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Review

Nerve growth factor as an important possible component of novel therapy for cancer, diabetes and cardiovascular diseases

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Abstract: Nerve growth factor (NGF) is protein discovered by Rita Levi Montalcini in the 1950s. It plays a crucial role in the development of nervous system. NGF may be produced by a variety of cells even beyond nervous system. NGF modulate cell metabolism by binding to $p75^{NTR}$ and TrkA receptors. NGF is involved in psychological processes and may be the possible therapeutical agent for diabetes, cancer and cardiovascular diseases, which will be described in the article.

Key words: Nerve growth factor; Cancer; Diabetes; Cardiovascular diseases.

Historical background

Nerve growth factor (NGF) was discovered, as a first neurotrophin, in the 1950s by the Nobel prize winner Rita Levi Montalcini. Firstly it was known as an important factor for the embryonic development of peripheral nerves, nowadays we know innumerable properties of this molecule. Studies were conducted on chick embryo, which had mouse sarcoma cells transplanted into it. After this procedure, hyperinnervation was observed. Montalcini hypothesized that cancer tissue is releasing growth factor. Further studies proved her theory (1). Montalcini cooperated with Hamburger while conducting her studies. In fact, Hamburger was the first one who noticed the NGF-like effect, but his interpretation was not accurate (2).

Introduction

NGF is a secreted protein, representative of neurotrophins. Except for NGF, the neurotrophin family consists of brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5) and neurotrophin-6 (NT-6). The molecular weight of NGF is 140kDa, and its gen is located on chromosome 1 (3). As all neurotrophins, NGF is produced as a precursor proNGF and then cleavage to an active form (4). NGF consist of two α, one β, two ϒ subunits (α2βϒ2) and zinc anion(s). The β chain is associated with the activity of NGF, whereas ϒ has proteolytic activity. α chain does not present any enzymatic properties and is homological to ϒ subunit (3).

NGF is involved in development, survival and programmed death of neurons in both central (CNS) and peripheral nervous system (5). Despite NGF is being produced in CNS, mainly in hippocampus and cortex (6), it cannot penetrate through blood-brain-barrier and intracerebral administration produces severe side effects (7). Origin of NGF is hard to determine precisely, but the richest source of NGF seemed to be anterior pituitary (8) and salivary glands (9). NGF is produced or stored by various type of cells such as astrocytes (10), mast cells (11), lymphocytes T CD4+ (12), Schwan cells (13), macrophages (14), platelets (15), smooth muscle cells (16) and other, it also circulates in blood serum. Although a role of NGF in physiology and development of the nervous system is well documented, there is growing evidence of the role of this peptide beyond neurons and in pathophysiology. NGF is linked with regulation of immunology response (12), learning processes (17), pain (18), Alzheimer disease (19), psychiatric abnormalities such as schizophrenia (20), inflammatory colon diseases (21) and others physiological processes and disorders.

Nerve growth factor is not only linked to maintaining homeostasis. Nowadays, we know that NGF plays a role in the psychology of humans. NGF level in blood plasma is significantly higher in those individuals which are in a romantic relationship but for short period of time (less than a year) (22). Other neurotrophins' level changes were not observed (22), which suggests a multifunction and primary role of NGF in metabolism. NGF level is also raised in stressful situations (23). That is why it can be called a 'social molecule'.

The main aim of this review is to focus on the role of NGF in pathophysiology and its role as a potential cure for selected diseases with growing morbidity. Tumours, type 2 diabetes, and cardiovascular disease will be discussed further. These conditions were chosen subjectively but epidemiology and current knowledge status were taken in concern.

Receptors and signaling

Two types of NGF's membranous receptors: high-affinity tyrosine kinase receptor (TrkA) and p75NTR

are known (7). p75NTR is a transmembranous glycoprotein, member of the tumor necrosis factor (TNF) receptor superfamily (24). Trk superfamily includes not only TrkaA but other receptors with kinase activity such as TrkB and TrkC (25). NGF does not bind to TrkB and TrkC receptors, but other neurotrophins do. Trk receptors have only a trophic role, whereas p75NTR has a wide range of effects from tropism to programmed cell death (24).

TrkA receptor is mainly activated by NGF or NT-3. p75NTR is less specific and may be activated by any neurotrophins (25). NGF dimer binds to TrkA dimer with activation loop on the cell membrane (18). Releasing binding activation loop activates tyrosine kinase activity, which phosphorylates tyrosine in amino acids residues (18). This process leads to phosphorylation of intracellular proteins which are involved in transducing the signal to the nucleus(18). The common signaling pathway triggered by NGF involves Ras, phosphatidylinositol-3-kinase (PI3-K), phospholipase Cϒ1 and their effectors (20,24–29) (Figure 1). TrkA receptor is linked with cancer cell survival. It was shown that GW441756, inhibitor of TrkA exerts antiprolifertaive effect on sarcoma and prostatic cancer cells (30,31).

When TrkA receptors are absent, high doses of neurotrophins induce cell apoptosis in oligodendrocytes, smooth muscle cells, and neurons, by binding to p75NTR (4). However, this way of signaling is less effective in comparison with other receptors of TNF superfamily (4). p75NRT is a less known receptor. However studies performed with PD90780 have proven that the effect of stimulation of p75NRT depends on co-expression of TrkA (32). PD90780 is a non-peptide inhibitor of NGF and p75NRT connection, but PD90780 do not influence binding other neutrophines to p75NRT (33). If p75NTR is the only neutrophine receptor of the cell, it triggers apoptosis. However if both TrkA, and p75NRT receptors are present on the surface of the cell, p75NRT receptor triggers pro-survival signaling (32–34). This may suggest superior role of TrkA receptor. However, scientific data about p75NRT are inconsistent. It was proven that presence of p75NRT inhibitor (TAT-PEP5) (35) promotes axonal regeneration in damaged optic nerve (36), but in other study it was shown that TAT-PEP5 did not have an impact on neurit outgrowth in PC12 cells (37).

NGF exerts its effects not only by influencing metabolism but also by modifying gene expression. NGF post-transcriptionally regulates the level of tumor growth factor β -1 (TGF β) by increasing gene transcription and stability of transcript (24). Moreover, in PC12 cells (pheochromocytoma of the rat adrenal medulla), by activation of MAPK, NGF also increases the level of M4 muscarinic receptor's mRNA (38). Role of NGF in gene expression is poorly examined (only on PC12 cell line), further investigation should be done to fully understand this process.

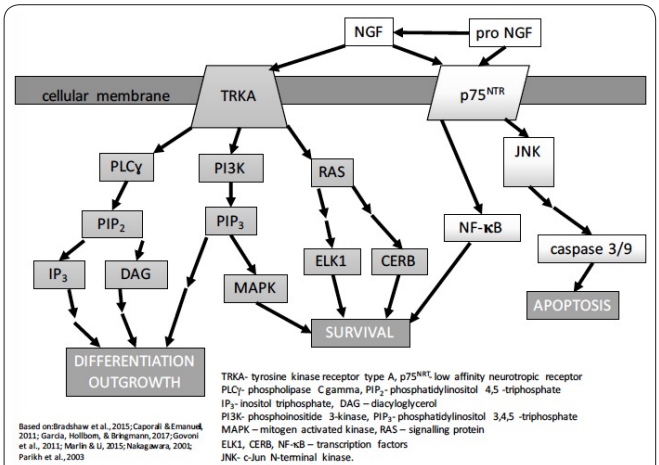

Figure 1. Simplified scheme of NGF metabolic pathways.

Intervention in NGF signaling pathways, as was proven in several studies, may have potential clinical application. For example three anti-NGF monoclonal antibodies Tanezumab, Fulranumab and Fasinumab are beneficial in chronic pain (39–42). In clinical trails all of mentioned antibodies was effective in pain-reveling in osteoarthritis (42). However, Ekman et al., have proven that Tanezumab is a better pain reliever than placebo, but worse than naproxen in osteoarthritis (39). Contrarily Kivitz et al., have shown that Tanezumab is a better analgesic than placebo and Naporxen in chronic back pain (40). Tanezumab is also useful in treatment of cancer and visceral pain, other anti-NGF antibodies do not have this effect (42).

Neuronal and non-neuronal tumors

NGF plays an important role in neuronal and non-neuronal tumors. For example NGF seems to be important in suppression of neuroblastoma. Neuroblastoma is a neoplasm which typically occurs in youth, and in high stage is linked with poor prognosis (43). However, it is proven that expression of NGF's receptors TrkA and p75NRT on tumor cells is good prognostic factor and may led to spontaneous regression of the tumor (44). Moreover, in *in vitro* studies conducted on SH-SY5Y cell line it was shown that NGF via TrkA may trigger TRAIL (TNF-related apoptosis including ligand) (45). Typically apoptosis is triggered by NGF via p75NRT receptor (24). Another tumor with proven connection to NGF is glioblastoma, which is an aggressive and often treatment-resistant neoplasm (46). It is proven that glioma cells expresses TrkA receptor and produces NGF (46). However role of NGF in this tumor is questionable, Singer et al., have proven that NGF may induce proliferation of glioma cells (47), whereas Weis et al., have shown that NGF may inhibit growth and promote differentiation of glioma cells (48). In other study it was shown that activation of TrkA receptor promotes authophagy in human glioblastoma cells (46). Furthermore p75NRT receptor is thought to be essential for proliferation of brain tumor cells (49). p75NRT receptor is also involved in invasion of tumor (50,51). Lastly, in medulloblastoma which is an aggressive tumor with poor treatment, the expression of TrkA receptor is linked with good outcome, same as in neuroblastoma (52). Ohta et al., have proven that TrkA receptor, after stimulation by NGF may trigger medulloblastoma cells apoptosis (52). Similar result was obtained by Li et al, however they proposed different mechanism of cell death - macropinocytosis (53). Role of NGF in neuronal tumors is enigmatic and the scientific data, as was shown above are inconsistent. Thus it is impossible to precisely determine role of NGF in central nervous system tumors.

Angiogenesis, defined as forming new vessels from pre-existing ones, is crucial for the embryological development of human. There are also physiological processes in adults that include angiogenesis (e.g. formation of corpus luteum). Nonetheless, it is its role in pathology (e.g. cancer development) that is crucial. Unlike "classical", well-described angiogenic factors, NGF plays (yet) enigmatic role in angiogenesis (54). Several studies have shown the importance of neurotrophins in both cancer and non-cancer angiogenesis. Even though all of them are almost equally important, only effects linked with NGF will be shown below.

Angiogenesis is vital for tumor growth and metastasis, as it appears that limited blood flow might be a suppressing factor in those processes. Furthermore, it is proven that tumor blood vessels have no innervation and when cancer grows, a progressive loss of perivascular innervation is observed (55). Perivascular reinnervation caused by NGF is thought to be a suppressive factor for prostatic cancer progression (56). Goda et al. conducted studies which proved that NGF is a suppressive factor to the growth of human prostatic cells (DU145 cell line) which have TrkA and p75NTR receptors. However, NGF did not influence DU145 cells viability (56). In nude mice infected with prostatic cancer, NGF increased survival rate, even after discontinuity of the therapy (56). In this particular case, it is more probable that NGF exerts anti-cancer effects rather by promoting smooth cell migration than through stimulating angiogenesis itself, but the evidence is unclear. NGF may be a prospective therapeutic agent, though there is not enough data to enunciate conclusion about the mechanism of NGF actions.

Ovarian tissue is one of a few tissues in which angiogenesis in adult life is physiological; it is crucial for regular changes in histology of female uterine tissue. In epithelial ovarian cancer cells level of TrkA's mRNA is significantly higher than in regular ovarian cells, whereas there is no difference in the level of NGF's mRNA (57). NGF increases the level of VEGF isoforms at a dose-dependent rate (57). VEGF is a "classical" angiogenic factor, which stimulates angiogenesis in most tissues (54,57). NGF indirectly stimulates angiogenesis in epithelial ovarian cancer, which stimulates the growth of a tumor. Presence of TrkA receptors is a poor prognostic factor in epithelial ovarian cancer (58).

NGF increases VEGF level in non-small cell lung cancer as well, where its actions are similar to the ones in epithelial ovarian cancer (59). Angiogenic effect of NGF is also observed in breast cancer, where usage of anti-NGF antibody decreases cancer cell-induced angiogenesis by half (60).

Epidemiology of that neoplasm is appalling. Given that prostatic cancer is responsible for 8% (2nd most common) of all men's cancer-caused deaths, and ovary cancer for 5% (5th most common cause of neoplastic women's deaths) further studies should be performed (61). NGF-connected treatment of this types of cancer may save thousands of lives (in 2016 in US 40 360 people died of prostatic and ovary cancer (61)). Reverse macroscopic effects of NGF in pathophysiology are strong difficulties in preparing the studies and, later, in the clinical application of findings. Therefore, in epithelial ovarian cancer NGF is unwanted, contrarily to prostatic cancer. However, there are some similarities. Both in the prostatic and ovarian cell, which most often undergoes the neoplastic transformation, physiologically the expression of TrkA receptors is poor or there are no receptors at all (62). Notwithstanding we have strong evidence on NGF effects, we stand in front of yet unexplored field of studies. Complex further studies, including clinical ones, should be performed, the main goal is defining precise mechanism of NGF actions and assessing its clinical usefulness.

Type 2 diabetes

Diabetes emerges as the most important metabolic disease of XXI century because it caused the death of 1.5 million people in 2012 (63). The main symptom of diabetes mellitus is an abnormally high level of glucose in blood serum, which leads to severe consequences. Since 1980, the worldwide population of sick ones grew four times (from 108 million in 1980 to 422 million in 2014) (63). According to International Diabetes Federation, 642 million people will be suffering from diabetes by 2040 (64). Apart from epidemiology, that disease is a major economic problem. Treating diabetes and coping with its complications consume 12% of world health expenses (61). Prophylactics, proper treatment of diabetes and preventing its complications will not only increase the quality of people's lives but it also helps reduce health expenses.

Dysfunction of pancreatic β-cells is one of the several pathomechanisms of diabetes. Variety of factors, such as inflammatory cytokines or high glucose level in plasma,

Table 1. Summary of role of NGF in tumors.

Type of tumor	Role of NGF or its receptors	References
Neuroblastoma	expression of p75NRT and TrkA good prognostic factor	41
	ambiguous role	
Glioblastoma	NGF may induce proliferation of the cells	44
	NGF may inhibit growth and promote differentiation	45
Medullobastoma	expression of TrkA → good prognostic factor	49
	NGF may trigger apoptosis or macropinocytosis	49, 50
Prostatic cancer	perivascular reinervation caused by NGF suppresses cancer	53
Epithelial ovarian cancer	expression of TrkA is poor prognostic factor	55
	increased expression of TrkA than in non-cancer ovarian cells	54
Non-small cell lung cancer	NGF increased level of VEGF	56
Breast cancer	NGF is angiogenic factor	57

Table 2. Summary of role of NGF in DM2.

Role of NGF or its receptors	References
lower NGF level in the pancreas → apoptosis	62,63,65
decreased expression of TrkA, increased expression of p75NRT	63
NGF may prevent neuropathy	67, 69
decreased NGF's mRNA, NGF level → diabetic cystophaty	70,71

are thought to cause this process (65). Studies conducted on Sprague Dawley rats with streptozotocin-induced diabetes has proven that NGF level in the pancreas is significantly lower in diabetic rats and it is followed by down-regulation of TrkA and increased expression of p75NTR receptors (66). Opposite effects were observed in CNS of diabetic rats. In hippocampus and pituitary gland, on diabetic rats, amount of NGF is significantly higher, in other parts of the brain level of NGF is either higher, but without statistical significance, or there is no change at all (66). Expression of TrkA receptors was lower only in cortex and hypothalamus, same as in pancreas (66). Pancreatic β-cells are known for NGF synthesis (67). Several studies have shown the correlation between NGF level and Langerhans islet cells apoptosis. Decreased level of NGF induces apoptotic death in pancreatic β-cells not by influencing gene expression, but by having an impact on metabolism (65,68). This mechanism is relatively rapid (68). It is accomplished by influencing enzyme inhibition of PI3-K and protein kinase B, activation of c-Jun kinase and by reduction of Bcl-Xl protein (68). Enzymes ERK's, p38 mitogen-activated kinase and protein called Bcl-2 were observed and no changes happened (68). Moreover, blockage of NGF pathways caused atrophy of insulin secretors (69). Significant changes in NGF level is present in several other diabetic tissues and organs.

One of the most common complications of diabetes is neuropathy, which leads to severe complications. Some studies have shown that axonal retrograde transport is impaired in diabetic rats (70), which might be one of the factors promoting neuropathy. NGF was thought to be the prospective therapeutic agent in neuropathy. Promising animals studies led to clinical trials. Unfortunately, in phase 3 clinical trial, no positive effects were observed (71). People were administered with rhNGF in a dose of 0.1 μg/kg three times a week, which was 30-50 times less than animals' doses. This might be a probable reason for failure. Perspective treatment should be based not on the systematic administration of neurotrophins, but rather stimulating or inducing its synthesis. Goss et al. used viral vector-mediated gene to treat diabetic neuropathy (72). Herpes simplex virus was used as a vector (72). Expression of NGF was observed, which prevented diabetic neuropathy in mice (72).

Both levels of NGF's mRNA and NGF itself are lower in diabetic rats' bladders than in a control group (73,74). Decreased NGF level is thought to cause diabetic cystophaty, a severe complication of diabetes. Impaired function of Aσ and C type afferent nerve (parts of the autonomic nervous system) function is the most probable cause of cystopathy (74).

NGF might be a prospective therapeutic agent in diabetes. Regretfully, there are severe difficulties to be faced up. The major ones to be mentioned are short half-life time (75) or not fully examined side-effects. Biotechnology and genetic engineering may be answers to this problem. Further studies should focus on clinical application of NGF. Possible side effects should be spotted and a way to eliminate it should be discovered. More patient-friendly and long-lasting treatment will be preferable. If we consider dramatic increase of new cases of diabetes, more effective treatment is more than necessary.

Heart disorders

One of the major problems in novel cardiology are ischaemic heart diseases (e.g cardiac arrest), because they are the main reason for heart muscle cells necrosis, which may lead to heart failure. NGF level in blood plasma is significantly lower than in a control group in patients with the acute coronary syndrome (76) and in patients with congestive heart failure (77,78), which made NGF interesting as a therapeutic agent. The heart is extensively regulated by the sympathetic nervous system, which acts by secretion of norepinephrine (NE). Several cardiovascular diseases lead to ischemia of heart. One of the reasons may be improper working of the sympathetic nervous system. NGF seemed to be important but not fully examined factor in heart ischemia and may be used in therapy for this disorders.

NGF improves cardiac function and decreases myocardial apoptosis and fibrosis in a mouse model of ischemia/reperfusion injury of heart (79). NGF exerts its effect by accelerating autophagic flux and attenuate protein ubiquitination in myocardial ischemic/reperfused heart (79). On the animal model, both endogenously and exogenously released, NGF protects sympathetic cardiac innervation against ischemia (80). There are two novel substances, which may protect neuronal cells, and have positive effect in treatment of heart disorders. Lin et al., have described two molecules with neuroprotective effect 2-(6-chloro-1H-indol3-yl)-5-(2-cyclopropyl-1H-indol-3-yl)-3,6-dihydroxy-[1,4]benzoquinone (1H5) and 2,5-dimethoxy-3-(7-fluoro-1H-indol-3-yl)-[1,4]-benzoquinone (5E5) (81). They conducted studies on PC12 cells (pheochromocytoma) in which NGF – dependent neuronal differentiation is proven (81). Interestingly 1H5 (30 μM) is an activator of TrkA receptor (its effect is equal to the 50% of the effect of 100 ng/mL NGF) (81). In absence of neurotrophines, PC12 cells undergo programmed cell death, but 1H5 via TrkA receptor can protect cells from apoptosis (81). 5E5 (30 μM), which activity is 200% of 100 ng/mL NGF. NGF is a stronger TrkA activator than 1H5(81). Furthermore, low doses of 5E5 with presence of high doses of NGF endorse neuronal differentiation in PC12 cells (81). It seems that 1H5 and 5E5 may have positive impact on sympathetic cardiac innervation in ischemic condition. Described effects were present in non-toxic concentrations of those molecules (81), however further evalua-

Table 3. Summary of role of NGF in heart disorders.

Condition	Role of NGF or its receptors	References
Acute coronary syndrome	decreased NGF's plasma level	73
Congestive heart failure	decreased NGF's plasma level	74, 75
Ischemia/reperfusion	NGF protects sympathetic cardiac innervation	77
	NGF inhibits neuronal and myocardial cells death	76, 78
Heart failure	decreased NGF level → reduction of sympathetic innervation	86

tion should be made.

NGF may be secreted from heart muscle cells, in healthy human heart cardiomyocytes NGF is expressed and released (77,82). NGF triggers PI3K/AKT prosurvival pathway (83). However, NGF level in the heart is not constant. Studies conducted on animals have shown that in rat's ventricles, a significant decrease in NGF expression was observed from neonatal to young rats and a significant increase from young to old rat (84). Regardless of rat's age NGF expression is significantly higher in ventricles than in atria (84). In some age group in left part of the heart, NGF expression was significantly lower than in the right one (84).

The connection between NGF and NE seemed to be crucial for the understanding of heart disease. Some studies have shown that norepinephrine decreases the level of NGF and its mRNA in iris and brown adipose tissue (24). But there are several studies which show an opposite effect in fibroblast (85) and vascular smooth muscle cells (86). In vivo studies conducted on dogs and rats have shown that NE reduces NGF level in the heart (87,88). Decreased adrenoceptor stimulation in heart failure rats reduces NGF expression by the cardiac parasympathetic neuron (78). Preclusive evidence disenables forming a conclusion without performing advanced research.

Heart failure is one of the most life-threatening cardiovascular disorder. It is an effect of myocardial damage or/and cardiomyocytes loss (89). Proper heart rhythm is an effect of interaction between sympathetic and parasympathetic nervous system on the heart. The mechanism involved in this regulation is likely driven by paracrine releasing NGF (90). This opposite regulatory system is impaired in congestive heart failure (CHF) (91). Excessive activation of the sympathetic nervous system is associated with greater risk of death as a complication of disease (92). The main reason for that condition seemed to be the reduction of sympathetic innervation density as an effect of decreased NGF level (89). Such observation was made both on humans and animals. In the experimental environment, NGF reduces incidence by 50% and death by 65% in aristolochic acid-induced heart failure (93). Studies conducted on larval zebrafish have proven that NGF exerts its effect by stimulating cardiomyocytes proliferation (93).

NGF is an important factor in several different pathomechanisms of cardiovascular disorders such as ischemia of heart muscle or cardiomyocytes apoptosis. However, we should expand our knowledge, especially in pathway signaling. Sympathetic and parasympathetic innervation is closely related to NGF but is it all? Precise mechanisms of NGF actions are still unrevealed, what made clinical trials and use of that molecule as a drug impossible.

Conclusions

NGF – related processes are still being discovered. despite we know a lot of them, we do not possess full understanding. The versatility of NGF actions made this molecule interesting and important for translational medicine. It may be the answer to many questions and there is no doubt that understanding of NGF will help in better understanding of human physiology and pathophysiology.

Another important aspect is defining NGF level changes in sick individuals in comparison with healthy ones. It will help to choose NGF-dependent conditions. However, we should define if the NGF's level abnormalities are the symptom or a cause of disease.

As it was shown, NGF plays a crucial, but not yet fully revealed the role in maintaining homeostasis and in pathology. Although we know NGF for almost 70 years, it is still enigmatic. Unfortunately, as many other trophic factors, NGF is present in a very low concentration which makes it hard to detect. Moreover, NGF influences metabolism and gene expression, what makes defining signaling pathways a formidable challenge.

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Interest conflict

We claim no conflict of interests.

Author's contribution

Karol Steckiewicz- drafting the manuscript, revising the manuscript, conception of work, acquisition of data, data analysis and interpretation, critical review, final approval of the manuscript.

Ewelina Barcińska – critical review, revising the manuscript, conception of work, final approval of the manuscript.

Michał Woźniak – critical review, revising the manuscript, conception of work, final approval of the manuscript.

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