

## Review Article

# The neuroimmune guidance cue netrin-1: a new therapeutic target in cardiovascular disease

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**Abstract:** Netrins are a family of proteins involved in cell migration and axon guidance during embryogenesis. The different functions and mechanisms of action of this family of proteins have been better characterized with the study of the netrin-1. They are chemotropic and act as a bifunctional regulator of neuron migration. Apart from its role in the central nervous system, researchers have proven that netrin-1 plays a role in the development and formation of non-neural tissue, thus netrin-1 is involved in regulation of cancers, cardiovascular diseases, kidney diseases and other diseases. Concerning the cardio-vascular realm, netrin-1 promotes angiogenesis and accelerates atherosclerosis, protects the heart against ischemia-reperfusion injury and reduces the infarct size. These findings make the neuroimmune guidance cue netrin-1 an important therapeutic target. This work seeks to review the subject based on studies that have been conducted over the past decade to identify the perspectives and extent of the research on this protein in the field of cardiology.

**Keywords:** Netrin-1, atherosclerosis, angiogenesis, cardioprotection, ischemia-reperfusion injury

## Introduction

Netrins are a class of proteins involved in cell migration and axon guidance during the development. They are named after the Sanskrit word “netr” which means “one who guides” [1]. Netrin was first described in the nematode *Caenorhabditis elegans* in 1990, and named UNC-6, according to standard *Caenorhabditis elegans* naming protocol [2]. The first mammalian homologue of UNC-6 was discovered in 1994, where it was found to be a vital guidance cue for rodent commissural axons in the spinal cord [3]. As of 2009, five mammalian netrins have been identified; Netrins 1, 3, and 4 are secreted proteins, whereas G1 and G2 are membrane bound proteins tethered by Glycophosphatidylinositol tails [4]. The different functions and mechanisms of action of this family of proteins have been better characterized with the study of netrin-1 which is encoded by the NTN1 gene [5]. Structurally, netrin resembles the extracellular matrix protein laminin; with the amino-terminal domains named VI and V. Domain VI, at the amino termi-

nus, is globular. It is followed by domain V, which is composed of three epidermal growth factor repeats. Domains VI and V bind to the Deleted in Colorectal Cancer (DCC) and UNC-5 families of netrin-1 receptors; however, the precise molecular details of the interaction have not been determined. The remaining carboxy-terminal sequence of netrins 1 to 4 (the C-domain) is enriched in basic amino acids [6, 7]. Netrins are chemotropic; a growing axon will either move towards or away from a higher concentration of netrin. Though the detailed mechanism of axon guidance is not fully understood, it is known that netrin serve as bifunctional signals: attracting some neurons while repelling others during the development of brain. Netrin attraction is mediated through UNC-40/DCC cell surface receptors and repulsion is mediated through UNC-5 receptors [1]. In the absence of netrin-1, these receptors are known to induce apoptosis [8]. They also act as growth factors, encouraging cell growth activities in target cells. Mice deficient in netrin fail to form the hippocampal commissure or the corpus callosum [1]. Netrin-1 is found in the floor plate and neuroepithelial

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cells of the ventral region of the spinal cord, as well as other locations in the nervous system including the somatic mesoderm, pancreas and cardiac muscle. It functions as an attractive or repellent guidance cue for a number of neuronal cell types in the vertebrate CNS (central nervous system), including dopaminergic neurons (attraction) [9] and cerebellar granule neurons (repulsion) [10]. In addition to directing axon guidance, netrin-1 also acts as a bifunctional regulator of neuron migration [10]. Although originally understood to be specifically involved in axonal guidance in the CNS, new research has linked netrin to cancer regulation (netrin-1 has been found to be upregulated in tumors, and recent research has been done to identify netrin-1 as a biomarker to indicate the onset of cancer in the human body. It was found that netrin can be found in excess in the blood plasma for patients who are positive for renal, liver, prostate, meningioma of brain, pituitary adenoma, glioblastoma and breast cancer) [11-13]. Netrin-1 also plays a role in the development and formation of non-neural tissue, in cardiovascular diseases [14, 15], kidney diseases [16, 17], and other diseases [4].

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Netrin-1 has been discovered to play an important role in atherosclerosis, angiogenesis and ischemia-reperfusion injury by acting as cardioprotective agent.

### Netrin-1 in angiogenesis

Angiogenesis designates the formation of new vessels from preexisting ones, and occurs mainly during development [18]. Blood vessels and nerves often follow parallel trajectories, suggesting that distal targets use common cues that induce vascularization and innervations [15]. Netrin-1 stimulates proliferation, induces migration, and promotes adhesion of endothelial cells and vascular smooth muscle cells with a specific activity comparable to vascular endothelial growth factor and platelet-derived growth factor [18]. *Nguyen and Cai* [19] demonstrated the mechanism by which netrin-1 induces angiogenesis; in fact, netrin-1 induction of Angiogenesis is NO dependent and netrin-1 Stimulation of NO Requires ERK1/2 and DCC. Netrin-1 activates DCC to result in activation of ERK1/2 and subsequently endothelial NO production from serine 1179

phosphorylated eNOS. NO also contributes to ERK1/2 activation, forming a feed-forward cycle. NO then mediates netrin-1-induced enhancement in endothelial cell growth and migration. Utilization of NO in promoting angiogenesis categorizes netrin-1 into the family of potent endothelial mitogens including growth factors. The discovery of this pathway offered an important perspective for therapeutic angiogenesis in patients with ischemic coronary artery disease. A recent study demonstrated that Netrin-1 expression decreases apoptosis in endothelial cells and induces angiogenesis to reduce ischemia-reperfusion injury [20]. Another study in the adult mice brains proved that Netrin-1 enhanced focal neo-vascularisation, reduced infarct size, and improved long-term functional recovery after transient focal cerebral ischemia [21]. These findings suggest that netrin-1 can serve as an innovative agent for the treatment of strokes. Likewise, a study in rats showed that netrin-1 is implicated in angiogenesis in the placenta, making it vital to the survival of the fetus [22]. This finding has implications in the future treatment of vascular disease in the placenta. But interestingly, other studies have showed controversial results. In fact, they proved that the endothelial tip cells also express UNC5b, which netrin-1 can bind to, inhibiting angiogenesis [23, 24]. This, therefore, suggests that netrin-1 is either a pro or an anti-angiogenic factor.

### Netrin-1 in atherosclerosis

Atherosclerosis is a disease of chronic inflammation that is distinguished by the persistence of cholesterol-engorged macrophages in arterial plaques [25]. Arterial inflammation is initiated by the sub-endothelial retention of plasma low-density lipoprotein (LDL), and enhanced by oxidative modification of these lipoproteins, which triggers an influx of monocytes [26]. Unlike other inflammatory states, atherosclerotic inflammation does not readily resolve and cholesterol-laden macrophages persist in the arterial wall. These macrophages also known as the major source of foam cells cause expansion of the plaque through recruitment of additional leukocytes and vascular smooth muscle cells, and contribute prominently to plaque instability through the secretion of extracellular matrix-degrading proteases and cytotoxic factors. Notably, it has been shown that atherosclerotic plaques that cause clinical events are

characterized by high macrophage content [27]. Resolution of acute inflammation typically involves emigration of monocyte-derived cells out of the inflamed site through nearby lymphatic vessels [28]. This process appears to be impaired in atherosclerosis and has been attributed, in part, to the cholesterol loading of macrophages which shifts these cells to a more sessile phenotype [29]. Studies in transplant-based mouse models of atherosclerosis regression have shown that reducing plasma non-HDL cholesterol and/or increasing high-density lipoproteins (HDL), promotes emigration of macrophages from lesions to regional and systemic lymph nodes [30-32]. These findings indicate that macrophage emigration from the plaque is actively inhibited during hypercholesterolemia. Studies established that netrin-1 inhibits migration of monocytes, neutrophils and lymphocytes via its receptor UNC5b [33-35] and recently, studies has extended these findings to show that netrin-1 is abundantly expressed by macrophage foam cells formed *in vitro* and *in vivo*, and in atherosclerotic lesions [36]. In fact, these studies demonstrated that netrin-1 expressed by foam cells, differentially regulated the cellular constituents of atheroma. In this research, netrin-1 inactivated macrophages migration and supported chemoattraction of coronary artery smooth muscle cells. Thus, expression of netrin-1 in plaques would be predicted to simultaneously prevent inflammatory cell egress and induce smooth muscle cells recruitment into the intima, thereby promoting lesion progression. In support of their hypothesis, they demonstrated that deletion of netrin-1 in myeloid cells severely reduced atherosclerosis lesion size and complexity in mice and was associated with macrophage emigration from plaques [36]. The molecular mechanism by which netrin-1 and its receptor UNC5b are expressed in atherosclerotic plaques has been discovered in a more recent study. In the latter, HIF-1 $\alpha$  induces netrin-1 and UNC5b expression under hypoxic conditions in sustaining inflammation (knowing that hypoxia is intimately linked to chronic inflammation) by inhibiting the emigration and promoting the survival of lesional macrophages [37]. Through these studies, researchers discovered a new culprit in atherosclerosis that is netrin-1 and therefore a novel target for future therapeutic intervention for the treatment of atherosclerosis and other cardiovascular diseases. It offers

great promise to reducing the occurrence of fatal cardiac events; knowing that cardiovascular diseases remain a major public health problem worldwide.

### Netrin-1 in Ischemia-reperfusion injury

Reperfusion therapy of jeopardized myocardium is the most effective method for reducing infarct size and improving the outcome in patients with ST-segment elevation myocardial infarction. However, the restoration of coronary blood flow can paradoxically induce additional myocardial damage, making reperfusion therapy a "double-edged sword" [38]. Reperfusion injury is a complex phenomenon mediated by several factors, including oxidative stress, intracellular calcium accumulation, rapid restoration of pH, and inflammation, and involves at least partial opening of the so-called mitochondrial permeability transition pore [39]. Clinically identified features of this reperfusion injury may be reversible and transient, such as arrhythmias or myocardial stunning, or irreversible, such as myocardial infarction or microvascular obstruction [40]. It has been demonstrated that netrin-1 protect the heart from ischemia-Reperfusion Injury by two mechanisms. Firstly, this is done by the stimulation of NO production from cardiac endothelial cells and myocytes. This potent effect is mediated by a DCC/ERK1/2/Enos (s1177)/NO/DCC feed-forward mechanism in both cell types [41]. Netrin-1 promotes angiogenesis via the same pathway [19]. Secondly, by a reduction of NOX4 expression, to the restoration of NOS function, to improving mitochondrial function and ultimately decrease in infarct size resulting from ischemia-reperfusion injury. This pathway is known as oxidative stress pathway which requires netrin-1 in its regulation [42]. Post-conditioning has shown to be an effective and easy strategy that can protect the heart against ischemia-reperfusion injury and reduce the infarct size [43]. Ischemic and remote post-conditioning are effective in reducing infarct size and myocardial edema during angioplasty procedures [44]. In relation to pharmacological post-conditioning, reducing infarct size by pharmaceutical interventions as an adjunct to classical reperfusion interventions is an attractive therapeutic principle. Thus, studies and clinical trials are ongoing and some have been completed. *Hof-mann U. et al* [45] assessed the sphingosine-1-phosphate receptor modulator

FTY720 effect in rats without positive results but rather an increase in mortality. A recent study in animal model shown that post-conditioning with Netrin-1 protects against Myocardial Ischemia-Reperfusion Injury and reduces infarct size by increasing NO bioavailability [46]. Furthermore, netrin-1 perfusion may also inhibit coronary restenosis via its production of NO [41]. Likewise, *Durrani S. et al* [20] have shown that by the combination of its pro-angiogenic effect and decreasing apoptosis of cardiomyocytes, netrin-1 effectively reduces ischemia-reperfusion injury to preserve global heart function. Taken together, these findings suggest that netrin-1 can serve as an innovative and powerful agent for acute treatment of myocardial infarction; thereby reducing the mortality associated with this condition.

### Conclusion

In addition to its role in axon guidance and cell migration in the nervous system during embryogenesis, netrin-1 also plays an important role in other systems and fields inter alia in oncology, nephrology, cardiology etc. In the cardiovascular field, netrin-1 is involved in angiogenesis, promotes atherosclerosis and protects the heart against ischemia-reperfusion injury. Pro and anti-angiogenic factor, cardioprotective and atherogenic factor: Therein lies the interest of this protein which offers real and important therapeutic perspectives. It will be a matter of determining which application will be most beneficial for the patients; because netrin-1 appears to be “a double-edged sword” especially in coronary artery disease.

### Abbreviations

CNS, central nervous system; DCC, deleted in colorectal cancer; ERK, extracellular signal-regulated kinase; HDL, high-density lipoprotein; HIF, hypoxia-inducible transcription factor; LDL, low-density lipoprotein; NO, nitric oxide; NOS, nitrous oxide systems; NOX, nitrogen oxides; UNC5b, uncoordinated-5-b receptor.

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