Acta Pharmacologica Sinica (2016) 37: 571–579
www.nature.com/aps

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Review

Metrnl: a secreted protein with new emerging functions

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Secreted proteins play critical roles in physiological and pathological processes and can be used as biomarkers and therapies for aging and disease. Metrnl is a novel secreted protein homologous to the neurotrophin Metrn. But this protein, unlike Metrn that is mainly expressed in the brain, shows a relatively wider distribution in the body with high levels of expression in white adipose tissue and barrier tissues. This protein plays important roles in neural development, white adipose browning and insulin sensitization. Based on its expression and distinct functions, this protein is also called Cometin, Subfatin and Interleukin 39, which refer to its neurotrophic effect, adipokine function and the possible action as a cytokine, respectively. The spectrum of Metrnl functions remains to be determined, and the mechanisms of Metrnl action need to be elucidated. In this review, we focus on the discovery, structural characteristics, expression pattern and physiological functions of Metrnl, which will assist in developing this protein as a new therapeutic target or agent.

Keywords: secreted proteins; Metrnl; Cometin; Subfatin; adipokine; neurotrophin; insulin sensitization; white adipose browning

Acta Pharmacologica Sinica (2016) 37: 571–579; doi: 10.1038/aps.2016.9; published online 11 Apr 2016

Introduction

Secreted proteins play critical roles in physiological and pathological processes and can be used as biomarkers and therapies for aging and disease[1-7]. Because adipose tissue is the largest endocrine organ, we have focused on adipose-derived secreted proteins (also known as adipokines) and explored their functions over the past decade[8-16]. In addition to clarifying the roles and molecular mechanisms as well as possible clinical applications of known adipokines (such as NAMPT/Visfatin)[15–28], we have made efforts to discover new adipokines. Most recently, we identified a novel adipokine, Metrnl, also known as Meteorin(Metrn)-like, Cometin and Subfatin, and revealed its insulin sensitizing action, which may translate it into a promising therapeutic target for insulin resistance[29, 30].

Metrnl is a protein homologous to the neurotrophic factor Metrn. Although the expression and functions of Metrn have been explored extensively, studies on Metrnl are quite limited. In this review, we summarize the discovery, structural characteristics, expression pattern, functions and mechanisms of action of Metrnl that have been reported to date.

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Received 2015-12-21 Accepted 2016-01-24

Discovery of Metrnl

The human genome contains approximately 20 687 protein-coding genes[31], but most of their expression patterns and functions remain unknown. To discover novel functional proteins, advanced bioinformatic techniques show great potential. Using these techniques, a number of research groups noticed the Metrnl gene before the subsequent identification of the protein[29, 32-34].

The Metrnl gene is located on mouse chromosome 11qE2 and human chromosome 17q25.3[29, 35]. Its specific location on the q-arm terminal end of human chromosome 17 has recently attracted attention because a cyto-molecular analysis of a case of ring 17 syndrome showed that the breakpoints are very close to the telomeric ends, thus making Metrnl a candidate gene that is potentially involved in some of the phenotypic features related to the ring chromosome 17[33].

The protein homologous to Metrnl, Metrn, was reported by Nishino *et al* in 2004[36]. Considering the obvious role of Metrn in the central nervous system and following the demonstration of the Metrnl gene being a new, direct target of PAX2/5/8

for otic development[32], Jorgensen *et al* first described Metrnl protein and demonstrated its function as a neurotrophic factor similar to Metrn in 2012[35]. Our lab screened for new adipokines in a global gene expression profiling of different adipose depots with bioinformatic methods in 2007 and early 2008. We identified Metrnl as a novel adipokine[29]. Although Jorgensen *et al* and we discovered Metrnl protein in entirely independent ways, both labs verified Metrnl as a secreted protein[29, 35].

Metrnl as a novel secreted protein homologous to Metrn

Bioinformatic analysis predicts that the Metrnl proteins encoded in the mouse, rat, and human genomes contain 311 amino acids, with a NH_2-terminal signal peptide of 45 amino acids and without any transmembrane region, suggesting a mature protein that contains 266 amino acids (~30 kDa) when secreted. The secretion of Metrnl has been verified by both Jorgensen *et al* and us, independently. In brief, a C-terminally His-tagged version of mouse Metrnl was cloned into a eukaryotic expression vector, which was transfected into HEK293, COS-7[29] or HEK293F[35] cells. Metrnl was detected in both cell lysate and serum-free medium of transfected cells by western blotting with a Metrnl antibody[29, 35].

It is predicted that 239 amino acids (77%) are identical in the mouse and human Metrnl proteins (Figure 1). Moreover, orthologues for Metrnl are found in all vertebrates, including zebrafish and the frog *Xenopus tropicalis* (Figure 1), but not in invertebrates such as the fruit fly (*Drosophila melanogaster*) and the nematode (*Caenorhabditis elegans*)[29, 35, 37].

Metrnl shows approximately 40% amino acid identity with Metrn, with all ten cysteine residues in the mature sequence conserved. Structurally unrelated to other known proteins, Metrnl and Metrn constitute a new evolutionarily conserved two-member protein family[29, 35, 37] (Figure 2).

The gene product encoded by Metrn was first described by Nishino and co-workers in 2004 and was shown to play an important role in the regulation of glial cell differentiation and in the induction of axonal extension. Thus, the initial name of this protein, Meteorin, was a vivid description of its function in transforming glial cells into cells with an elongated tail that look like meteors[36]. As the homologous protein, Metrnl protein was accordingly annotated as Meteorin-like in public databases at that time because neither its expression nor its functions had been reported[29, 35].

Unlike Metrn, the prediction of N-glycosylation sites in Metrnl indicates a single potential N-glycosylation site at amino acid 103 in mouse Metrnl. This was proven by both Jorgensen *et al* and us using recombinant mouse Metrnl-his_6 protein. In both laboratories, the recombinant protein was purified by affinity chromatography and then separated by SDS-PAGE. Metrnl purified by this method appeared much heavier than the calculated molecular weight of Metrnl-his_6 lacking the signal peptide[29, 35]. In our case, we observed two bands between 34 and 43 kDa, which were recognized by anti-Metrnl and anti-His_6 antibodies in SDS-PAGE analysis, and we identified both these bands as Metrnl by mass spectrometry[29]. In contrast, Jorgensen *et al* observed one band of approximately 34 kDa by SDS-PAGE analysis, which, when analyzed by MALDI-TOF MS, had a mass of 33.8 kDa with a shoulder at 33.4 kDa[35]. Because the bioinformatic prediction suggests

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Human     MRGAARAAWGRAGQPWPRPPAPGPPPPP---LPLLLLLAGLLGGAGAQYSSDRCSWKGSGLTHEAHRKEVEQVYLRCAA   77
Mouse     MRGAVWAARRRAGQQWPRSPGPGPGPPPPPPL---LLLLLLLLGGASAQYSSDLCSWKGSGLTREARSKEVEQVYLRCSA   77
Rat       MRGVVWAARRRAGQQWPRSPGPGPGPPPPPPL---LLLLLLLLGGASAQYSSDLCSWKGSGLTREAHSKEVEQVYLRCSA   77
Cattle    MRGATRAAGGRAGQLWPRPPAPGPGPPP---L---LLLLAVLLGGAGAQYSSDLCSWKGSGLTHEAHRKEVEQVYLRCSA   74
Frog      MLRRV--------------------------L-LSFFMVILMDRGTSQQYSSDMCNWKGSGLTHEGHTKDVEQVYLRCSE   53
Zebrafish MLS-------------------------PFL-AYLLSVVLLCRIARSQYSSDQCSWRGSGLTHEGHTRGVEQVYLRCAQ   53

                                 *
Human     GAVEWMYPTGALIVNLRPNTFSPA-RHLTVCIRSFTDSSGANIYLEKTGELRLLVPDGDGRPGRVQCFGLEQGGLFVEAT  156
Mouse     GSVEWMYPTGALIVNLRPNTFSPA-QNLTVCIKPFRDSSGANIYLEKTGELRLLVRDIRGEPGQVQCFSLEQGGLFVEAT  156
Rat       GSVEWMYPTGALIVNLRPNTFSPA-QNLTVCIKPFRDSSGANIYLEKTGELRLLVRDVRGEPGQVQCFSLEQGGLFVEAT  156
Cattle    GTVEWMYPTGALIVNLRPNTFSPS-RNLTLCIKPLRGSSGANIYLEKTGELKLLVRDGDLGPGQAPCFGFEQGGLFVEAT  153
Frog      GSVEWLYPTGAMIINLRPNTLTSAYKHLTVCIKPFKDSKGANIYSEKTGELKLVVPDGENNPHKVYCFGLDQRGLYIEAT  133
Zebrafish GFLEWLYPTGAIIVNLRPNTLSPAASLLSVCIKPSKESSGTHIYLDRLGKLRLLLSEGDQAEGKVHCFNIQDGALFIEAV  133

Human     PQQDIGRRTTGFQYELVRRHRASDLHELSAPCRPCSDTEVLLAVCTSDFAVRGSIQQVTHEPERQDSAIHLRVSRLYRQK  236
Mouse     PQQDISRRTTGFQYELMSGQRGLDLHVLSAPCRPCSDTEVLLAICTSDFVVRGFIEDVTHVPEQQVSVIYLRVNRLHRQK  236
Rat       PQQDISRRTTGFQYELMSGQRGLDLHVLSAPCRPCSDTEVLLAICTSDFVVRGFIEDVTHVPEQQVSVIHLRVSRLHRQK  236
Cattle    PQQDISRRTTGFQYELTSRRTGPDLHALLAPCRPCSHTEVLLAVCTSDFVVRGSIQKVTHEPERQESAIHLNVSRLYRQK  233
Frog      PQQDISRKITGFQYELISQRTLSDLHTVSDPCRPCSDTEVLLAVCISDFVVKGTIGTVTNDEELQESLIGVTVDKLYRQK  213
Zebrafish PQRDISRKITAFQYELVNHRPGADPQSLSAPCQPCTDAEVLLAVCTSDFVARGRILGVSEEDEQ--TSVTVSLSHLYRQK  211

Human     SRVFEPVPEGD-GHWQGRVRTLLECGVRPGHGDFLFTGHMHFGEARLGCAPRFKDFQRMYRDAQERGLNPCEVGTD  311
Mouse     SRVFQPAPE-DSGHWLGHVTTLLQCGVRPGHGEFLFTGHVHFGEAQLGCAPRFSDFQRMYRKAEEMGINPCEINME  311
Rat       SRVFQPAPE-DSGHWLGHVTTLLQCGVRPGHGEFLFTGHVHFGEAQLGCAPRFSDFQKMYRKAEERGINPCEINME  311
Cattle    SRVFRPAPEGEGGGWRGRVSTLLECGVRPGHGEFLFTGHMHFGEAWLGCAPRFKDFQRMYRDAEERGLNPCEMGTE  309
Frog      SKIFLPKEN---GGWEGTIRTPRECGVKAGSGSFLFTGRMHFGEPRLGCTPRYSDFTRIYLEAKKQGLNPCEISTD  286
Zebrafish TQVFVSGGG-RAKRWTGFVKMSRQCGVKPGDGEFLFTGTVRFGEAWLSCAPRYKDFLRVYQDARQQGTNPCHLETD  286
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Figure 1. Amino acid sequences of Metrnl precursors in several vertebrates. Identical amino acids are marked in black, and similarity is marked in gray. The putative NH_2-terminal signal sequences are indicated by the red frame, and the potential glycosylation site is indicated by a red star.

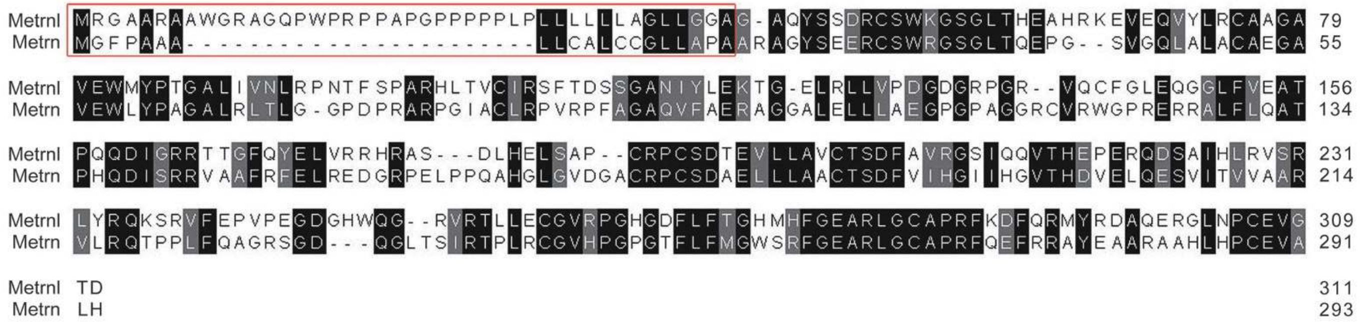

Figure 2. Amino acid sequences of human Metrnl and Metrn. Identical amino acids are marked in black, and similarity is marked in gray. The putative NH_2-terminal signal sequences are indicated by the red frame.

that the mouse Metrnl contains one potential N-glycosylation site, the two labs then incubated the purified recombinant protein with glycopeptidase F[29] or N-glycanase[35], both obtaining a single band with a decreased molecular weight closer to the calculated size. These experimental results from both groups indicate that the larger molecular weight proteins represent glycosylated forms of Metrnl, thus proving that Metrnl is indeed post-translationally glycosylated, which may facilitate its secretion from the cells[38].

However, the N-glycosylation site in mouse Metrnl is not conserved in Metrn or human Metrnl (Figure 1), suggesting that the glycosylation of mouse Metrnl may not play a central role in its function.

Metrnl expression in various tissues

Homologous to Metrnl, Metrn was discovered earlier and is mainly expressed in the nervous system both during development and in adult mice[37]. It has been reported that during embryonic mouse development, Metrn is widely expressed in undifferentiated neural progenitors and in the astrocyte lineage, including radial glia[36]. In the adult mouse brain, Metrn is highly expressed in Bergmann glia and in a few discrete neuronal populations, with low levels of Metrn in astrocytes distributed ubiquitously throughout the brain[37, 39, 40].

In view of Metrn expression being closely associated with the brain, there is now great interest in the Metrnl expression pattern, especially concerning whether this novel protein is also expressed mainly in brain and whether it plays a similar role as a neurotrophic factor in neurogenesis like Metrn.

However, the structural similarity of Metrnl and Metrn do not seem to grant a similar expression pattern. Recent studies of Metrnl have demonstrated that it is widely expressed in adult mouse tissues at differing expression levels, but its highest expression is obviously not in the brain[29].

In the nervous system

Assessed by *in situ* hybridization (ISH), Metrnl expression is found in very restricted sites within the brain during development. Metrnl mRNA expression appears weak in the otic vesicle of medaka embryos[32], but during early mouse development, this transcript is found exclusively in the floor plate, and from E13.5, it is also found in dorsal root ganglions (DRG) and the inner ear but apparently not in the adult nervous system[35].

Nevertheless, Metrnl is reported to be expressed in adult mouse brain, with a much lower expression level than that found in other tissues, such as white adipose tissue and skin[29, 41].

In adipose tissue

Examined by real-time PCR, the highest expression level of Metrnl is in subcutaneous white adipose tissue of both rodents and humans, with expression also detected in various tissues, including liver, spleen, muscle, heart, thymus, forebrain, midbrain, hindbrain, omental adipose tissue, subcutaneous adipose tissue, perivascular adipose tissue and interscapular adipose tissue[29].

Metrnl protein is easily detected in the incubation medium of white adipose tissue by Western blot and ELISA[29, 30], which is consistent with its secretion. To further clarify the cell types in which Metrnl is mainly expressed, Metrnl expression was first compared between adipocytes and stromal cells separated by collagenase digestion of fat tissue, with no significant difference observed[29]. Upon comparing Metrnl expression between RAW264.7 macrophages and 3T3-L1 adipocytes, much lower expression was found in the macrophages[29]. These results indicate that both adipocytes and stromal cells but not unactivated macrophages are the main cell types expressing Metrnl. Immunohistochemical analysis of the fat tissue shows that Metrnl is distributed throughout the adipose tissue, except in the lipid droplets[29].

In mucosal tissues, skin and activated macrophages

According to Metrnl expression data from the human BIGE database, the highest expression of Metrnl is in activated monocytes, followed by digestive and respiratory mucosal tissues and the skin[41]. Our unpublished results on the real-time PCR detection of Metrnl expression in various tissues also showed that Metrnl is highly expressed in digestive tract and lung (data not shown).

With strong expression in activated human monocytes

according to the BIGE database, Metrnl was further found to be produced by alternatively activated macrophages and macrophage colony stimulating factors (M-CSF)-stimulated bone marrow macrophages[41].

In addition, under resting conditions, Metrnl is expressed in fibroblasts but not in keratinocytes or peripheral blood mononuclear cells, whereas its expression is increased in IFNγ-treated keratinocytes[41]. Moreover, significant up-regulation of Metrnl expression was observed in familial primary localized cutaneous amyloidosis (FPLCA)[42], psoriasis, prurigo nodularis, actinic keratosis and atopic dermatitis and in synovial membranes of human rheumatoid arthritis, thus suggesting a potential role of Metrnl in both innate and acquired immune responses[41].

Function of Metrnl

Metrnl acts as a neurotrophic factor

Many secreted proteins, including NGF, GDNF and Metrn, are neurotrophic factors that nourish neurons and play central roles in neuronal development, maintenance and regeneration[36, 37, 43–47]. Recently, Jorgensen *et al*[35] reported the neurotrophic activity of Metrnl in neurite outgrowth and neuroblast migration *in vitro* and in the survival of spiral ganglion neurons *in vivo*.

Using Metrn as a positive control, both proteins were tested on cultures of dissociated dorsal root ganglia. In the Metrnl treated group, neurite outgrowth was dose dependent and not significantly different from that induced by Metrn. Moreover, simultaneous treatment with Metrnl and Metrn showed an additive effect, with significantly more neurites compared to treatment with either factor alone[35]. Either the Jak inhibitor I (JAKiI) or the MEK inhibitor U0126 abrogates the Metrnl induced neurite outgrowth, indicating that both the Jak-STAT3 and MEK-ERK pathways are involved in the effect of Metrnl[35]. In rat subventricular zone explants, Metrnl induced a significant increase of neuroblast migration, with a similar effect to that induced by the positive control, stromal cell-derived factor 1a[35]. In a further investigation of the possible role of Metrnl in the adult inner ear, Jorgensen *et al* used a guinea pig model whose auditory sensory cells had been destroyed. The hearing-impaired animals treated with recombinant Metrnl exhibited a significant therapeutic effect in retaining electrical responsiveness of the auditory neurons, which was further supported by a stereological analysis indicating more spiral ganglion neurons in the treated animals than in the control group[35].

Later, Watanabe *et al*[48] reported that Metrnl is a latent process (LP) gene. The expression of these genes is upregulated during the latent process, a preparation step for cellular function, and this expression is required for subsequent neurite extension. Small interfering RNA targeting of Metrnl significantly inhibits NGF-induced neurite extension of PC12 cells, an adrenal chromaffin cell line that is a well-characterized model of nerve cells. This inhibition is partially prevented by Metrnl rescue constructs, which indicates an indispensable role of Metrnl in neurite extension. The effect of small interfering RNA knockdown of Metrnl expression on neurite extension is also observed in primary dissociated hippocampal neurons of rats, indicating that the role of Metrnl in neurite extension in primary neurons is consistent with that in the PC12 cell line[48]. Given that persistent activation of ERK is required for the NGF-induced differentiation of PC12 cells, Metrnl expression was shown to be dependent on ERK activity[48]. Further, the neurotrophic factors, *ie*, pituitary adenylate cyclase-activating peptide (PACAP) and forskolin, induce weaker ERK phosphorylation but greater Metrnl expression than NGF does, suggesting that Metrnl expression is regulated not only by ERK but also by other signaling pathways, at least in response to PACAP and forskolin[48].

Metrnl induces white adipose browning

In mammals, there are two main types of adipose tissue: white adipose tissue (WAT), which stores energy, and brown adipose tissue (BAT), which dissipates energy[49–51]. WAT and BAT differ at the functional, morphological, and molecular levels[51–53]. However, upon thermogenic stimuli, WAT possesses the capacity to generate brown-like adipocytes (also called beige adipocytes), and this process is termed "browning" or "beiging"[53, 54]. Due to its great therapeutic potential in developing new therapies for metabolic diseases, the browning of WAT has received much attention.

Recently, Rao *et al*[55] showed a role for Metrnl in the browning of white adipose tissue. Metrnl, which Rao *et al* identified as a PGC-1α4-dependent myokine in skeletal muscle, can be induced in muscle after exercise, a physiological stimulus that also increases PGC-1α4 expression[55]. The authors demonstrated that muscle-specific expression of PGC-1α4 promotes browning of the subcutaneous and epididymal white adipose tissue[55], a process that correlates with the ability to defend body temperature in cold environments[53, 56–58]. These results suggest that Metrnl mediates muscle-fat crosstalk to promote the expression of genes that are associated with browning of the white adipose tissue[55].

Rao *et al* also showed that increasing circulating Metrnl in mice, either by delivering a Metrnl-expressing adenoviral vectors to the liver or through the administration of recombinant Metrnl, produces remarkable increases in the expression of genes associated with beige fat thermogenesis and anti-inflammatory cytokines in white adipose tissue[55]. Metrnl was also shown to induce a thermogenic phenotype when expressed locally in adipose tissue *in vivo*. However, it should be noted that this phenotype can only be maintained for a short period (fewer than 10 days).

Examination of mRNA profiles from the subcutaneous white fat of mice with increased circulating Metrnl shows significant increases in several genes associated with alternative macrophage activation. Metrnl expression also increases the production of IL-4 and IL-13 as well as catecholamines in the adipose tissue. This suggests that Metrnl induces a phenotypic switch in adipose tissue macrophages *in vivo*, along with production of pro-thermogenic catecholamines, possibly via the induction of the M2-regulatory cytokines IL-4

and IL-13[55]. The browning response induced by Metrnl is IL-4/13 dependent because disruption of IL-4/13 signaling in STAT6 knockout mice causes no change in alternative macrophage activation but attenuates the effects of IL-4/13 on the regulation of thermogenic or β-oxidation genes and reduces the content of catecholamines[55]. The primary source of IL-4/13 upon Metrnl treatment is shown to be eosinophils, the number of which increases in the adipose tissue when circulating Metrnl is elevated[55].

Finally, blocking Metrnl actions *in vivo* by anti-Metrnl antibody results in reduced mRNA expression of IL-4/13 along with a reduced number of eosinophils induced upon acute cold exposure for 24 h. The anti-Metml antibody also significantly inhibits the expression of genes that are characteristic of M2 macrophages and adipose thermogenesis induced by 72 h of cold exposure, thereby implicating the role of Metrnl in cold adaptation[55].

Metrnl antagonizes insulin resistance

In addition to being the largest reservoir for energy storage, adipose tissue is a highly active endocrine organ that synthesizes and secretes proteins/peptides termed adipokines[10, 59]. Adipokines participate in the regulation of multiple physiological functions, including metabolism, insulin sensitivity, cardiocerebrovascular function, immunity and inflammation[10, 60]. Dysregulated production or secretion of adipokines is associated with the pathogenesis of obesity-linked disorders[61, 62]. With potential clinical relevance, adipokines are promising candidates as new therapeutic compounds or targets in the treatment of obesity and its related diseases[2, 61].

Our lab has identified Metrnl as an adipokine that is abundantly expressed in rat, mouse and human subcutaneous white adipose tissue, with relatively lower expression levels found in brown adipose tissue and a much lower expression level in the brain[29]. In addition, Metrnl is downregulated in white adipose tissue of caloric restriction rats but is dramatically upregulated during white adipocyte differentiation and in the white adipose tissue of diet-induced obese mice[29]. These results suggested a role for Metrnl in white adipose biology and metabolic homeostasis, leading us to explore the function of Metrnl in white adipose tissue.

Adipogenesis, the differentiation of fibroblast-like mesenchymal stem cells into adipocytes, plays a central role in the regulation of whole body energy metabolism[63]. Metrnl is detectable both in the incubation medium of white adipose tissue and in the culture medium of either primary adipocytes or 3T3-L1 adipocytes[29, 30]. To study the adipogenic potential of Metrnl, we employed gain-of-function and loss-of-function experiments using 3T3-L1 adipocytes as an *in vitro* model of adipogenesis to demonstrate that Metrnl promotes lipid accumulation and upregulates markers specific to mature adipocytes[30]. Importantly, the expression of PPARγ, which is the key regulator of adipocyte differentiation[64–66], is induced by Metrnl protein in a concentration-dependent manner, indicating that secreted Metrnl promotes adipocyte differentiation[30].

We further demonstrate an insulin sensitizing role of Metrnl using genetically engineered mouse models. Tested by various methods, insulin resistance induced by a high-fat diet (HFD) is exacerbated in adipocyte-specific Metrnl knockout mice, whereas transgenic mice overexpressing Metrnl specifically in adipocytes were protected from diet-induced insulin resistance. Moreover, the overexpression of Metrnl in adipose tissue also antagonizes insulin resistance in mice with leptin deficiency[30].

We provide evidence that adipose Metrnl is most likely to ameliorate overall insulin resistance through its action on local adipose tissue in an autocrine or paracrine fashion[30]. First, though the phenotypes with regards to insulin resistance in these mouse models are obvious and unambiguous, their serum Metrnl concentrations remain unchanged compared to the corresponding control mice. Second, the insulin-stimulated phosphorylation of AKT is enhanced by adipocyte Metrnl in white adipose tissue, but not in other major metabolic tissues (brown adipose tissue, muscle and liver). Third, increasing the circulating Metrnl levels via the intravenous administration of recombinant Metrnl for 1 week is unable to rescue insulin resistance in adipose-specific Metrnl knockout mice fed a HFD. Moreover, acute intravenous injection with recombinant Metrnl has no hypoglycemic action in HFD-fed C57 obese mice or in leptin knockout obese mice[30].

Both *in vitro* and *in vivo*, Metrnl promotes adipocyte differentiation, which is a key factor in forming functional fat for insulin sensitivity and lipid metabolism. We have detected gene markers related to adipocyte differentiation and lipid metabolism in white adipose tissue of both HFD-fed Metrnl knockout mice and Metrnl transgenic overexpression mice and found that Metrnl upregulates key transcription factors for adipocyte differentiation (PPARγ, C/EBPα) and lipid metabolism genes for lipid transport (FABP4, CD36), lipogenesis (ACC, FASN), lipolysis (Lipe, PNPLA), and lipid storage (Perilipin). In addition, the overexpression of Metrnl decreased the proportion of small adipose cells[30], which is consistent with insulin sensitization[67–69].

The transcription factor PPARγ is the key regulator of the fully differentiated and insulin-sensitive adipose cell phenotype, with consequences for the proper functioning of the adipose tissue and whole-body insulin sensitivity[64–66]. The expression of PPARγ is increased markedly in adipose tissue of Metrnl transgenic mice, in agreement with the *in vitro* results. We subsequently demonstrated that PPARγ plays a critical role in Metrnl-mediated beneficial effects by using long-term treatment with two different small-molecule inhibitors of PPARγ as well as the knockdown of PPARγ. The inhibition or knockdown of PPARγ completely abolished the insulin-sensitizing effect of Metrnl in HFD-fed Metrnl transgenic mice, indicating that Metrnl-mediated insulin sensitization occurs through the PPARγ pathway[30].

We also investigated many other factors that are possibly related to the insulin sensitization of Metrnl[30]. For example, adipocyte Metrnl prevents an increase in TNF-α by chronic HFD but not acute LPS, indicating a role of Metrnl in the inhibition of HFD-induced adipose inflammation. Adipocyte

Metrnl attenuates HFD-induced hypertriglyceridemia but not HFD-induced hypercholesterolemia or the accumulation of liver triglyceride. Metrnl enhances serum triglyceride clearance during acute lipid overload test and elevates the expression and activity of lipase in adipose tissue. These results all indicate a role of Metrnl in the activation of adipose lipid metabolism. However, no changes have been observed in body weight, food intake, lean/fat mass, distribution of adipose tissue, or energy expenditure in either Metrnl adipose-specific knockout or transgenic overexpression mice compared to control mice[30]. In particular, considering a physiological role for Metrnl in cold adaption as a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis, as reported by Rao *et al*[55], we explored whether this mechanism is involved in our transgenic mouse models. We did not observe any significant changes in IL-4/13 expression, M2 macrophage activation, eosinophil accumulation, or thermogenic gene expression in the white adipose tissue of these transgenic mouse models, indicating that no browning of white adipose tissue occurs[30]. This discrepancy between these studies may be caused by the different animal models and intervention methods, for instance, the relatively acute models used by Rao *et al* versus the chronic models used in our study.

We have also noted a meeting abstract reporting that Metrnl is mainly expressed in undifferentiated osteoblasts and hypertrophic chondrocytes and reduces the terminal differentiation of human osteoblastic MG63, which may be associated with the inhibition of Metrnl on transcription activity of AP-1[70].

Concluding remarks

Metrnl is a novel secretory protein with three new emerging functions (Figure 3). First, Metrnl acts as a neurotrophic factor that promotes neurite outgrowth and neuroblast migration *in vitro* and supports the survival of spiral ganglion neurons *in vivo*. Second, Metrnl is involved in cold adaption by regulating immune-adipose interactions to increase beige fat thermogenesis. Third, Metrnl plays an important role in the biology of white adipose tissue, improving adipose function and antagonizing obesity-induced insulin resistance. With its neurotrophic activity and beneficial metabolic effect, this protein could be expected to provide novel therapeutic strategies for certain diseases. However, the utilization of this newly described protein as a therapeutic target or agent requires much work to be done to understand its functional spectrum in health and disease, especially the key mechanisms that

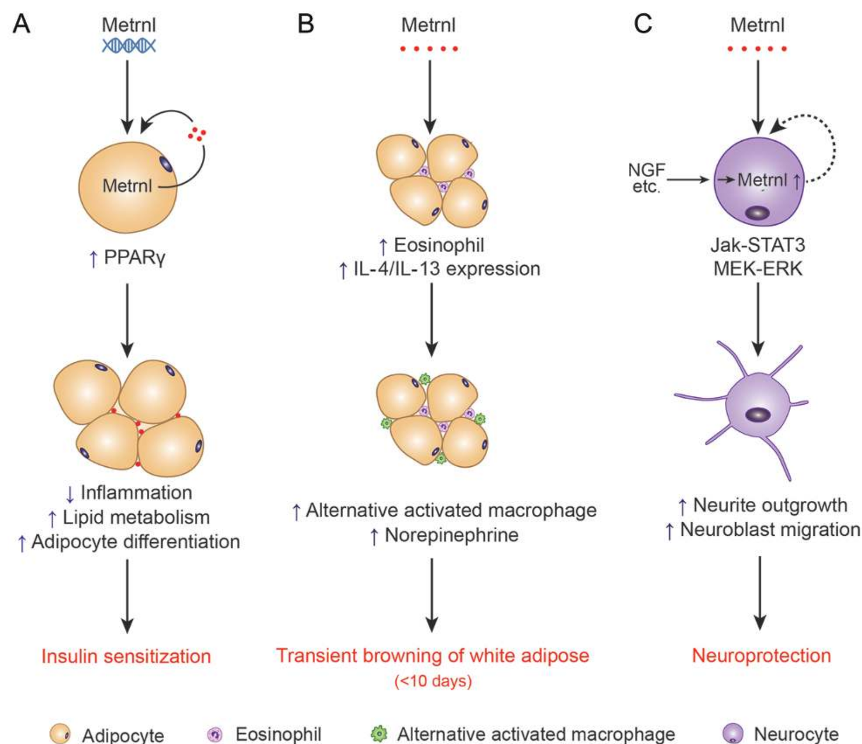

Figure 3. Known functions of Metrnl in white adipose tissue and neurocytes. (A) Overexpression of Metrnl upregulates PPARγ in adipocytes via an autocrine/paracrine mechanism, which inhibits adipose inflammation, enhances adipocyte differentiation, activates lipid metabolism, and ultimately reduces insulin resistance. (B) Administration of abundant Metrnl causes trafficking of eosinophils into white adipose tissue and increases local IL-4/13, which promotes alternative macrophage activation along with increased norepinephrine. Through this mechanism, Metrnl transiently induces white adipose browning for less than ten days. (C) Nerve growth factor (NGF), pituitary adenylate cyclase-activating peptide (PACAP) and forskolin induce Metrnl expression in PC12 cells. Metrnl protein promotes neurite outgrowth and neuroblast migration via the Jak-STAT3 and MEK-ERK pathways and plays a neuroprotective role *in vivo*.

initiate its actions. Important questions exist, such as what is the role of Metrnl in barrier tissues; how does Metrnl influence nerve system function; and what, if any, specific receptor exists for Metrnl?

Because research into this novel bioactive protein, Metrnl, has only just begun, its function and mechanisms of action remain largely unknown. As many as five names (Metrnl, Meteorin-like, Cometin, Subfatin, IL-39) have been proposed for this novel protein. Meteorin-like, simply highlighting Metrnl's homology with Meteorin (Metrn), was the initial name of the protein annotated in public databases at the time when neither its expression nor its functions had been reported. The most recent studies on Metrnl have revealed that the expression patterns and functions of Metrnl appear to be quite different from those of Meteorin (Metrn). Thus, the name "Meteorin-like" is not appropriate, which has led to new names being proposed, including Cometin, Subfatin and IL-39, based on the expression features and functions of this protein. However, one name for each function of the same protein is inappropriate, makes further work complicated for researchers, authors and readers, and is unfavorable for broad studies of this novel protein. To solve this issue, we suggest Metrnl, identical to the gene symbol, as the protein designation in the future literature. With the gene symbols being unique, Metrnl could uniquely represent the encoded protein, thus allowing for clear and unambiguous reference to Metrnl protein in scientific communications.

Acknowledgements
This work was supported by grants from the National Natural Science Foundation of China (No 81130061, 81202572, and 81373414).

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