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Mesencephalic astrocyte-derived neurotrophic factor (MANF), a new player in endoplasmic reticulum diseases: structure, biology and therapeutic roles

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Abstract

Mesencephalic astrocyte-derived neurotrophic factor (MANF), a newly identified 18 kDa soluble protein, localizes to the luminal endoplasmic reticulum (ER). ER stress can stimulate MANF expression and secretion. In *Drosophila* and zebrafish, MANF regulates dopaminergic neuron development. In contrast, in mice, MANF deficiency leads to diabetes and activation of the unfolded protein response. Recent studies in rodent models have demonstrated that MANF mitigates diabetes, exerts neurotrophic function in neurodegenerative disease, protects cardiomyocytes and neurons in myocardial infarction and cerebral ischemia, respectively, and promotes immune cell phenotype switch from proinflammatory macrophages to prorepair anti-inflammatory macrophages. The cytoprotective mechanisms of MANF upon ER stress are currently under active investigation. In addition, for the first time we have discovered that MANF can potentially serve as a urinary ER stress biomarker in ER stress-mediated kidney disease. These studies have underscored the diagnostic and therapeutic importance of MANF in ER diseases.

Introduction

The endoplasmic reticulum (ER) is the central site for folding, post-translational modifications, and transport of secretory and membrane proteins. Environmental, aging and genetic factors disrupting ER function lead to accumulation of misfolded and unfolded proteins in the ER lumen, termed ER stress¹. The adaptive response to ER stress is the unfolded protein response (UPR), which is initiated by three ER transmembrane proteins, inositol-requiring enzyme-1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6)². IRE1 is a dual activity enzyme with an endoribonuclease and

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Conflicts of Interest: A patent application entitled "Mesencephalic astrocyte-derived neurotrophic factor (MANF) as a urine biomarker for endoplasmic reticulum (ER) stress-related kidney disease, methods and uses therefor" has been filed by Y.M. Chen and Washington University Office of Technology Management (application number 14730465, filed on June 4, 2015). All authors have read the journal's policy on disclosure of potential conflicts of interest.

a kinase domain. IRE1-mediated cleavage of XBP1 leads to a spliced XBP1 mRNA (XBP1s), encoding a potent transcriptional activator³. IRE1 also recruits TRAF2 (tumor necrosis factor receptor-associated factor 2) and activates Jun N-terminal kinase (JNK), thus transmitting apoptotic signals⁴. PERK phosphorylates eukaryotic initiation factor-2 α (eIF2 α), leading to attenuation of protein translation and induction of activating transcription factor 4 (ATF4)⁵. ATF6 activates transcription of chaperones after being cleaved by S1P/S2P proteases in Golgi from 90 kDa to the active 50 kDa ATF6 (p50ATF6)⁶. Cells rendered dysfunctional due to severe or prolonged ER stress are eliminated from the organism by the proapoptotic UPR, mediated by CHOP, JNK, or caspase 12 (Fig 1). The dysregulation of the UPR pathway can result in various diseases such as diabetes, inflammation, and neurodegenerative disorders including Alzheimer's disease and Parkinson's disease, which are collectively known as "ER diseases"⁷. Mounting evidence has also demonstrated that ER stress contributes to the development and progression of renal glomerular and tubular diseases⁸.

Podocyte ER stress and dysfunction induced by podocyte gene mutations have been shown to play an important role in the pathogenesis of proteinuria. In cell culture studies, certain nephrin or podocin missense mutants are trapped inside the ER and activate ER stress^{9–11}. Moreover, in mouse models, podocyte ER stress induced by *LAMB2* C321R¹², *ACTN4* K256E¹³, or *COL4A3* G1334E¹⁴ leads to podocytopathy. Meanwhile, in diverse sporadic nephropathies including focal segmental glomerulosclerosis, membranous nephropathy, minimal change disease, and diabetic nephropathy, multiple studies have linked podocyte ER stress to the pathogenesis of these diseases in both experimental models^{15–17} and human kidney biopsies^{18, 19}. Similarly, in renal tubular cells, ER dysfunction induces tubular injury and apoptosis and plays a pathogenic role in the onset and progression of renal tubulointerstitial disease, which ultimately advances to end stage renal disease^{20–24}.

Mesencephalic astrocyte-derived neurotrophic factor (MANF), also known as arginine-rich mutated in early tumors, or ARMET, was first discovered by Dr. Commissiong's group in 2003 as a new dopaminergic neurotrophic factor in astrocyte-conditioned medium²⁵. Emerging studies have demonstrated that MANF is a novel ER soluble protein and that its cytoprotective effects are not limited to neurons. In the current review, we will provide an up-to-date overview about the molecular structure of MANF, molecular mechanisms regulating MANF upregulation and secretion, important functions of MANF in different disease models, utility of MANF as an ER stress biomarker, and its translational potential.

MANF Structure

Cloning and sequencing of the MANF cDNA verified that MANF encoded by a 4.3 kb gene with 4 exons is located on human chromosome 3²⁵. The MANF primary transcript encompasses 1109 bp, which codes for a predicted 179 amino acid protein. The N-terminal 21 amino acids serve as the signal peptide, targeting nascent MANF to the ER. The secreted form of MANF is the full length protein without the signal sequence, i.e. 158 amino acids with a molecular weight of 18 kDa²⁶. Nuclear magnetic resonance (NMR) spectroscopy of the three-dimensional solution structure of human MANF reveals that MANF is composed of an N-terminal saposin-like domain (residues 1–95) and a C-terminal SAP (SAP-A/B,

Acinus and PIAS) domain (residues 104–158), which are connected with a short linker (residues 96–103)²⁷ (Fig 2).

The N-terminus of MANF can bind membrane and free lipids²⁸. Importantly, the C-terminus is homologous to the SAP domain of Ku70, a well-known inhibitor of proapoptotic Bax protein²⁷. It has been shown that the small C-terminal domain is responsible for the intracellular protection against the Bax-dependent apoptosis in the sympathetic superior cervical ganglion (SCG) neurons²⁷. It has also been shown that the C-terminal SAP-like domain of MANF interacts with the DNA binding domain of NF- κ B p65 subunit after translocation of MANF into nucleus under inflammation and ER stress, which negatively regulates NF- κ B signaling²⁹. MANF contains eight conserved cysteines that form four intramolecular disulfide bonds including two in CXXC sequences²⁸. Although the CXXC motif can be found in thiol-disulfide oxidoreductase^{26, 30}, MANF is not involved in redox reactions. The CKGC disulfide bridge in the MANF C-terminus is crucial for MANF's neuroprotective activity³⁰. In addition, the four amino acids, RTDL at the extreme C terminus of MANF (Fig 2) can bind to the KDEL receptors (KDELRs), which is localized primarily in the *cis*-Golgi, to facilitate the cycling of MANF from the Golgi to the ER^{30, 31}. *In vitro* deletion of RTDL in SCG neurons almost completely abrogates the intracellular pro-survival effect of MANF by causing it to localize to the Golgi complex³⁰. Interestingly, the interaction between RTDL and KDELRs can also be found at the plasma membrane of SH-SY5Y cells, which is required for MANF binding at cell surface³¹.

MANF Expression and Upregulation upon ER Stress

MANF mRNA and protein are widely expressed in both neurons and nonneuronal tissues. In the brain, relatively high MANF levels are detected in the cerebral cortex, hippocampus and cerebellar Purkinje cells. In nonneuronal tissues, high MANF expression is detected in the adult liver, testis and salivary gland³². *In vitro*, MANF expression is upregulated in cell lines derived from bone tissue (U2OS), kidneys (HEK293), neuroblastoma (SH-SY5Y) and embryo fibroblasts (NIH 3T3) in response to ER stress triggered by tunicamycin (TM), thapsigargin (TG), and lactatystin^{26, 33}. *In vivo*, MANF expression is induced in chondrocytes following ER retention of the mutant cartilage extracellular matrix protein in mouse knock-in models of chondrodysplasia caused by matrilin-3 or type $\times$ collagen mutations³⁴, in pancreatic β cells following misfolding of mutant proinsulin in Akita mouse model carrying an insulin C96Y mutation²⁶, and in synoviocytes following inflammation mediated-ER stress in a rabbit antigen-induced arthritis model²⁹. MANF expression is also increased in response to ischemia-induced ER stress both *in vitro* and *in vivo*^{32, 33, 35–37}.

Related to ER stress-mediated kidney diseases, we have shown that in our podocyte ER stress-induced hereditary nephrotic syndrome (NS) mouse model in which podocyte ER stress is activated by the C321R mutation of the glomerular basement membrane constituent laminin β 2 (LAMB2), MANF is induced and secreted by ER-stressed podocytes^{12, 38}. We have also shown that in acute kidney injury (AKI) triggered by ER stressor TM or ischemia/reperfusion (I/R), MANF is upregulated and secreted by ER-stressed tubules³⁸.

MANF expression has also recently been studied in human diseases involving ER stress. Chen LJ. et al. reports that compared with healthy controls, MANF mRNA is dramatically upregulated in peripheral white blood cells from patients with autoimmune inflammatory disease, including rheumatoid arthritis and systemic lupus erythematosus²⁹. Galli E. et al. shows that high serum MANF concentrations are found in children 1–9 years of age close to the diagnosis of type 1 diabetes (T1D), but not in older children and adolescents 10–17 years of age with recent onset T1D or adults with longer-term T1D³⁹. The increased MANF concentrations in 1–9-year-old patients with T1D are inversely correlated with C-peptide levels that are an indirect measure of functional β cell mass, but not associated with diabetes-predictive autoantibodies³⁹. These results suggest that elevated serum MANF level at the onset of T1D may reflect ER stress in the remaining β cells strained with increased demand for insulin production due to reduced β cell mass.

ER stress-induced transcriptional upregulation of MANF is driven by an ER stress response element (ERSE)-II in the MANF promoter, which is immediately downstream of –160 bp in the promoter^{26, 36}. ERSE-II (ACGTGGNCCAAT) contains two transcriptional factor recognition sequences: ACGTGG is recognized by ATF6 or XBP1, whereas CCAAT is recognized by nuclear transcriptional factor (NF)-Y. It has been shown that both binding sites are required for the MANF induction by ER stress²⁶. Moreover, ATF6 α most strongly increases the MANF promoter activity via ERSE-II, while the effects of ATF6 β and splicing XBP1 (XBP1s) are moderate in Neuro2a cells⁴⁰. In agreement with these reports, MANF expression is upregulated in the hearts of ATF6 transgenic mice that constitutively express a tamoxifen-activated form of ATF6 in cardiomyocytes⁴¹.

ER Stress-Enhanced MANF Secretion

The trafficking and secretion of MANF is regulated by ER stress. Enhanced protein secretion is not a general cellular response to ER stress. However, a robust secretory response of MANF to TG-induced ER stress, but not to TM or DTT was observed in SH-SY5Y, cardiomyocytes and HeLa cells^{31, 42}. MANF secretion is influenced by its N- and C-terminal sequences. The signal peptide directs MANF to the ER during protein synthesis and allows access to the secretory pathway³². The C-terminal sequence RTDL of MANF (Fig 2), unlike most ER stress response (ERSR) proteins that contain the canonical ER retention signal KDEL, functions as an ER retention signal by binding to the KDELR in the Golgi. Removal of the RTDL sequence promotes MANF secretion^{31, 42, 43} whereas KDELR overexpression reduces MANF secretion³¹. However, RTDL has a lower affinity for the receptor than KDEL in averting secretion. Thus, under non-stressed conditions, MANF is efficiently retained in the ER and not secreted. In contrast, upon ER stress, ERSR proteins with both KDEL and RTDL are induced while KDELR levels do not change. It has been proposed that due to the differential affinities of canonical and non-canonical ER retention sequences for the KDELR, proteins with KDEL motifs compete better for binding to the KDELR than MANF with RTDL motif, thus fostering MANF secretion⁴⁴. There are opposite findings regarding whether the RTDL motif regulates TG-induced MANF secretion. Henderson et al. showed an RTDL-dependent induction of MANF secretion in response to TG in SH-SY5Y cells³¹. On the contrary, Glembotski et al. found an RTDL-independent secretory response of MANF to Tg in cardiac myocytes and HeLa cells⁴². The

KDEL composition and levels in different cell types may account for the observed differences, which remains to be resolved. Meanwhile, MANF secretion is also regulated by Ig binding protein (BiP) via calcium-dependent interaction of MANF and BiP in the ER (Fig 1)⁴². BiP overexpression inhibits secretion of MANF, independently of the C-terminal RTDL^{42, 43}. Additionally, the BiP-MANF complex is TG-sensitive and the complex levels decrease in coordination with ER calcium depletion⁴². Finally, Oh-hashii et al. reported that MANF secretion is almost completely abolished by overexpression of a dominant negative Sar1 by impairing COPII-mediated transport from the ER to Golgi, indicating that the secretion of MANF is the COPII pathway dependent⁴³.

MANF Functions (Table 1) and Underlying Mechanisms

MANF deficiency and diabetes mellitus (DM)

To investigate the physiological role of MANF *in vivo*, Lindahl et al. generated MANF global knockout mice (*Manf*^{-/-}). In contrast to neuronal phenotypes observed in knockout of *Manf* in *Drosophila*⁴⁵ and knockdown in zebrafish⁴⁶, *Manf*^{-/-} mice developed DM postnatally⁴⁷. The diabetic phenotype is due to progressive reduction of β cell mass resulting from decreased proliferation and increased apoptosis⁴⁷. Moreover, MANF ablation *in vivo* leads to generalized activation of the UPR in β cells⁴⁷. Differently from the insulin-deficient phenotype of *Manf*^{-/-} mice, a MANF gene homozygous mutation (*IVS1 + 1G>T*) in a human patient presents with type 2 diabetes and obesity⁴⁸. In line with these studies, using a transgenic T1D non-obese diabetic (NOD) mouse model that expresses hen egg lysozyme in β cells and thus increases the basal load of unfolded protein stress, a recent study has shown that reduced islet expression of transcription factor *Glis3* caused by genetic variation or high fat diet and the resultant defect in *Manf* upregulation lead to enhanced susceptibility to apoptosis and β cell failure⁴⁹. The same transcriptional relationships are also observed in human islets⁴⁹. Remarkably, recombinant human MANF induces β cell proliferation *in vitro* and overexpression of MANF by adeno-associated virus (AAV) serotype 6-mediated transduction in the pancreas of diabetic mice promotes β cell regeneration⁴⁷. Thus, MANF could be a therapeutic candidate to treat DM.

Neurotrophic function in neurodegenerative diseases

Parkinson's disease (PD) is a progressive degenerative brain disease characterized by bradykinesia, resting tremor, muscle rigidity, and postural abnormalities. The movement disorder is mainly a consequence of degeneration of dopamine neurons projecting from the substantia nigra (SN) to the caudate-putamen (dorsal striatum). MANF and conserved dopamine neurotrophic factor (CDNF) form a novel, evolutionarily conserved neurotrophic factor family⁵⁰ and are structurally distinct from other neurotrophic factors including glial cell line-derived neurotrophic factor (GDNF) and neurturin. Low to intermediate levels of MANF are expressed in the striatum and SN³². *In vitro*, MANF was shown to selectively protect nigral dopaminergic neurons, but not GABAergic or serotonergic neurons²⁵. In *Drosophila*, loss of the MANF ortholog leads to a deficiency in dopaminergic neuron development, which can be rescued by fly and human MANF⁴⁵. In a rat model of PD induced by 6-hydroxydopamine (6-OHDA)-caused degeneration of dopamine neurons, intrastrially injected MANF restores the function of the nigrostriatal dopaminergic system

when administered either 6 hours before or 4 weeks after 6-OHDA administration in the striatum. It was hypothesized that the neurotrophic effect of MANF against 6-OHDA-induced neurotoxicity may be attributable to inhibition of ER stress⁵¹.

MANF is also enriched in cerebellar Purkinje cells³². In a genetic mouse model of spinocerebellar ataxia (SCA) arising from mutant TATA box binding protein (TBP)-mediated Purkinje cell degeneration (SCA17 knock-in mice), MANF expression is reduced in the mutant mouse cerebellum⁵². More importantly, overexpression of MANF either by cerebellum injection of lentivirus expressing MANF or by transgenic MANF expression specifically in the central nervous system ameliorates severe loss of Purkinje neurons and ataxic gait in SCA17 mice, which is dependent on protein kinase C signaling⁵².

Protection against cerebral and cardiac ischemia

Brain ischemia induces neuron death through ER stress^{53, 54}. It has been shown that in a rat model of focal cerebral ischemia induced by ipsilateral middle cerebral artery occlusion (MCAO), MANF protein is significantly induced in ischemic cortical neurons at the early stage after ischemia, which occurs prior to C/EBP homologous protein (CHOP) activation and histologic evidence of infarction^{33, 37}. Under focal cerebral ischemia, MANF expression is also upregulated in rat glial cells, including astrocytes³⁵. Similarly, following global forebrain ischemia caused by bilateral occlusion of the common carotid arteries in combination with hypotension, MANF mRNA is increased in the hippocampus and cerebral cortex³². *In vitro* study shows that human recombinant MANF inhibits ER stress-induced apoptosis in cultured primary neurons³⁷. More importantly, *in vivo* studies demonstrate that intracerebral injection of human recombinant MANF 20 minutes before MCAO or intracortical delivery of AAV serotype 7 encoding human MANF one week before MCAO in rats reduces infarct volume and facilitates motor recovery^{55, 56}. The neuroprotective effect of MANF is mediated, at least in part, through attenuation of ischemia-mediated cell apoptosis⁵⁶. In addition, ipsilateral ventricle injection of human recombinant MANF 2 hours after MCAO rescues the cerebral ischemia-induced neuron loss and promotes behavioral recovery in rats, which is associated with inhibition of ischemia-induced upregulation of ERSR proteins including BiP, phosphorylated IRE1 and XBP1s, as well as suppression of caspase 3 cleavage⁵⁷.

Myocardial infarction (MI) also activates ER stress in the surviving myocytes adjacent to the damaged region *in vivo* and simulated ischemia (sI) or simulated ischemia/reperfusion (sI/R) induces ERSR in cultured rat and mouse ventricular myocytes *in vitro*^{41, 58, 59}. Correlating with ER stress induction, MANF expression is shown to be upregulated in cardiac myocytes of mice subjected to MI *in vivo* and in cultured neonatal rat ventricular myocytes subjected to sI in an ATF6 and XBP1-dependent manner *in vitro*³⁶. miRNA-mediated knocking down of endogenous MANF in cultured cardiac myocytes increases cell death upon sI/R, whereas overexpression of MANF by adenovirus or addition of recombinant MANF to medium protects cardiac myocytes from serum starvation or sI- or sI/R-mediated cell death³⁶. Furthermore, infusion recombinant MANF into mice 24 hours prior to subjecting them to 30 minutes of myocardial ischemia has cardioprotective effect⁴².

Immune modulation and inhibition of inflammation

Immune modulation by MANF plays an important role in the repair and regeneration of retina. Regenerative therapies based on cell replacement hold promise for the treatment of a range of degenerative retina diseases, such as macular degeneration or retinitis pigmentosa. However, the proinflammatory microenvironments negatively affect integration and repair. A recent study has shown that in *Drosophila* or mouse, the damaged retina secretes Pvf-1 (platelet-derived growth factor (PDGF)- and vascular endothelial growth factor-related factor 1) and PDGF-A, respectively. These damage signals induce MANF expression in innate immune cells and MANF activation promotes a phenotype switch of macrophages from pro-inflammatory to anti-inflammatory, thereby improving tissue repair in both invertebrates and vertebrates, thus enhancing retinal regenerative therapy⁶⁰.

MANF can also repress proliferation of inflammatory fibroblast-like synoviocytes in a rodent methylated bovine serum albumin-induced arthritis model²⁹. Furthermore, MANF can alleviate oxygen–glucose deprivation-induced cell damage and inflammation in rat primary astrocytes⁶¹, as well as suppress lipopolysaccharide-induced inflammatory response in neural stem cells⁶².

The molecular mechanisms by which MANF attenuates inflammation remain to be fully understood. Recent data demonstrate that MANF in the nucleus interferes with the binding of NF- κ B p65 subunit to its target gene promoters and subsequently suppresses the expression of NF- κ B-dependent inflammatory genes²⁹. MANF also inhibits p38 mitogen activated protein kinase (MAPK) pathway⁶².

Utility of MANF as an ER stress biomarker

Although ER stress underpins the initiation and/or development of a variety of ER diseases, ER stress biomarkers that can be applied in human patients are still lacking to date. For the first time, we demonstrate that MANF can potentially serve as a urinary biomarker for detecting ER stress in podocytes or renal tubular cells, respectively³⁸.

In the podocyte ER stress-induced NS mouse model that we have developed, transgenic expression of C321R-LAMB2 in podocytes via the podocyte-specific mouse nephrin promoter on the *Lamb2*^{-/-} background (Tg-C321R mice) recapitulates features of human NS patients carrying the C321R mutation. As a control, transgenic expression of wild type-LAMB2 in podocytes is sufficient to rescue the NS phenotype in *Lamb2*^{-/-} mice^{12, 63}. In the first postnatal month, when Tg-C321R mutants exhibit trace proteinuria without notable renal histologic alternations, podocyte ER stress as manifested by the upregulation of BiP and MANF that is induced by the C321R misfolded protein is evident. Most importantly, MANF is easily detected in urine specimens from C321R mutants at the incipient stage of NS, but not in the controls. In addition, urinary MANF excretion increases during disease progression in the C321R mutants³⁸.

Similarly, in the AKI mouse model triggered by TM or I/R, significant upregulation of MANF at both transcriptional and translational levels is observed in the ER-stressed renal tubules before obvious renal histopathologic changes or elevation of serum creatinine occur.

Moreover, urinary MANF excretion concurrent with tubular cell ER stress precedes histologic or clinical manifestations of the corresponding disease, respectively³⁸.

Our study will help identify subgroups of patients associated with ER dysfunction who can be treated with ER stress modulators in a highly-targeted manner. We will continue to validate MANF as a urinary ER stress biomarker in human patients with ER stress-mediated kidney diseases.

Conclusion

In summary, MANF, a novel ER soluble protein, is upregulated and secreted in response to experimental ER stress in various cell and rodent models. Accumulating evidence has demonstrated important protective and immunomodulatory effects of MANF in different disease models. Thus, MANF may have significant therapeutic potential to treat ER stress-mediated diseases including kidney disease. To date, knowledge about the intracellular and extracellular actions of MANF is still limited and the receptor for MANF remains to be identified. Further studies are required to dissect the MANF downstream signaling pathways, investigate how MANF regulates the UPR, and validate whether MANF can be utilized as a noninvasive ER stress biomarker in human ER diseases.

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Abbreviations

AAV	adeno-associated virus
AKI	acute kidney injury
ARMET	arginine-rich mutated in early tumors
ATF6	activating transcription factor 6
BiP	Ig binding protein
CDNF	conserved dopamine neurotrophic factor
CHOP	C/EBP homologous protein
DM	diabetes mellitus

ER	endoplasmic reticulum
ERSE	ER stress response element
ERSR	ER stress response
GDNF	glial cell line-derived neurotrophic factor
I/R	ischemia/reperfusion
IRE1	inositol-requiring enzyme 1
KDEL	KDEL receptor
LAMB2	laminin β 2
MANF	mesencephalic astrocyte-derived neurotrophic factor
MAPK	mitogen activated protein kinase
MCAO	middle cerebral artery occlusion
MI	myocardial infarction
NF	nuclear transcriptional factor
NMR	Nuclear magnetic resonance
NOD	non-obese diabetic
NS	nephrotic syndrome
6-OHDA	6-hydroxydopamine
PD	Parkinson's disease
PDGF	platelet-derived growth factor
PERK	PKR-like ER kinase
Pvf-1	PDGF- and vascular endothelial growth factor-related factor -1
SAP domain	<u>S</u> AF-A/B, <u>A</u> cinus and <u>P</u> IAS domain
SCA	spinocerebellar ataxia
SCG	superior cervical ganglion
sI	simulated ischemia
sI/R	simulated ischemia/reperfusion
SN	substantia nigra
T1D	type 1 diabetes

TBP	TATA box binding protein
TG	thapsigargin
TM	tunicamycin
UPR	unfolded protein response
XBP1s	splicing XBP1

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Fig 1. The UPR pathway and possible interaction between MANF and BiP
The image is modified from Xu et al.⁶⁴ with permission.

Fig 2. NMR solution structure of human MANF

Table 1

Protective functions of MANF in various rodent models of ER diseases

1. Diabetes
<ul style="list-style-type: none">• AAV-mediated MANF overexpression in pancreas attenuates diabetes in a diabetic mouse model⁴⁷
2. Neurodegenerative disease
<ul style="list-style-type: none">• Intrastriatally injected MANF restores the function of the nigrostriatal dopaminergic system in a rat model of parkinson disease caused by 6-OHDA⁵¹• MANF overexpression in cerebellum ameliorates spinocerebellar ataxia in a genetic mouse model arising from mutant TATA box binding protein-mediated Purkinje cell degeneration⁵²
3. Ischemia
<ul style="list-style-type: none">• Intracerebral administration of MANF before or after ischemic insult reduces infarct volume and promotes behavioral recovery in a rat model of cerebral ischemia induced by ipsilateral middle cerebral artery occlusion⁵⁵⁻⁵⁷• Infusion of recombinant MANF prior to cardiac ischemia protects the heart from ischemic damage⁴²
4. Retina degeneration
<ul style="list-style-type: none">• Subretinal injection of recombinant MANF at the onset of retinal degeneration enhances the efficiency of retinal regenerative therapies in the mouse model⁶⁰