 Stem Cells Translational Medicine logo Stem Cells Transl Med. 2021 Feb 15;10(7):1008–1020. doi: 10.1002/sctm.20–0290 Nerve growth factor (NGF) and NGF receptors in mesenchymal stem/stromal cells: Impact on potential therapies Kangkang Zha 1,2,3, Yu Yang 4, Guangzhao Tian 1,2,3, Zhiqiang Sun 1,2,3, Zhen Yang 1,2,3, Xu Li 5, Xiang Sui 2, Shuyun Liu 2, Jinmin Zhao 4,, Quanyi Guo 2, Author information  PMCID: PMC8235142 PMID: 33586908

Abstract Mesenchymal stem/stromal cells (MSCs) are promising for the treatment of degenerative diseases and traumatic injuries. However, MSC engraftment is not always successful and requires a strong comprehension of the cytokines and their receptors that mediate the biological behaviors of MSCs. The effects of nerve growth factor (NGF) and its two receptors, TrkA and p75NTR, on neural cells are well studied. Increasing evidence shows that NGF, TrkA, and p75NTR are also involved in various aspects of MSC function, including their survival, growth, differentiation, and angiogenesis. The regulatory effect of NGF on MSCs is thought to be achieved mainly through its binding to TrkA. p75NTR, another receptor of NGF, is regarded as a novel surface marker of MSCs. This review provides an overview of advances in understanding the roles of NGF and its receptors in MSCs as well as the effects of MSC‐derived NGF on other cell types, which will provide new insight for the optimization of MSC‐based therapy. Keywords: cellular therapy, mesenchymal stem/stromal cell, nerve growth factor, P75NTR, TrkA In this study, the modulatory effect of exogenous nerve growth factor (NGF) on mesenchymal stem/stromal cell (MSC) functions and the paracrine effects of MSC‐derived NGF on other cell types are discussed. In particular, the cytological functions and superiority of p75NTR+ MSCs as the basis for utilizing p75NTR as a surface marker to identify MSCs is presented. graphic file with name SCT3‐10‐1008‐g002.jpg Significance statement. Mesenchymal stem/stromal cells (MSCs) have shown great promise in regenerative medicine, and their functions are regulated by various cytokines. This review provides an overview of the roles of nerve growth factor and its receptors in MSCs to potentially identify strategies to optimize the therapeutic effects of MSCs. 1. INTRODUCTION Mesenchymal stem/stromal cells (MSCs) exhibit fibroblastic morphology as well as self‐renewal and multiple differentiation potentials. 1 The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has established the following minimal standard criteria for human MSCs: (a) must be plastic adherent (PA) under standard culture conditions; (b) must express CD105, CD73, and CD90 and lack surface expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA‐DR; and (c) must be able to differentiate into chondrocytes, osteoblasts, and adipocytes in vitro. 2 MSCs exist in various tissues, such as bone marrow (bone marrow‐derived MSCs [BMSCs]), 3 and as other MSC‐like populations, such as adipose tissue‐derived MSCs (ADSCs), 4 skin‐derived MSCs (SSCs), 5 Wharton's jelly‐derived MSCs (WJMSCs), 6 umbilical cord blood‐derived MSCs (UCBSCs), 7 placental‐derived MSCs (PSCs), 8 and dental pulp stem/stromal cells (DPSCs). 9 However, biological characteristics of MSCs vary based on the in vivo environment as well as the

isolation and expansion methods. Gene expression analysis has indicated that a considerable number of genes are differently expressed in MSCs derived from different origins. Cellular properties, including proliferation, multiple differentiation, migration and immunomodulatory activity, also vary among MSCs isolated from different tissues. Besides, MSCs tend to gradually lose their biological functions after in vitro expansion. Usually at passages 8 to 15, MSC begin to degenerate and show some cellular aging signs, such as larger cell size, reduced confluency, slower proliferation rate, attenuated multiple differentiation potential, and changes in molecular profiles. 10 , 11 In fact, plasticity is a fundamental property of MSCs related to a whole sequence of cytological functions and even to the fate of MSCs. 12 A number of microenvironmental changes and molecular signaling pathways have been studied to elucidate the roles of growth factors, cytokines, and chemokines on the functions of MSCs. For example, transforming growth factor-beta (TGF-β) has been proposed to have a substantial effect on the properties of MSCs, including their proliferation, multiple differentiation, and immunomodulatory capacities. 13 Some cytokines that are widely used in the study of other cell types also display a certain regulatory action on MSCs, which has attracted increasing attention from researchers. Nerve growth factor (NGF) is a member of the neurotrophic factor family that was first discovered by Levi-Montalcini in the 1950s. He conducted a study in which mouse tumors were transplanted into chicken embryos and produced NGF to stimulate the growth of sympathetic and sensory nerve cells and fibers. 14 , 15 An antiserum targeting this factor was able to destroy most of the sympathetic ganglia in various newborn mammals. 16 Later, its crucial roles in regulating the production of neuropeptides and neurotransmitters and the survival, growth, and differentiation of neurons in the peripheral and central nervous systems were revealed. 17 , 18 , 19 Moreover, NGF has displayed a superior nerve injury repair capacity in animal models 20 , 21 and in clinical trials. 22 A recent case report demonstrated that intranasal NGF administration improved the functional assessment and electrophysiological and clinical conditions of a patient with traumatic brain injury (TBI). 23 In addition, it was suggested that NGF also played significant roles in pain regulation via the stimulation of sensory and sympathetic nerve formation during the fetal period and elicitation of sensitization in both peripheral and spinal cord nerves during adulthood. NGF inhibitors could be used as new analgesics to prevent refractory chronic pain. 24 , 25 , 26 NGF has two membrane receptors: TrkA, a 140-kDa transmembrane tyrosine kinase that exhibits a high affinity for NGF and can be phosphorylated on tyrosine residues after binding to NGF, 27 and p75 neurotrophin receptor (p75NTR), also known as CD271, a 75-kDa glycoprotein belonging to the TNF receptor superfamily that shows a low affinity for NGF and can also bind to other neurotrophins. 28 , 29 The functions of NGF are not restricted to the nervous system, and numerous studies have shown that NGF has biological effects on MSCs. 30 , 31 , 32 The effects of NGF on MSCs are thought to be mediated mainly by the high-affinity receptor TrkA, 33 while the low-affinity receptor p75NTR is more likely to serve as a novel marker of MSCs. 34 In this review, we focus first on the modulatory effect of exogenous NGF on MSC functions, the paracrine effect of MSC-derived NGF on other cell types, and the activation of the NGF/TrkA signaling pathway in MSCs. Then, we discuss the possibility of utilizing p75NTR as a surface marker for identifying MSCs as well as the cytological functions and superiority of the p75NTR+ MSCs. Finally, we summarize the applications of the p75NTR+ MSCs for MSC-based therapy in various

diseases. 2. EFFECT OF NGF ON MSCs Previous studies have demonstrated that NGF exerts diverse cytological effects on neuronal cells, and such effects are now being observed in studies on MSCs (Table 1). Kolli et al investigated the effect of NGF on limbal stem cells (LSCs) and found that NGF and its receptors were expressed in LSCs, while NGF and p75NTR were downregulated throughout the differentiation period. In contrast, the expression of TrkA was variable but did not obviously decrease during the culture process. Blocking the action of NGF with an anti‐NGF antibody led to reductions in DNA replication, colony‐forming capacity and expression of the LSC markers ABCG2 and C/EBP but to higher expression of the corneal epithelial cell marker CK3. Given these findings, the authors suggested that NGF played a key role in maintaining the stemness of LSCs. 35 In another study, Lu et al reported that the proliferation of BMSCs was promoted after treatment with NGF, as shown by a higher DNA content and increased hematoxylin and eosin (HE) staining. 36 However, Gronthos et al demonstrated that NGF did not support colony growth of MSCs under serum‐deprived conditions, indicating that NGF was not able to initiate fibroblast colony‐forming unit (CFU‐F) colony formation in MSCs. 37 Thus, NGF may promote MSCs proliferation through increasing its sensitivity to surrounding stimulating factors, rather than directly inducing the proliferation of MSCs. TABLE 1. Effects of NGF on different types of MSCs Cell type Cell source Passage number Treatment Results Year Ref LSCs Human Not mentioned Treated with an anti‐NGF antibody Exhibit reductions in DNA replication, colony‐forming capacity and expression of the LSC markers but higher expression of the corneal epithelial cell marker 2019 35 BMSCs Rabbit Not mentioned Treated with different concentration of NGF (0, 1.5, 3, 6 g/mL for in vitro study and 0, 10 ng/mL for in vivo study) Cells treated with 3 g/mL NGF show highest proliferation and chondrogenesis abilities in vitro; cells treated with 10 ng/mL NGF show a better therapeutic effect on rabbits with cartilage damage in vivo 2017 36 BDSCs Rabbit P3 Treated with 3 g/mL NGF Display enhanced proliferation and chondrogenic differentiation abilities 2019 39 BMSCs Mice P3 Treated with NGF Show greater osteoblastic differentiation and mineralization capacities 2018 40 BMSCs Rat P3‐4 NGF gene modification Enhance its neurogenic differentiation 2016 41 BMSCs Rat P3‐5 Treated with different concentration of NGF (0, 50, 100, 200 g/L) Cells treated with 200 g/L exhibit the most obvious reduction in apoptosis 2019 50 CSPCs Human Not mentioned Treated with an anti‐NGF antibody Exhibit reduced matrix remodeling activity 2015 38 ADSCs Human P3‐4 Treated with NGF encapsuled in chitosan nanoparticles Enhance its neurogenic differentiation capacity 2019 42 UCBSCs Human Not mentioned Treated with different concentration of NGF (0, 12.5, 25, 50 100 ng/mL) Cells treated with 100 ng/mL NGF show the most enhanced neurogenic differentiation ability 2017 43 DPSCs Human P3 Treated with 100 ng/mL NGF Enhance its neurogenic differentiation 2017 44 Open in a new tab Abbreviations: ADSCs, adipose tissue‐derived mesenchymal/stromal stem cells; BMSCs, bone marrow‐derived mesenchymal/stromal stem cells; CSPCs, cartilage stem/progenitor cells; DPSCs, dental pulp‐derived mesenchymal/stromal stem cells; LSC, limbal stem cells; MSCs, mesenchymal stem/stromal cells; NGF, nerve growth factor; UCBSCs, umbilical cord blood‐derived mesenchymal/stromal stem cells. After chondrogenic induction, NGF‐treated BMSCs produced more GAG and type II collagen and expressed higher levels of cartilage‐specific genes, such as Aggrecan, SOX9, and COL II, than untreated BMSCs in both monolayer cultures and 3D cultures. When used to

repair damaged cartilage in rabbits, NGF‐treated BMSCs showed a greater therapeutic effect than untreated BMSCs. 36 Furthermore, Jiang et al found that the expression of NGF was increased in osteoarthritic cartilage and in vitro‐cultured chondrocytes exposed to interleukin (IL)‐1. NGF could serve as a chemokine to promote the migration of cartilage stem/progenitor cells (CSPCs). In addition, treatment with an anti‐NGF antibody significantly affected the matrix remodeling activity of CSPSs. 38 This result was similar to the findings of Miao and colleagues, who suggested that NGF was better than TGF‐1 at enhancing the proliferation and chondrogenic differentiation abilities of MSCs. 39 Moreover, Cui et al reported that the ALP levels and calcium nodule formation ability were significantly enhanced in NGF‐treated BMSCs compared to untreated BMSCs, suggesting that NGF promotes the osteoblastic differentiation and mineralization capacities of BSMCs isolated from mice with diabetes. 40 In addition, NGF can reportedly induce the neurogenic differentiation of a variety of MSCs, including BMSCs, 41 ADSCs, 42 UCBMSCs, 43 and DPSCs, 44 in vitro. Moattari et al proposed using NGF‐treated MSCs to repair peripheral nerve injuries in rats. They demonstrated that better therapeutic effects were achieved with NGF in combination with MSCs than with MSCs alone, as shown by behavioral, electrophysiological, and histological assessments. 45 In fact, MSC differentiation is a complex process involving interactions between cells and extracellular environment. NGF may not serve as an inducer of MSC differentiation because it can promote the differentiation of MSCs into different cell types, including chondrocytes, osteoblasts, and neural cells. A more reasonable explanation is that NGF improves the responsiveness to differentiation inducers of MSCs, thus increase its differentiation tendency. To elucidate whether NGF has an impact on BMSC angiogenesis and the possible mechanisms, Wang et al cultured BMSCs in Matrigel and treated them with different concentrations of NGF. They found that tube formation was significantly promoted in MSCs treated with 50g/L NGF and that this effect was associated with the enhancement of MSC proliferation but not with vascular endothelial growth factor (VEGF) expression. 46 In addition, it has been proposed that NGF acts as a pro‐survival factor in various types of cells. 47 , 48 , 49 Wang et al found that NGF treatment increased the viability of BMSCs and suppressed hexanedione‐induced apoptosis of BMSCs in vitro. They suggested that NGF could be used to prevent the apoptosis of BMSCs to improve their transplantation into damaged tissues for regenerative therapy. 50 3. MSCs AND NGF: PRODUCTION AND FUNCTION Cytokine secretion, which varies based on tissue origin, is regarded as one of the most important functions of MSCs. 51 Both BMSCs and ADSCs reportedly produce NGF, while BMSCs produce significantly more NGF than ADSCs. 52 Crigler et al found significant differences in NGF release in different MSC clones, suggesting that the expression of NGF was restricted to specific MSC subpopulations. 53 In addition, Peng et al induced WJMSCs into Schwann‐like cells in vitro and found that differentiated WJMSCs were capable of producing neurotrophic factors, including NGF, and stimulating neurite outgrowth of PC12 cells. 54 Bai et al reported that NGF could perfectly mimic the antiapoptotic effect of conditioned BMSC medium and that this effect was abolished by intervention with an anti‐NGF antibody, indicating that NGF was involved in the anti‐apoptotic effect induced by BMSCs. 55 Interestingly, the concentration of NGF was higher in BMSC and neural stem cell (NSC) cocultures than in BMSC or NSC monocultures. 56 Thus, MSC transplantation may be an excellent tool for the local delivery of NGF into damaged tissues to

promote the survival and repopulation of host neurons. Wang et al. injected BMSCs into 2,5‐hexanedione‐treated rats via their tail vein. After 4 weeks, they found that 2,5‐hexanedione‐induced neuronal apoptosis in the spinal cord was significantly attenuated due to an increased concentration of NGF. 57 Wu et al transfected the NGF gene into UCMSCs to improve the efficiency of NGF synthesis. Then, they intrathecally injected these transfected cells into the spinal cord to treat cystopathy in rats with diabetes and found that their voiding function improved as the NGF concentration increased. 58 In another study, Jo et al utilized BMSC transplantation to alleviate olfactory dysfunction. The study revealed that the expression of NGF and brain‐derived neurotrophic factor (BDNF) was significantly increased at week 2 and slightly reduced at week 4. The thickness and composition of the olfactory epithelium were close to normal, and olfactory function was improved greatly. The authors suggested that BMSCs possessed therapeutic potential for olfactory dysfunction due to their paracrine actions, especially the secretion of NGF and BDNF. 34 4. SIGNALING TRANSDUCTION BY NGF AND TrkA IN MSCs In neurons, TrkA is expressed on the plasma membrane with a extracellular ligand that could bind to NGF. Then NGF/TrkA signaling begins across into the intracellular cytoplasm and recruits those pro‐differentiation and pro‐survival signaling molecules, which are mainly signaling cascades including phosphatidylinositol 3‐hydroxy kinase (PI3K)‐protein kinase B (Akt), Ras‐mitogen‐activated protein kinase (MAPK), and phospholipase C gamma (PLC )‐protein kinase C (PKC). As a result, the survival and differentiation of neural cells are enhanced. 33 (Figure 1A). FIGURE 1. FIGURE 1 Open in a new tab Overview of NGF/TrkA signaling pathways in neurons and MSCs. A, NGF binds to the extracellular ligand of TrkA and then actives PI3K‐Akt, Ras‐MAPK and PLC ‐PKC signaling pathways to promote the survival and differentiation of neurons. B, After binding to TrkA, NGF can activate the PI3K/Akt and MAPK/Erk signaling pathways in MSCs. Sirt is also involved in the activation of Akt and Erk, both of which can stimulate the neural differentiation of MSCs. In addition, the activation of Akt can promote the proliferation, chondrogenic differentiation and osteogenic differentiation of MSCs and prevent their apoptosis. MSCs, mesenchymal stem/stromal cells; NGF, nerve growth factor Although the NGF receptors TrkA and p75NTR are both expressed by MSCs, NGF binds more specifically to TrkA and then activates intracellular signaling pathways such as PI3K/Akt and MAPK/Erk (Figure 1B). Previous studies have demonstrated that Akt is involved in the growth and differentiation of MSCs. 59 Spontaneously, NGF promotes the proliferation of BMSCs by activating the PI3K/Akt signaling pathway. Treatment with NGF significantly enhances the Akt phosphorylation and proliferation of BMSCs, both of which are blocked by intervention with the specific PI3K inhibitor LY294002. 46 Bad is a downstream target of Akt that can be phosphorylated and inactivated by Akt, 60 while dephosphorylated Bad induces the apoptotic caspase‐3 cascade. 61 NGF treatment significantly reduces the apoptosis of BMSCs and caspase‐3 activity, and this effect is counteracted by the Akt inhibitor MK‐2206. Thus, the protective effect of NGF against BMSC apoptosis may be achieved through the Akt/Bad pathway. 50 Sirt1 is a member of the Sir2 family that plays roles in neuroprotection, cell senescence, apoptosis, and the inflammatory response by interacting with proteins in a variety of signaling pathways. 62 Recent research has indicated that Sirt1 activation induces the neuronal differentiation of BMSCs. 63 Zhang et al reported that NGF could induce the neural differentiation of DPSCs by increasing the expression of Sirt1. In addition,

after treatment with NGF, phosphorylation of Akt and Erk is promoted in DPSCs, and this effect can be reversed by a Sirt1 inhibitor, indicating that the Akt and Erk signaling pathways may also be involved in the neuronal differentiation of DPSCs induced by NGF. 44 However, it is not clear whether the NGF–induced upregulation of Sirt1 expression occurs universally in MSCs or is restricted to DPSCs. Moreover, NGF can more robustly activate the PI3K/Akt signaling pathway than TGF–1 during the process of MSC chondrogenesis. 39 Since the PI3K/Akt signaling pathway is commonly recognized to be ini