Targeting transglutaminase 2 as a potential disease modifying therapeutic strategy for synucleinopathies

Jie Zhang, Hilary Grosso Jasutkar[†], M. Maral Mouradian[*]

Synucleinopathies are a group of progressive neurodegenerative disorders characterized by the accumulation of α-synuclein (α-Syn) aggregates in Lewy bodies (LBs) and Lewy neurites (LNs) in Parkinson's disease (PD) and dementia with Lewy bodies (DLB), and in glial cytoplasmic inclusions in multiple system atrophy (MSA). α-Syn is a 140 amino acid intrinsically disordered protein, which tends to self-aggregate and form fibrils in these neuropathological hallmark inclusions. Several lines of evidence suggest that misfolding and aggregation of α-Syn is a critical step leading to neuronal dysfunction and death. Additionally, propagation of α-Syn aggregates across neurons to synaptically connected brain regions correlates with the progressive nature of synucleinopathies and the emergence of additional clinical manifestations as the disease advances over time. Currently, there is no cure for these disorders. Available treatments for PD improve the motor symptoms but do not halt the underlying neurodegeneration. Thus, from the therapeutic perspective, identifying factors that initiate or promote misfolding of α-Syn is critical to developing strategies that reduce or prevent the formation of pathologic aggregates and thereby slow disease progression. In addition to well established genetic factors such as disease linked point mutations and SNCA gene locus multiplication leading to elevated levels of α-Syn, a number of exogenous and endogenous factors have been identified that can contribute to the formation of these aggregates including oxidative stress, exposure to neurotoxins, hyper-phosphorylation of α-Syn, and protein cross-linking. Several lines of evidence have attributed the latter to the activity of transglutaminase 2 (TG2).

TG2 is one of nine members of the family of transglutaminases (TGs). It catalyzes a calcium-dependent acyl transfer reaction between the γ-carboxamide group of peptide-bound glutamine and the ε-amino group of peptide-bound lysine resulting in the formation of ε-(γ-glutamyl) lysine isopeptide bonds (Grosso and Mouradian, 2012). TG2 is the most abundant and best-studied isoform of TGs in the brain. In addition to its cross-linking activity, TG2 is highly versatile functionally, with multiple domains allowing for other activities including as a transamidating enzyme, a GTPase, a protein disulfide isomerase, a protein kinase, and an isopeptidase. Through these various activities, it is involved in a number of biological processes, including transcription regulation, autophagy, and inflammation (Grosso and Mouradian, 2012). TG2 is widely distributed in many tissues including the brain, spinal cord and peripheral nerves. In the brain, it is broadly expressed and can be found in the frontal and temporal cortex, hippocampus and substantia nigra, primarily in neurons but also in glial cells.

Accumulating evidence suggests that TG2 can contribute to the pathogenesis of neurodegenerative diseases including synucleinopathies through its transamidation activity leading to aggregation of the pathogenic protein (**Figure 1**). In an *in vitro* study, purified TG2 catalyzes α-Syn cross-linking, resulting in the formation of insoluble high molecular weightaggregates in a calcium dependent manner (Junn et al., 2003). This phenomenon is replicated in a cell culture model, where it leads to the formation of intracytoplasmic inclusions. Further, expression of a catalytically inactive mutant isoform (C277S) of TG2 fails to generate α-Syn aggregates, indicating the requirement of TG2 cross-linking activity in forming these high molecular weight species of α-Syn (Junn et al., 2003). Additionally, increased transamidation of α-Syn by TG2 is found in SH-SY5Y cells challenged with the mitochondrial complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP), and the TG2 inhibitor Z006 blocks this effect (Verhaar et al., 2011). In human postmortem studies, multiple observations support the role of TG2 in PD and DLB. For example, there is an upregulation of TG2 mRNA and protein expression in the substantia nigra of PD brains compared to control subjects (Citron et al., 2002). TG2 protein concentration is higher in the cerebrospinal fluid of patients with PD compared with that of control subjects (Vermes et al., 2004). Further, the isopeptide bonds formed by TG2 colocalize with α-Syn immunoreactivity in Lewy bodies in PD and DLB brains (Junn et al., 2003), and TG2 and α-Syn coimmunoprecipitate in lysates of the substantia nigra from patients with PD (Andringa et al., 2004). In addition, several pathogenic aberrations found in neurodegenerative disease brains, including increased generation of reactive oxygen species, elevated calcium, and ATP depletion, can activate TG2 (Grosso and Mouradian, 2012).

The above body of evidence raises the hypothesis that TG2 can contribute to the pathogenesis of PD and related disorders by cross-linking α-Syn, leading to aberrant aggregation and toxicity. Support for this hypothesis comes from studies in genetically modified mice that are transgenic for α-Syn and either over-express TG2 ($TG2^{Tg}/Syn^{Tg}$) or have deletion of the TG2 gene ($TG2^{-/-}/Syn^{Tg}$) (Grosso et al., 2014; Zhang et al., 2020). In $TG2^{Tg}/Syn^{Tg}$ mice, there is an increase in high molecular weight insoluble species of α-Syn and proteinase K-resistant aggregates in the brain compared with SynTg mice, whereas in $TG2^{-/-}/Syn^{Tg}$ mice there is a reduced amount of α-Syn aggregates and fewer proteinase K-resistant α-Syn aggregates. These observations suggest that TG2 promotes α-Syn aggregation, replicating a previous *in vitro* study (Junn et al., 2003). In addition, high molecular weight species of α-Syn are only seen in the synaptic vesicle fraction of brain homogenates from $TG2^{Tg}/Syn^{Tg}$ mice, consistent with the hypothesis that cross-linking of α-Syn by TG2 leads to a stable interaction with vesicle membranes. Given that α-Syn may exert its toxicity in synaptic terminals, a more stable interaction between α-Syn and synaptic vesicles may further contribute to the toxicity of α-Syn. As further evidence that cross-linking of α-Syn by TG2 contributes to toxicity, the degree of aggregation in double genetically modified mice correlates with downstream neuropathologic consequences. First, compared with SynTg mice, $TG2^{Tg}/Syn^{Tg}$ mice show an exaggerated neuroinflammatory reaction with more astrocytes and microglia, whereas this response is attenuated in $TG2^{-/-}/Syn^{Tg}$ mice. Second, neuronal damage, suggested by morphological disruption of nerve fibers, is more severe in $TG2^{Tg}/Syn^{Tg}$ mice but is alleviated in $TG2^{-/-}/Syn^{Tg}$ mice when compared to SynTg mice. In addition, c-Fos, an immediate early gene used as a surrogate marker for neuronal activity, shows decreased immunoreactivity in hippocampal neurons of $TG2^{Tg}/Syn^{Tg}$ mice compared with Syn^{Tg} mice. Finally, compared with Syn^{Tg} mice motor performance is impaired in $TG2^{Tg}/Syn^{Tg}$ mice while it is preserved in $TG2^{-/-}/Syn^{Tg}$ mice.

Due to its multiple functions, TG2 plays an important role in many biological processes, which may contribute to neuronal toxicity beyond its ability to cross-link α-Syn into insoluble aggregates (Grosso and Mouradian, 2012). For example, TG2 could play a role in neuroinflammation through activation of NF-κB and TNF-α. Alternatively, TG2 may contribute to toxicity through disrupted regulation of autophagy, although the exact mechanism by which it does so remains to be clarified. Thus, to further demonstrate that it is the cross-linking activity of TG2 that specifically contributes to α-Syn toxicity, selective inhibition of its transamidating activity without any effect on its other functions is needed. In the TG2 structure, cysteine 277 (C277) is the essential nucleophile for transamidation and composes a catalytic triad with histidine 355 (H335) and aspartate 358 (D358); mutation of this cysteine to serine (C277S) has been commonly used to block TG2 transamidating activity (Liu et al., 2002). However, C277S mutation also greatly impairs the GTP/GDP binding capability of TG2 resulting from a conformational change (Begg et al., 2006), which makes it unsuitable for studying its transamidating activity. Besides the catalytic triad, tryptophan 241 (W241) is also critical for transamidating activity due to its ability to stabilize the enzyme-thiol intermediate that forms during catalysis, and mutating W241 to an alanine (W241A) knocks out transamidating activity but retains full GTP binding function (Murthy et al., 2002). This property of mutant W241A makes it a preferred genetic tool to specifically study the role of TG2 transamidating activity in α-Syn toxicity *in vitro* and *in vivo*.

Given the evidence that TG2 contributes to the

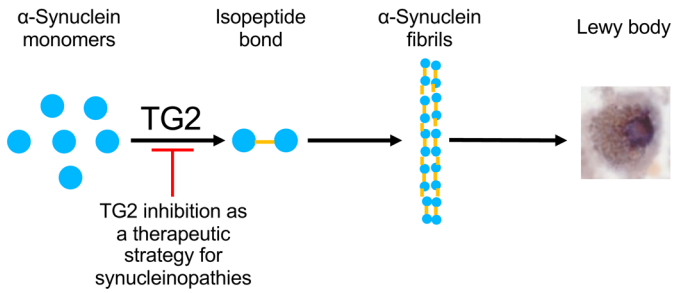

Figure 1 | **Schematic of the role of TG2 in cross linking α-synuclein monomers.**
Following cross-linking by TG2, early α-synuclein oligomers may progress to fibrilizate and accumulate in Lewy bodies, which double stain for isopeptide bonds and α-synuclein in the brain of a Parkinson's disease patient. Inhibition of TG2 has the potential to prevent this aggregation process at its very early steps, leading to neuroprotection. TG2: Transglutaminase 2.

pathogenesis in synucleinopathies, pharmacological inhibition of TG2 is a plausible strategy and a tractable approach to be tested for disease modification in these disorders (**Figure 1**). Many factors must be considered in developing a TG2 inhibitor with therapeutic utility in these disorders. Besides general considerations such as solubility, *in vivo* potency, toxicity, tolerability and the ability to traverse the blood-brain barrier (BBB), current challenges in developing TG2 inhibitors are (1) selectively targeting TG2 but not other members of the TG family, and (2) specifically inhibiting TG2 crosslinking activity without altering its other functions. For example, cystamine and its disulfide form cysteamine are thiols that inhibit TG activity. Cystamine/cysteamine have been proposed as a candidate therapeutic for neurodegenerative diseases including PD. Cystamine functions by forming a mixed disulfide link with a TG, and blocking the active site of the TG. However, cystamine has actions beyond TG2 inhibition, including inhibition of caspases and other TGs. As a result, there is the possibility of off-target effects of this drug. Additionally, there are at least four TGs (TG1, 2, 3, and TG6) found in the human brain (Iannaccone et al., 2013), and as such, a non-selective inhibitor of TGs may lead to adverse effects that may result from broad inhibition of TG activity. Thus, although cystamine attenuates the phenotype of animal models of Huntington's disease (HD) and PD (Grosso and Mouradian, 2012), and cysteamine is approved by the US Food and Drug Administration (FDA) for the treatment of nephropathic cystinosis, several side effects of cysteamine including nausea, weight loss, halitosis and motor impairment have been reported in phase I studies in patients with HD (Dubinsky and Gray, 2006), which would potentially limit its utility in clinical practice.

Although there are challenges for TG2 inhibitor development, many efforts have been made. To date, there is only one TG2 inhibitor in clinical trial (phase1b). Zedira has developed ZED1227 (EudraCT Number: 2017-002241-30), a promising peptidomimetic irreversible inhibitor of TG2, for the treatment of coeliac disease (Yoosuf and Makharia, 2019). It functions by blocking the TG2-mediated deamidation of gliadin peptides. ZED1227 is a potent compound with an IC_{50} of 45 nM, and is considered to be a highly selective compound since its corresponding IC_{50} values for TG1, TG3, TG6 and FXIII are at least 100-fold higher than that for TG2 (Keillor and Apperley, 2016). However, ZED1227 does not cross the BBB and is, therefore, not suitable for testing in diseases of the brain. Nevertheless, the development of this drug is a proof of concept that a selective inhibitor of TG2 can be made, and thus offers promise that other selective inhibitor compounds that can cross the BBB may be on the horizon.

In conclusion, accumulating evidence supports the notion that TG2 plays a pathogenic role in synucleiopathies by promoting aggregation of α-Syn. Currently available data indicate that it is the cross-linking function of TG2 that leads to its pathologic activity. Therefore, pharmaceuticals that specifically inhibit the transamidating activity of TG2 without interfering with its other functions may be of value for synucleinopathies. Considering the importance of TGs in the body, these pharmaceuticals must also be highly selective for TG2 and be able to penetrate the BBB. Compounds harboring these properties will allow proof of concept testing in animal models of synucleinopathy. Meanwhile, several approaches to enhance drug delivery across the BBB are under development. Among them, nanoparticles (lipid, polymeric, magnetic, and carbon-based nanoparticles, etc.) are the most promising methods to deliver therapeutics to the central nervous system target site that otherwise have poor BBB penetrance. Whether future innovative drug delivery techniques can overcome such limitations remains poorly understood but might broaden research and development of TG2 inhibitors.

MMM is the William Dow Lovett Professor of Neurology and is supported by grants from the Michael J. Fox Foundation for Parkinson's Research (ID 12350), the American Parkinson Disease Association, the New Jersey Health Foundation, and by NIH grants NS101134, NS096032, and NS116921.

Jie Zhang, Hilary Grosso Jasutkar[†], M. Maral Mouradian[*]
Robert Wood Johnson Medical School Institute for Neurological Therapeutics, and Department of Neurology, Rutgers Biomedical and Health Sciences, Piscataway, NJ, USA
[†]Current address: Department of Neurology, Columbia University Medical Center, New York, NY, USA
***Correspondence to:** M. Maral Mouradian, MD, m.mouradian@rutgers.edu.
https://orcid.org/0000-0002-9937-412X (M. Maral Mouradian)
Date of submission: September 14, 2020
Date of decision: November 13, 2020
Date of acceptance: December 8, 2020
Date of web publication: January 7, 2021

https://doi.org/10.4103/1673-5374.303027
How to cite this article: *Zhang J, Jasutkar HG, Mouradian MM (2021) Targeting transglutaminase 2 as a potential disease modifying therapeutic strategy for synucleinopathies. Neural Regen Res 16(8):1560-1561.*

References

Andringa G, Lam KY, Chegary M, Wang X, Chase TN, Bennett MC (2004) Tissue transglutaminase catalyzes the formation of alpha-synuclein crosslinks in Parkinson's disease. FASEB J 18:932-934.
Begg GE, Carrington L, Stokes PH, Matthews JM, Wouters MA, Husain A, Lorand L, Iismaa SE, Graham RM (2006) Mechanism of allosteric regulation of transglutaminase 2 by GTP. Proc Natl Acad Sci U S A 103:19683-19688.
Citron BA, Suo Z, SantaCruz K, Davies PJ, Qin F, Festoff BW (2002) Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration. Neurochem Int 40:69-78.
Dubinsky R, Gray C (2006) CYTE-I-HD: Phase I dose finding and tolerability study of cysteamine (Cystagon) in Huntington's disease. Mov Disord 21:530-533.
Grosso H, Mouradian MM (2012) Transglutaminase 2: biology, relevance to neurodegenerative diseases and therapeutic implications. Pharmacol Ther 133:392-410.
Grosso H, Woo JM, Lee KW, Im JY, Masliah E, Junn E, Mouradian MM (2014) Transglutaminase 2 exacerbates alpha-synuclein toxicity in mice and yeast. FASEB J 28:4280-4291.
Iannaccone M, Serretiello E, De Vivo G, Martin A, Stefanile A, Titta F, Gentile V (2013) Transglutaminase inhibition as a possible therapeutical approach to protect cells from death in neurodegenerative diseases. Recent Pat CNS Drug Discov 8:161-168.
Junn E, Ronchetti RD, Quezado MM, Kim SY, Mouradian MM (2003) Tissue transglutaminase-induced aggregation of alpha-synuclein: Implications for Lewy body formation in Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci U S A 100:2047-2052.
Keillor JW, Apperley KY (2016) Transglutaminase inhibitors: a patent review. Expert Opin Ther Pat 26:49-63.
Liu S, Cerione RA, Clardy J (2002) Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. Proc Natl Acad Sci U S A 99:2743-2747.
Murthy SNP, Iismaa S, Begg G, Freymann DM, Graham RM, Lorand L (2002) Conserved tryptophan in the core domain of transglutaminase is essential for catalytic activity. Proc Natl Acad Sci U S A 99:2738-2742.
Verhaar R, Jongenelen CA, Gerard M, Baekelandt V, Van Dam AM, Wilhelmus MM, Drukarch B (2011) Blockade of enzyme activity inhibits tissue transglutaminase-mediated transamidation of alpha-synuclein in a cellular model of Parkinson's disease. Neurochem Int 58:785-793.
Vermes I, Steur EN, Jirikowski GF, Haanen C (2004) Elevated concentration of cerebrospinal fluid tissue transglutaminase in Parkinson's disease indicating apoptosis. Mov Disord 19:1252-1254.
Yoosuf S, Makharia GK (2019) Evolving therapy for celiac disease. Front Pediatr 7:193.
Zhang J, Grosso Jasutkar H, Yan R, Woo JM, Lee KW, Im JY, Junn E, Iismaa SE, Mouradian MM (2020) Transglutaminase 2 depletion attenuates α-synuclein mediated toxicity in mice. Neuroscience 441:58-64.

C-Editors: Zhao M, Song LP; T-Editor: Jia Y