4) Although NGF binds to $p75^{NTR}$ with a lower affinity than to the complex of TrkA/$p75^{NTR}$, local NGF concentration can be high enough to activate $p75^{NTR}$ after injury or during inflammation (Heumann et al., 1987; Bengzon et al., 1992; Donovan et al., 1995; Widenfalk et al., 2001); 5) Moreover, the majority of small diameter sized nociceptors that express TrkA also co-express $p75^{NTR}$, which suggests an important role of $p75^{NTR}$ in nociceptive signaling.

The failure of nociception by NGF^{R100W} was confirmed in our *in vivo* study (Sung et al., 2018). Intraplantar injection of NGF^{WT} into the hind paws of adult rats induced thermal and mechanical hypersensitization by decreasing the pain threshold significantly. NGF^{R100W} at similar dosages did not induce both thermal and mechanical hyperalgesia (Sung et al., 2018). We also tested hyperaglesic priming, an indicator of the transition from acute to chronic pain: when neurons are unprimed, mild pro-nociceptive molecules cause hyperalgesia which disappears after 1 hour (Ferrari et al., 2015). On the other hand, when primed, this hyperalgesia can be prolonged to more than 4 hours. When tested for chronic pain, NGF^{R100W} was still able to induce priming even 7 days after intraplantar injection, suggesting long-lasting neuroplastic change conceivably involves NGF-TrkA downstream signaling (Sung et al., 2018). Neuroplastic change such as priming involves transcriptional change likely accompanied by retrograde trafficking. This could explain late onset of pain, since the axon can exceed the length of one meter. This provides further evidence that the R100W mutation in NGF^{R100W} does not appear to affect the retrograde TrkA signaling. Token together, our study has suggested that both TrkA and $p75^{NTR}$ contribute to potentiate pain response by wildtype NGF (**Figure 1A**). In the case NGF^{R100W}, its inability to activate $p75^{NTR}$ signaling may result in an increase in pain threshold (**Figure 1B**).

However, the question remains why HSAN IV patients lose pain sensation despite normal TrkA activation. Many studies support the notion that TrkA mediates nociceptive signaling. For example, intrathecal injection of antisense oligodeoxynucleotide for TrkA reduces NGF-induced hyperalgsia (Malik-Hall et al., 2005). Moreover, HSAN IV patients with CIPA (congenital insensitivity to pain with anhidrosis), which is associated with numerous mutations in TrkA, suggests that TrkA signaling does mediate hypersensitivity (Indo, 2001). Furthermore, many effectors downstream of TrkA have been implicated in transmitting NGF-induced hyperalgesia. Administration of Erk inhibitors, such as U0126, attenuates thermal hyperalgesia induced in a capsaicin dose

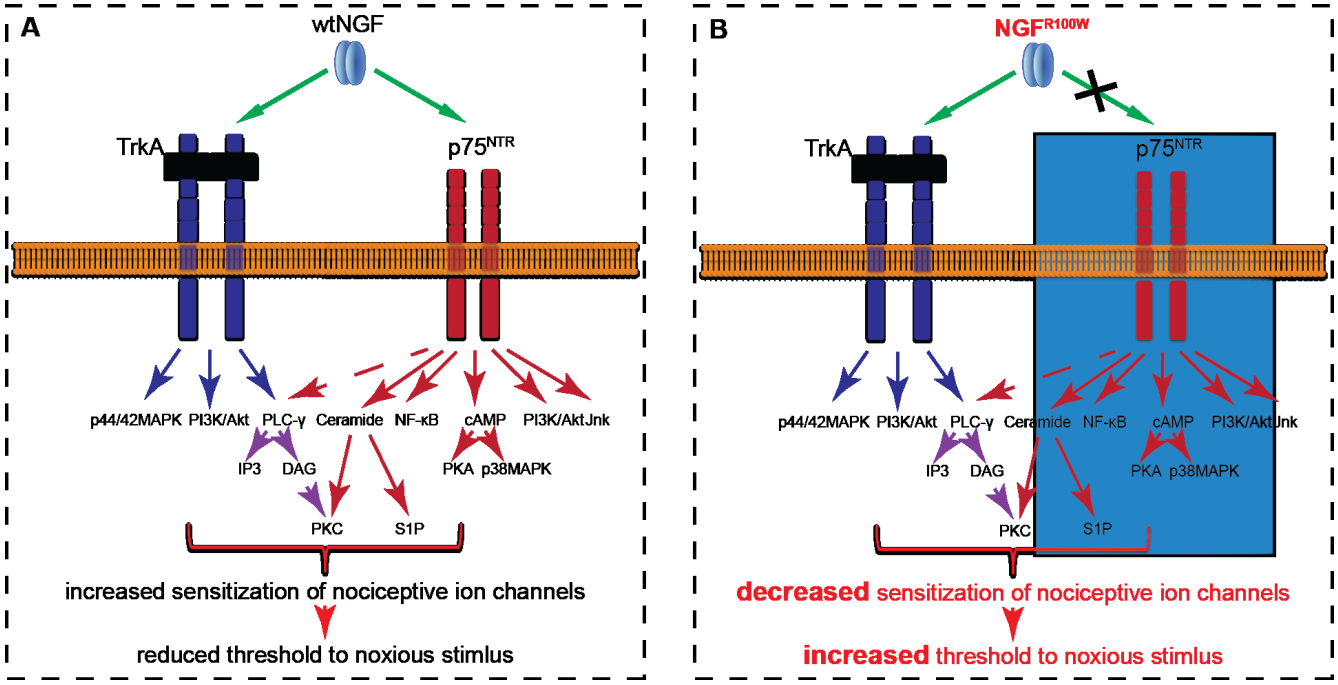

Figure 1 A proposed model underlying the loss of deep pain perception in HSAN V patients.
(A) NGF acts through both TrkA and $p75^{NTR}$ to induce intracellular signaling cascades such as PLC-γ to potentiate sensitization. (B) Although still capable of inducing TrkA signaling, NGF^{R100W} no longer activates $p75^{NTR}$ downstream signaling effectors, which results an increase in pain threshold. HSAN V: Hereditary sensory autonomic neuropathy type V; NGF: nerve growth factor; PLC-γ: phospholipase C gamma; TrkA: tyrosine receptor kinase A; wt: wildtype; NF-κB: nuclear factor kappa B; cAMP: cyclic adenosine monophosphate; IP3: inositol 1,4,5-trisphosphate; DAG: diacylglycerol; PKA: protein kinase A; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; Jnk: c-Jun N-terminal kinase; PKC: protein kinase C; S1P: sphingosine-1-phosphate; MAPK: mitogen-activated protein kinase; R100W: a change of residue from arginine to tryptophan.

dependent manner (Aley et al., 2001). Given that NGF^{R100W} activated Erk1/2 is similar to NGF^{WT}, it is unclear why NGF^{R100W} failed to induce acute sensitization. One possible explanation is that TrkA downstream pathways involved in sensing pain could be negatively affected by intertwined communication between $p75^{NTR}$ and TrkA. When we inhibited both TrkA and $p75^{NTR}$ in our *in vivo* study, we observed a synergistic effect in inhibiting both acute and chronic pain when compared to the use of single inhibitor. These studies suggest the crosstalk between $p75^{NTR}$ and TrkA helps to mediate and regulate the pain threshold. Based on these observations, we speculate that both NGF receptors, TrkA and $p75^{NTR}$, mediate pain signaling. But, their relative contribution to peripheral sensitization is most likely dependent on many variable conditions such as the ratio between TrkA and $p75^{NTR}$ at the cell surface.

Clinical studies of HSAN V patients showed orthopedic complications, such as bone fracture, due to loss of pain mainly in distal legs and arms (Einarsdottir et al., 2004; Minde et al., 2004). Their sural nerve biopsy also revealed a reduced number of C fibers and moderate loss of A delta neurons. Nerve fibers positive transient receptor potential cation channel (TRPV)1, TRPV2, TRPV8, calcitonin gene-related peptide and substance P were substantially reduced in both homozygous and heterozygous patients (Axelsson et al., 2009). Considering that production and secretion of mature NGF^{R100W} was educed in many sources (Axelsson et al., 2009; Covaceuszach et al., 2010; Capsoni et al., 2011; Sung et al., 2018), atrophy, or developmental deficit of peripheral sensory fibers, appears due to insufficient NGF availability.

Interestingly, the HSAN V patients still reported visceral pain, such as abdominal pain, despite of lack of pain in arms and legs (Einarsdottir et al., 2004). Visceral pains differ from somatic pain (inflammatory lesion of skin, muscle, joints, or peripheral nerve injury) in several points including: 1) pain could be induced by a psychophysical condition; 2) visceral pain is generally dull and its location hard to pinpoint. Taking those major differences into consideration, the mechanism underlying visceral and somatic pains are likely different. Therefore, the impact of NGF^{R100W} in HSAN V patients is expected to be mainly associated with loss of somatic pain, but not

much visceral pain.

In summary, the well-established pain-inducing effect of NGF is the predominant hurdle in using NGF to treat human diseases and conditions. Uncoupling pain from trophic function of NGF will thus allow various clinical trials to fully utilize the benefits of NGF as a therapeutic drug without unwanted side effects. Detailed characterization of NGF^{R100W} will undoubtedly help to uncover novel target(s) and means for accomplishing our eventual goal.

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