Neurobiology of Disease 97 (2017) 90–102

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

Unconventional neurotrophic factors CDNF and MANF: Structure, physiological functions and therapeutic potential

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ARTICLE INFO

Article history:
Received 2 March 2016
Revised 29 June 2016
Accepted 13 July 2016
Available online 15 July 2016

Keywords:
CDNF
MANF
Parkinson's disease
Diabetes
ER stress

ABSTRACT

Cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) promote the survival of midbrain dopaminergic neurons which degenerate in Parkinson's disease (PD). However, CDNF and MANF are structurally and functionally clearly distinct from the classical, target-derived neurotrophic factors (NTFs) that are solely secreted proteins. In cells, CDNF and MANF localize in the endoplasmic reticulum (ER) and evidence suggests that MANF, and possibly CDNF, is important for the maintenance of ER homeostasis. MANF expression is particularly high in secretory tissues with extensive protein production and thus a high ER protein folding load. Deletion of MANF in mice results in a diabetic phenotype and the activation of unfolded protein response (UPR) in the pancreatic islets. However, information about the intracellular and extracellular mechanisms of MANF and CDNF action is still limited. Here we will discuss the structural motifs and physiological functions of CDNF and MANF as well as their therapeutic potential for the treatment of neurodegenerative diseases and diabetes. Currently available knockout models of MANF and CDNF in mice, zebrafish and fruit fly will increase information about the biology of these interesting proteins.

Contents

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.07.009
0969-9961/

1. Introduction

Neurotrophic effects of CDNF and MANF in mammals have mainly been demonstrated in animal models of Parkinson's disease. Increasing evidence indicates that CDNF and MANF, when applied as extracellular proteins or delivered by viral vectors can protect and repair midbrain dopamine neurons *in vivo* (Airavaara et al., 2012; Back et al., 2013; Cordero-Llana et al., 2015; Lindholm et al., 2007; Voutilainen et al., 2011, 2009). Neuroprotective effects of MANF have also been shown in rodent models of cerebral ischemia and spinocerebellar ataxia (Airavaara et al., 2010; Yang et al., 2014). Importantly, cytoprotective effects of CDNF and MANF are not restricted to neurons. Infusion of MANF reduced tissue damage in myocardial infarction in mice (Glembotski et al., 2012). Deletion of MANF in mice resulted in diabetes indicating the importance of MANF for the functionality of pancreatic insulin-producing beta cells (Lindahl et al., 2014).

Although CDNF and MANF show neurotrophic activities, they are structurally clearly distinct from the classical secreted NTFs. The latter include glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) and neurotrophins, which belong to the family of cystine knot growth factors and bind transmembrane receptors to induce intracellular signaling cascades (Airaksinen and Saarma, 2002). GDNF functions by binding to its co-receptor GFRα1, thereby activating receptor tyrosine kinase RET and inducing intracellular signaling which promotes the survival and regeneration of neurons (Paratcha and Ledda, 2008). In contrast, the mechanism of CDNF and MANF cytoprotective action is still largely unclear, and their ability to bind transmembrane receptors has not been demonstrated. Although MANF and CDNF can be secreted from cells they are largely retained intracellularly in the ER (Apostolou et al., 2008). Interestingly, studies suggest that MANF is important for protein homeostasis in the ER since knockdown of MANF in cultured cells and knockout of MANF in mice and fruit fly results in the activation of UPR, a signaling pathway induced by ER stress (Apostolou et al., 2008; Lindahl et al., 2014; Palgi et al., 2012).

In the present review we will discuss the structural motifs of CDNF and MANF that are important for their function, animal models available to unravel MANF and CDNF biological roles *in vivo*, and summarize preclinical studies on potential therapeutic effects of CDNF and MANF for the treatment of neurodegenerative diseases. Our aim is to provide an up-to-date insight to the biology of CDNF and MANF trophic factors.

2. Molecular structure of CDNF/MANF protein family

MANF (also known as arginine-rich, mutated in early stage tumors; ARMET) is an evolutionary conserved protein present in vertebrate and invertebrate species, including *Drosophila melanogaster* and *Caenorhabditis elegans* (Petrova et al., 2003). CDNF is a paralog of MANF found in vertebrates (Lindholm et al., 2007). Amino acid sequence of CDNF/MANF family members reveals no homology with other proteins. MANF and CDNF are relatively small proteins with a molecular weight of 18 kDa, highly soluble and monomeric in neutral solution (Hellman et al., 2011; Hoseki et al., 2010; Latge et al., 2015; Lindholm et al., 2007; Mizobuchi et al., 2007). Their primary sequence contains an amino-terminal (N-terminal) signal peptide that directs them to the ER and when cleaved, results in a mature protein which can be secreted (Lindholm et al., 2007; Mizobuchi et al., 2007; Petrova et al., 2003) (Fig. 1A).

Originally, MANF was discovered as a survival promoting factor for midbrain dopaminergic neurons *in vitro* derived from the culture medium of rat type-1 astrocyte ventral mesencephalic cell line (Petrova et al., 2003). Sequence analysis of the active protein revealed a homology to a predicted human arginine-rich protein (ARP) of 234 amino acids (Shridhar et al., 1996). Based on sequence analysis of different organisms it was concluded that the putative arginine-rich region of human ARP is not translated and the protein was renamed MANF. According to the original report, human MANF is 179 amino acids long and contains a predicted signal peptide of 21 amino acids, cleavage of which results in a mature protein of 158 amino acids (Petrova et al., 2003) (Fig. 1A). Still, there is some discrepancy about the start methionine of human MANF. In the UniProt database (http://www.uniprot.org/), a sequence of human MANF (Acc. No. P55145) is 182 amino acids long with a signal peptide of 24 amino acids. It is unclear whether Met-1 or Met-4 is the initiator methionine in the MANF P55145 sequence.

CDNF was identified by analysis of database sequences homologous to MANF, cloned, purified and characterized (Lindholm et al., 2007). CDNF consists of 187 amino acids and contains a predicted signal peptide of 26 amino acids, cleavage of which results in a mature protein of 161 amino acids. Amino acid identity between the mature forms of human CDNF and MANF is 59%. CDNF and MANF proteins apparently lack the pro sequence for enzymatic activation that is common for classical NTFs including GFLs. Mature GFLs have seven cysteine residues in their primary structure whereas CDNF and MANF have eight cysteines with conserved spacing (Airaksinen and Saarma, 2002, Lindholm et al., 2007; Petrova et al., 2003) (Fig. 1A).

Human MANF was not glycosylated when expressed and secreted from transiently transfected cells (Apostolou et al., 2008; Lindholm et al., 2008). Human CDNF contains an *N*-linked glycosylation site (Apostolou et al., 2008) and an *O*-linked glycosylation site (Sun et al., 2011) and both glycosylated and non-glycosylated forms of CDNF are detected in overexpressing cells (Apostolou et al., 2008). However, glycosylation is not required for the neuroprotective activity of CDNF or its secretion (Lindholm et al., 2007; Sun et al., 2011).

2.1. Functions of the two domains

A characteristic feature of the primary sequence of CDNF/MANF family proteins is eight conserved cysteine residues which form four disulphide bridges (Hoseki et al., 2010; Lindholm et al., 2008, 2007; Parkash et al., 2009). Solving the crystal structure of human MANF revealed a two-domain protein in which the N-terminal domain is homologous to saposin-like proteins (SAPLIPs) (Parkash et al., 2009). Solution structure of MANF resolved by nuclear magnetic resonance (NMR) spectroscopy showed that the carboxy-terminal (C-terminal) domain of human MANF is homologous to SAF-A/B, Acinus and PIAS (SAP) protein superfamily (Hellman et al., 2011) (Fig. 1B). The structure of CDNF highly resembles that of MANF. Structure of the saposin-like N-terminal domain of CDNF has been determined by X-ray crystallography and NMR spectroscopy (Latge et al., 2013; Parkash et al., 2009). Recently a solution structure of full-length CDNF, including the C-terminal domain, was resolved by NMR (Latge et al., 2015). Intriguingly, the two domains of CDNF/MANF appear to have distinct functions and a flexible linker between the domains allows them a freedom of orientation in relation to each other which might be an important feature for their mechanism of action (Hellman et al., 2011; Hoseki et al., 2010; Latge et al., 2015).

SAPLIPs are versatile proteins with abilities to interact with lipids and membranes (Bruhn, 2005). The saposin-fold of MANF/CDNF N-terminal domain consists of five alpha helices and a 3_{10} helix in a globular "closed leaf" conformation with a hydrophobic core and three cysteine bridges stabilizing the structure (Hellman et al., 2011; Latge et al., 2013, 2015; Parkash et al., 2009). Porcine NK-lysin and human