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mitoNEET as a novel drug target for mitochondrial dysfunction

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Mitochondrial dysfunction plays an important part in the pathology of several diseases, including Alzheimer's disease and Parkinson's disease. Targeting mitochondrial proteins shows promise in treating and attenuating the neurodegeneration seen in these diseases, especially considering their complex and pleiotropic origins. Recently, the mitochondrial protein mitoNEET [also referred to as CDGSH iron sulfur domain 1 (CISD1)] has emerged as the mitochondrial target of thiazolidinedione drugs such as the antidiabetic pioglitazone. In this review, we evaluate the current understanding regarding how mitoNEET regulates cellular bioenergetics as well as the structural requirements for drug compound association with mitoNEET. With a clear understanding of mitoNEET function, it might be possible to develop therapeutic agents useful in several different diseases including neurodegeneration, breast cancer, diabetes and inflammation.

Introduction

Several diseases have been shown to have dysfunctional mitochondria as part of the disease etiology [1–6]. Mitochondria play an important part in energy homeostasis of cells. Several of the age-related diseases increasingly seen worldwide seem to share a common mitochondrial link [7,8]. Mitochondrial dysfunction has been suggested to play a crucial part in several neurodegenerative diseases from Alzheimer's disease to Parkinson's disease [9,10], and in several of the cell death pathways that result in tissue damage, especially via reactive oxygen species (ROS) and the resultant oxidative stress [11,12]. In addition, metabolic diseases such as type II diabetes have mitochondrial-associated abnormalities and changes in bioenergetics [13]. Targeting mitochondria as a therapeutic intervention to prevent cellular damage or cell death has been discussed increasingly in the literature, from cardiovascular to neurodegenerative diseases. Additionally, mitochondria are also thought to be a relevant target for cancer drug discovery, because hyperproliferative cells show higher metabolic demands than normal cells [14]. The role mitochondria have in different diseases is still not fully understood, especially because the complexities of the cellular environment impact on mitochondrial function and vice versa remains to be fully elucidated. Within the paradigm of developing drugs that modify mitochondrial bioenergetics, this review will focus on an emerging mitochondrial protein drug target: mitoNEET (gene: CISD1, CDGSH iron sulfur domain 1), recently discovered to have a significant role in cellular bioenergetics and the mitochondrial oxidation capacity [15–17].

Discovery and structure of mitoNEET

The mitochondrial protein mitoNEET was identified through its ability to bind pioglitazone [18]. Because several of the beneficial activities of thiazolidinediones (TZDs) could not be explained by activation of peroxisome proliferator-activated receptor (PPAR)-γ [19,20], Colca *et al.* used [^{3}H]-pioglitazone and a photo-affinity cross-linker to isolate a ~17 kDa protein that contained a NEET (Asn-Glu-Glu-Thr) sequence and was localized within mitochondrial fractions. This led to the naming of this novel protein as 'mitoNEET' [18]. The search for mitoNEET stemmed from an interesting observation that TZDs have several effects that could not be fully explained by their activity as PPAR-γ agonists [20]. These included the reduction in blood lipids by pioglitazone (Fig. 1) that was of greater magnitude than that of the more potent PPAR-γ agonist rosiglitazone.

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1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2014.05.001

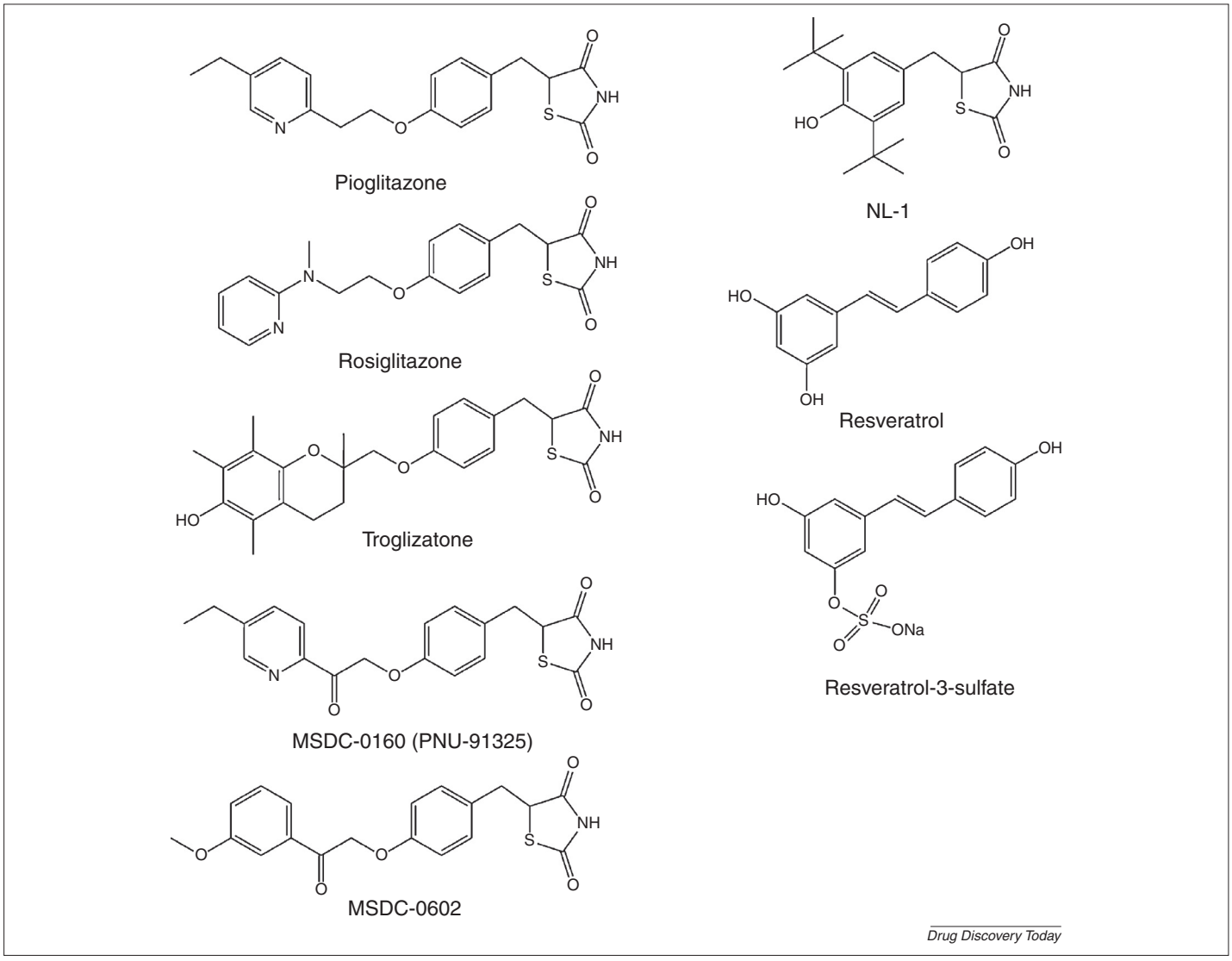

FIGURE 1

Structures of mitoNEET and mitochondrial target complex of TZDs (mTOT) ligands discussed in this review. *Abbreviation*: TZD, thiazolidinedione.

The crystal structure of mitoNEET was reported by two independent groups several days apart [21–23] (Fig. 2). From bioinformatics structural analysis it was shown that mitoNEET belongs to the zinc-finger family of proteins with a 39 amino acid sequence that is unique to this family: C-X-C-X2-(S/T)-X3-P-X-C-D-G-(S/A/T)-H [17]. Interestingly, mitoNEET does not contain any zinc ions, but rather iron contained in a 2Fe-2S (iron–sulfur) cluster [17]. The coordinating amino acids in the cluster are three cysteines and one histidine. mitoNEET is shown in the crystal structures to form a dimer with two iron–sulfur-cluster-binding domains. The topology of this dimer is distinctive in that the N-terminal β-strand is 'strand swapped' into the opposite domain. There is a striking similarity in structure between different species of mitoNEET. To date, mitoNEET crystal structures from mammals [21,22], thermophilic bacteria [24] and plants [25] reveal the same fold topology, demonstrating its importance as an evolutionarily conserved domain. Additional structural analysis from the protein sequence suggests that the amino acids 14–32 contain a transmembrane sequence [17] (Fig. 2).mitoNEET is thought to be redox sensitive, owing to the presence of the 3-Cys-1-His 2Fe-2S clusters in its structure [17,21,26]. The iron–sulfur cluster seems to be labile, because at low pH it can be released from mitoNEET and the cluster can be transferred to iron-accepting proteins such as apo-transferrin [22,27]. Pioglitazone, an antidiabetic drug, was shown to slow down the release of the iron–sulfur clusters from mitoNEET under acidic conditions, which suggests that mitoNEET can be stabilized by pioglitazone upon binding [22]. The release of the iron–sulfur cluster seems to be dependent on the oxidation state of the iron, where release occurs in the oxidized state. The release of the FeS cluster can be modulated by the mutation of the histidine to a cysteine (H87C), and this mutation has been shown to slow the transfer of the iron–sulfur cluster [27,28]. At first glance, the pH dependence of the stability and mitigation of this iron–sulfur sensitivity by the H87C mutant indicates that it is the titration of the imidazole from His87 (with a pK_a approaching 6) that leads to disruption of the iron–sulfur cluster rather than a pH-dependent destabilization of the domain fold. However, although the pH-dependent stability of H87C is over six-times greater at or close to pH 6 [28], there is still a modest loss of activity as the pH is lowered from 7 to 5. The H87 thus seems to play an important part in the

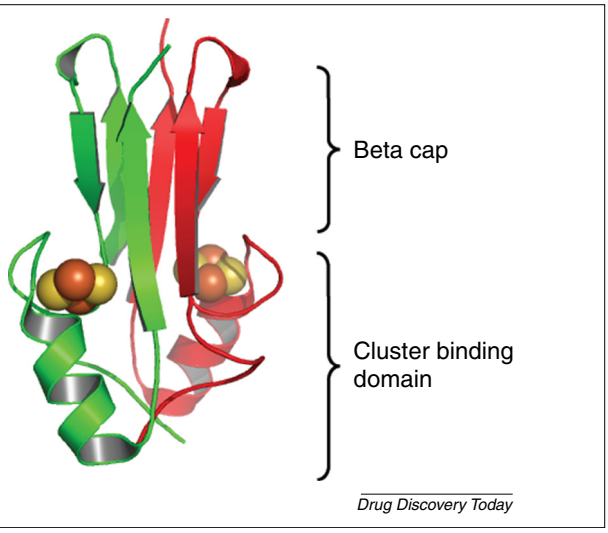

FIGURE 2

Structure of the mitochondrial outer membrane protein mitoNEET. Interestingly, this protein forms a dimer as a crystal structure and contains 2Fe-2S clusters that could play an important part in the biological functions. Figure created with 3REE.pdb using PyMOL Molecular Graphics System, Version 0.99 [61].

iron–sulfur cluster transfer and regulates the stability of mitoNEET [27].

Despite the three examples of mitoNEET structures from three different kingdoms and many known sequences across the domains of life, the phylogenetic origin of this protein is still somewhat unclear. For example, until recently it was unclear whether the sequence motif distinctive to this type of iron–sulfur cluster protein had emerged multiple times by convergent evolution or if all mitoNEET-related proteins stem from a common ancestral gene. A recent publication by Lin *et al.* has begun to answer this question and suggests a common evolutionary source [29]. Crystallization of different types of CDGSH iron–sulfur domain proteins revealed seven different types of proteins, with mitoNEET (CISD1) belonging to the first group. The CISD proteins can be classified into three primary groups based on the folds of the proteins, having a eukaryotic fold (types 1 and 2), a prokaryotic fold (types 3, 4 and 7) or a tandem-motif fold (types 5 and 6) [29]. Lin *et al.* conclude, based on phylogenetic data and their structural studies, that these three fold classes have arisen from a common ancestral homodimeric CISD with a single CDGSH motif in each protomer. Miner1 [gene: CISD2; nutrient-deprivation autophagy factor-1 (NAF-1)], found on the endoplasmic reticulum (ER) has been linked to Wolfram syndrome, whereby mice with a mutation in Miner1 have a shortened lifespan and mice overexpressing Miner1 have increased longevity, suggesting an important role in aging [30–36].

Biological activity

Our current understanding of the part mitoNEET plays in physiology is based on a combination of genetic studies and the effect of mitoNEET ligands on mitochondrial activity and protein expression levels (Fig. 3). The full biological activity of mitoNEET still remains to be elucidated, but several studies have shown this mitochondrial protein to have a significant effect in bioenergetics of cells [15,20,37], as well as possibly having an important role in mitophagy [38]. The bioenergetics of mitochondrial function refers to the mitochondrial function and dysfunction, which essentially refers to the ability of mitochondria to respond to cellular energy demands and need for ATP synthesis [39]. mitoNEET null (−/−) mice did not appear to have an apparent physical phenotype [17]. However, respiration studies performed on isolated heart mitochondria from mitoNEET (−/−) mice showed that there was a ~30% decrease in state 3 respiration (ADP stimulated respiration) and an uncoupling of respiration. By contrast, the state 4 respiration was unchanged in the mitochondria without mitoNEET [17]. Our group has also shown that a mitoNEET ligand, NL-1 (Fig. 1), can protect neuronal cells against the complex I toxin rotenone. Taken together, the data suggest that mitoNEET plays an important part in mitochondrial energy function, because in the mitochondria where mitoNEET was absent the regulation of oxidative phosphorylation and electron transport was significantly diminished [17].

Several insights into the biological activity of mitoNEET came from a landmark study by the Scherer group [15]. Obesity has several key factors that converge at the bioenergetics of cells. As can be seen in obesity as well as in type II diabetes, there is lower observable β-oxidation, electron transport chain activity and oxidative metabolism [15,40]. To determine the effect mitoNEET has on bioenergetics in obese mice, mitoNEET was overexpressed in the ob/ob mouse strain [15]. Interestingly, mice with overexpression of mitoNEET gained significant weight via an increase in adipose tissue. In contrast to normal obesity, it was found that insulin sensitivity was maintained in the mice overexpressing mitoNEET and a decrease in ROS generation from the mitochondria was observed. When obese mice had mitoNEET knocked out there was an increase in mitochondrial ROS and a loss of insulin sensitivity. It seems that mitoNEET could play a key part in regulating energy use in cells and their lipid metabolism [40].

The importance of mitoNEET in cellular bioenergetics was corroborated by findings in MDA-MB-231 breast cancer cells overexpressing mitoNEET [16]. In these cancer cells, the overexpression of mitoNEET led to a correlated increase in the mitochondrial electron transport chain complexes as well as increased tumor growth. Additionally, cells that overexpressed mitoNEET appeared to have resistance to autophagy under starving-induced conditions [16]. Although a clear link still has yet to be made between the mitoNEET levels and hyperproliferation, the notion that mitochondrial transport chain complexes and mitoNEET could have a central role in oncogenesis is an intriguing possibility [16]. Jennings and colleagues showed that mitoNEET plays an important part in the proliferation of breast cancer cells and tumor formation [41]. Taken together, this could explain the mechanism behind the observation that compounds that act as mitochondrial inhibitors, such as the complex 1 inhibitor metformin, prevent cancer prevalence in type II diabetic patients [42]. However, it should be noted that metformin itself does not bind to human recombinant mitoNEET (unpublished data), suggesting its mechanism of action on mitochondria does not include mitoNEET binding.

As mentioned, mitoNEET plays an important part in mitochondrial bioenergetics and TZD compounds have been shown to interact with mitochondria [43]. This led to the development of novel TZDs that do not bind PPAR-γ by targeting the mitochondrial target complex of TZDs (mTOT) [44]. Colca and colleagues developed a pioglitazone clinical derivative MSDC-0160 (PNU-91325) (Fig. 1)

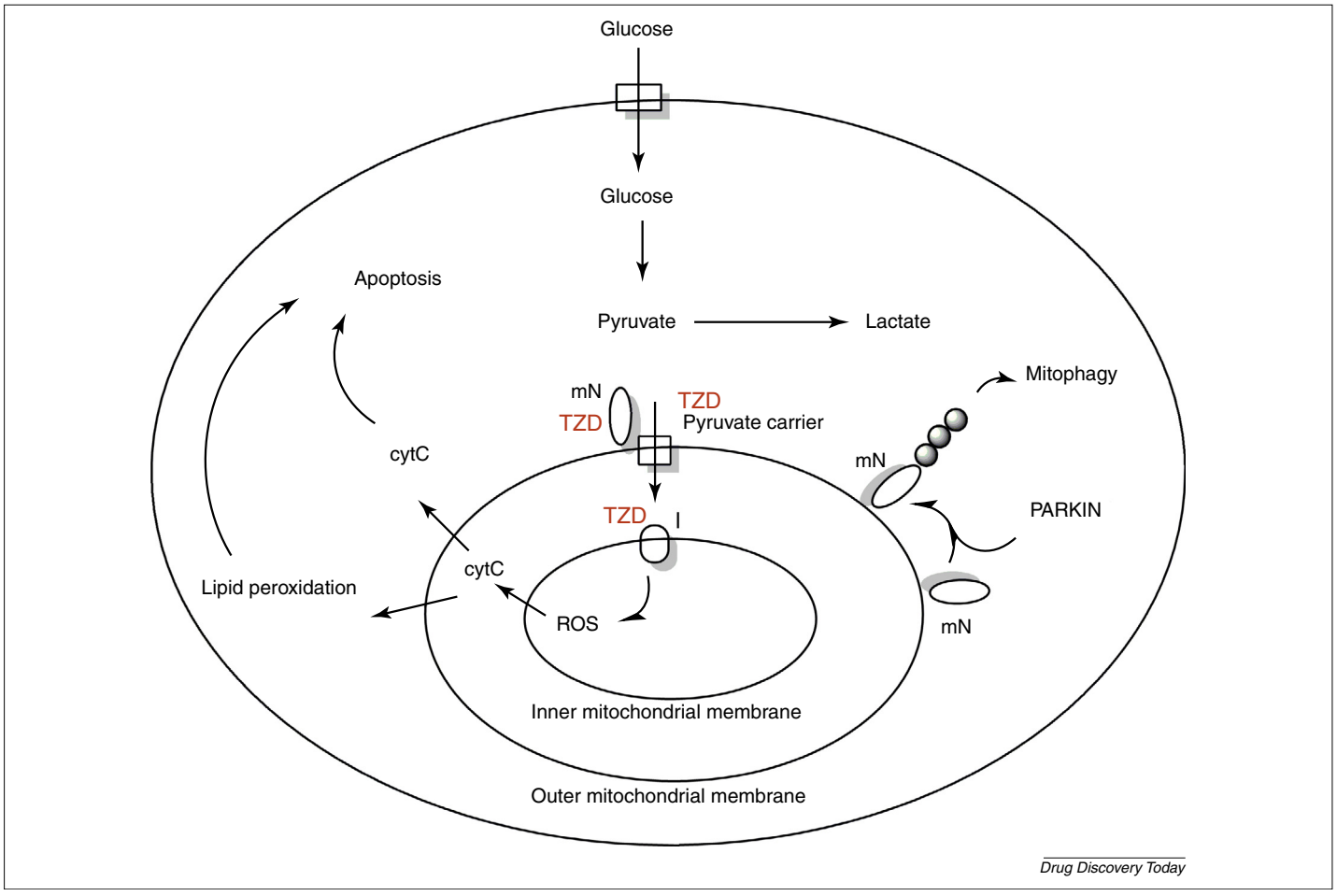

FIGURE 3

mitoNEET has been implicated to play a part in several pathways in cells, based on our current understanding. In this schematic representation, mitoNEET plays a part in mitochondrial bioenergetics as well as interacting with several other pathways. Recently, mitoNEET has also been identified to have a role in mitophagy, via its interactions with phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and PARKIN, and with glutamate dehydrogenase 1 (GDH1) and insulin regulation. Figure adapted, with permission, from [15,50,51]. *Abbreviations*: cytC, cytochrome C; mN, mitoNEET; ROS, reactive oxygen species; TZD, thiazolidinedione.

based on the metabolites of pioglitazone that do not activate PPAR-γ [45]. They showed that MSDC-0160 could bind to mitoNEET and mitochondria in a similar way to that of rosiglitazone and pioglitazone, as well as activate phosphoinositide-3-kinase-directed AKT phosphorylation. In addition, daily oral treatment with MSDC-0160 led to a significant increase in mitoNEET RNA expression as well as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) [45]. These data suggest that mitoNEET might be involved in signaling changes in mitochondrial bioenergetics. Similar results were found from another clinical candidate: MSDC-0602. This compound had the same positive effects on insulin sensitivity as seen with MSDC-0160, with lower rates of gluconeogenesis and lipogenesis. PPAR-γ null mice demonstrated the same effects indicating that the MSDC-0602 effects observed were not related to PPAR-γ [46]. Taken together, the beneficial effects of TZD drugs such as pioglitazone and rosiglitazone are in part due to their interactions with mitoNEET. MSDC-0160 was recently submitted to Phase IIb clinical trials to investigate its positive effects in lowering fasting glucose levels without demonstrating the negative side effects (e.g. edema formation) found with PPAR-γ agonists. One of the side effects normally associated with PPAR-γ agonist therapy is the development of fluid retention [47]. During a 12-week study, it was found that MSDC-0160 lowered blood glucose levels to the same extent as pioglitazone, but with a 50% reduction in edema formation [44]. Recently, MSDC-0160, pioglitazone and rosiglitazone were shown to interact with an additional component of the mTOT complex, specifically the mitochondrial pyruvate carrier [48,49]. This is consistent with the observation that ligands to mitoNEET can reduce pyruvate-driven respiration implicating mitoNEET as part of the pyruvate carrier complex. New insight into the mechanism of TZDs was recently brought to light when it was shown that pioglitazone can bind to complex I (subunits NADH dehydrogenase (ubiquinone) complex 1 assembly factor (NDUFA) NDUFA9, NDUFA6 and NDUFB6) and induce dissociation from complex I [50]. mitoNEET can also play a part in insulin regulation via interaction with glutamate dehydrogenase 1 (GDH1), a key enzyme in insulin regulation [51,52].

A link between mitoNEET and inflammation was also established [53,54]. Injection of lipopolysaccharide (LPS) into the striatum of rats led to an increase in mitoNEET levels, which pioglitazone was able to attenuate [53]. When lipopolysaccharide (LPS) was injected into the striatum the mitoNEET protein expression levels were

TABLE 1

Effect of compounds on mitoNEET and mitochondrial target complex of TZDs (mTOT) related pathways

Compound	Activity	Refs
NL-1	Human recombinant mitoNEET binding (7.3 μM) State 3 respiration (2.45 μM) Protection against complex I toxin rotenone	[60,62]
MSDC-0160 (PNU-91325)	Binding to mitoNEET (1–10 μM)[a]	[19]
Pioglitazone	mitoNEET binding (1 μM) State 3 respiration	[18,43,63,64]
Rosiglitazone	Human recombinant binding (700 nM) State 3 respiration Reduction in ROS	[43,50,60]
Resveratrol and resveratrol-3-sulfate	Reduction in ROS State 3 respiration PGC-1α	[16,65,66]

Abbreviations: PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ROS, reactive oxygen species; TZD, thiazolidinedione.
[a] Only the concentration range was given in citation.

increased, whereas co-treatment with pioglitazone attenuated LPS stimulation of mitoNEET expression. Similarly, tumor necrosis factor (TNF)α was found to mediate its inflammation effects through an interaction pathway with mitoNEET. Shulga and Pastorino found that TNFα causes a Stat3–Grim-19 complex to move to the mitochondria and bind to mitoNEET, leading to a release of the iron from mitoNEET. This release of iron in addition to overexpression with ethanol and fructose could in part underlie the mechanism of necroptotic cell death and the resultant hepatic steatosis [54]. Taken together with the work of others, for example TZD effect on survival kinases [55,56], it would suggest that mitoNEET could be playing a part in cell-survival–mitochondrial pathways and that inflammation stimuli activate a mitoNEET-based survival response. How the TZDs such as pioglitazone interact with this cell-survival–mitochondrial mechanism still remains to be fully elucidated. Some preliminary work done by our group has suggested that, under oxidative stress, mitoNEET could be releasing from the mitochondria into the cytosolic fraction, which could account for these different observations (data not shown) (Fig. 3).

As mentioned in the previous paragraph, mitoNEET could play a part in cell survival pathways. A recent report also showed that mitoNEET could be involved in the autophagy phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)–PARKIN pathway. The interaction between the E3 ligase PARKIN and the kinase PINK1 is crucial for the health of cells, where this protein pair plays an important part in the removal of damaged mitochondria from cells [57,58]. mitoNEET was found to be ubiquitinated by PARKIN, suggesting that it might play a part in autophagy similar to that of the ER protein Miner1 (CISD2) [30,31,59].

mitoNEET ligands

Several compounds have been identified as mitoNEET ligands since the initial identification with pioglitazone [18,19] and the follow-up work with TZD derivatives that bind to mitoNEET [60] (Table 1). Structurally, the TZD war-head moiety has thus far been the most explored structural feature in mitoNEET binding, but several other scaffolds have also been identified [18]. For instance, the dietary chemotherapeutic agent resveratrol has had one of its metabolites, resveratrol-3-sulfate (Fig. 1), shown to bind mitoNEET with an IC_{50} of 3 μM [61].

The first structure-based drug design mitoNEET ligand was the reported discovery of NL-1 [60,62]. By removal of the tail of pioglitazone, PPAR-γ activity was eliminated while retaining mitoNEET binding affinity. Additionally, NL-1 (mitoNEET binding IC_{50}: 7.36 μM) mildly uncoupled mitochondria (IC_{50} of uncoupling: 2.4 μM) and was able to protect neuronal cells (N2A) against the electron transport complex I toxin rotenone [62].

Concluding remarks

The central role mitochondria have in several aging and metabolic diseases has raised the possibility that drugs can be directed to modulate this activity. The beneficial effects of TZD compounds such as pioglitazone in diabetes and neuroprotection seem to be related to mitoNEET and activity on the mitochondrial function. mitoNEET represents a novel drug target for aging diseases and the ability to modulate the bioenergetics of cells. The development of novel mitoNEET ligands devoid of PPAR-γ activity represents a novel drug discovery approach to develop drugs capable of treating aging disorders.

Acknowledgments

The authors gratefully acknowledge the financial support from the Stark Community Foundation, Canton, OH.

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