Application of PROTAC strategy to TTR-Aβ protein-protein interaction for the development of Alzheimer's disease drugs

Nicoló Tonali, Susanna Nencetti, Elisabetta Orlandini, Lidia Ciccone*

Alzheimer's disease (AD) is a complex and multifaced neurodegenerative disorder for which the precise pathological molecular mechanisms are still not completely known.

In last years, rational multi-target drug design methods, which combine multiple molecules having complementary modes of action, have been increasingly used in the development of anti-AD drugs.

In the present perspective, a new multi-target therapeutic approach, based on an inspired proteolysis targeting chimera (PROTAC) construction, is examined as a future applicable strategy to modulate the favourable cross-interactions between transthyretin and Aβ 1–42 peptide.

The role of the positive cross-interaction between transthyretin (TTR) and β-amyloid (Aβ): AD is one of the most spread form of dementia affecting more than 35 million people worldwide. AD is characterized by a chronic and progressive neurodegenerative disorder associated with gradual impairment of cognitive functions. The main pathological features of AD are the deposition of Aβ-amyloid in extracellular senile plaques and the formation of intracellular neurofibrillary tangles derived from hyperphosphorylated tau protein.

It is acknowledged that an imbalance between production and clearance of Aβ peptides in the brain results in spontaneous self-association into soluble toxic oligomers and insoluble aggregates. This aspect makes the disease connected to Aβ aggregation.

Nowadays, the only approved drugs (Memantine and Donezepil) for AD are symptomatic and there is not an effective treatment of the disease. Moreover, the exact cause of AD is not known and therefore the development of alternative therapies is still controversial (Sarkar et al., 2016; Mutsuddi and Mukherjee, 2019).

Because AD is considered as a multi-factorial disorder with various pathogenic molecular mechanisms, a multifunctional strategy to create effective neuroprotective agents may be required to treat this disorder.

The Aβ formation can be considered as an abnormal protein-protein interaction process, during which the misfolded protein undergoes through a conformational change, thereby allowing the self-aggregation. Moreover, other amyloid proteins seam to participate in the progression of the pathology through a synergistic occurrence between amyloids (cross-interaction), which promotes mutually aggregations. These cross-interactions between Aβ and other amyloid proteins (such as Tau) are increasingly regarded as playing a critical role in AD pathogenesis.

However, together with these cross-interactions having a negative effect on the progression of the disease, it is possible to recognize other cross-interactions with a positive effect (TTR, CysC, ApoA1). The positive protein-protein cross-interactions are able to induce the inhibition of the formation of amyloid oligomers and fibrils, the reduction of the aggregates' toxicity, the promotion of the degradation and dissociation of the aggregates. TTR is, for example, one of the amyloid proteins participating in the Aβ clearance (Ciccone et al., 2020a).

TTR is mainly synthesized by the liver and the choroid plexus of the brain, in minor amounts in the retina and in human placenta. The tetramer is formed by four identical subunits (AA'/BB') which are assembled in couples of dimers that interact each other back to back to form the tetrameric structure. TTR is crossed along the 2-fold axis by a channel which forms two symmetric binding sites named thyroxine binding sites (T4-BS) for their binding with the endogen ligand thyroxine (**Figure 1A**).

The brain of AD patients is characterized by an imbalance of the metal ions levels which drastically increase. It has been reported that metal ions interact also with TTR, therefore it has been hypothesized that the TTR-Aβ interaction can be modulated by metal ions. The binding experiment between TTR and Aβ showed an increased complex stability when Cu^{2+} is added to the buffer solution. Moreover, the crystal structures of TTR obtained in presence of Cu^{2+} and Fe^{2+} showed a conformational change comparable to that found for the TTR-rhenium complex in which the distances between L110 and L110', two residues located in the binding pocket and implicated in TTR-Aβ interaction, increased up to 8.5 Å in one pocket inducing enlargement of the T4-BS. (Ciccone et al., 2016, 2018).

Even if the precise mechanism by which TTR binds to Aβ remains unknown, several *in vitro* and *in vivo* studies focused on TTR-Aβ interaction appeared in the last years, confirming the neuroprotective effect of TTR against Aβ amyloid deposition and toxicity (Ribeiro et al., 2012).

PROTACs as versatile multitarget therapeutic approach against Alzheimer's disease: PROteolysis-TArgeting Chimeras (PROTACs) recently emerged as a new therapeutic technology exploiting the intracellular ubiquitin-proteasome system to selectively degrade target proteins (Xi et al., 2019).

A PROTAC works by inducing selective intracellular proteolysis, demonstrating a good efficacy in inhibition of proliferation and promotion of apoptosis in cancer cells (Kargbo, 2019a) and already proved to be applicable for treating neurodegenerative disorders such as AD and Parkinson's disease (Kargbo, 2019b, 2020). Heterobifunctional PROTAC molecules consist of a ligand to the target protein, a ligand to the E3 ubiquitin ligase, and a linker connecting the two ligands (**Figure 1A**).

In recent years, small-molecule PROTACs with good pharmaceutical properties have been reported, especially for targeting undruggable proteins which lack of active sites and transfer signaling through interfaces (Xi et al., 2019).

Relying on PROTAC technology and by assuming the positive effect that TTR has on Aβ clearance, we outlook to design new PROTAC molecules able to exploit the positive cross-interaction between the two amyloid proteins. The formation of a stable ternary complex between the two amyloids, close together through the PROTAC construct, should improve the approach of the two proteins and allow the natural positive effect of the cross-interaction. We aim to promote the approach of TTR and Aβ and by consequence the stabilization of the Aβ native state. In this regard, Aβ could be easily metabolized in its monomeric state and its clearance could be maintained under physiological level (Chen et al., 2017).

The new PROTAC compounds consist of a ligand having affinity for the binding pocket of TTR, a peptidomimetic able to interact with Aβ, and a linker connecting the two ligands (**Figure 1B**). TTR tetramer is usually stable, exception when a single point mutation occurs, and so drastically decreases its stability, promoting amyloidosis onset.

One therapeutic strategy against TTR amyloidosis is the tetramer stabilization by small synthetic molecules or natural molecules (Ciccone et al., 2020b). In this regard, a good ligand candidate of TTR for PROTACs protein-protein interaction compounds could be a TTR-tetramer stabilizer.

Generally, the affinity with Aβ peptides is challenging because it requires the modulation of typically large, relatively flexible surface area. Small molecules often lack selectivity, and this is the reason why peptides and peptidomimetics are more often considered as promising therapeutics in the field of protein-protein interactions. They can afford selectivity and affinity thanks to their size in midway between small molecules and protein therapeutics (Robertson and Spring, 2018; Laxio Arenas et al., 2019). Thus, the ligand for Aβ could be a peptidomimetic foldamer, offering the possibility to mimic the secondary structure (α-helix and/or β-hairpin) of the

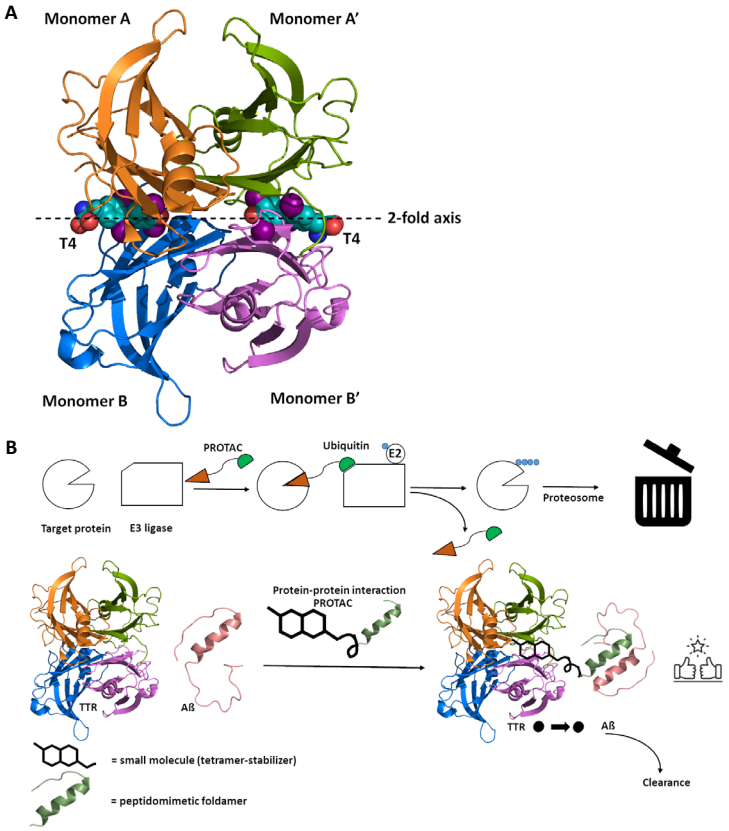

Figure 1 | **Transthyretin tetrameric structure and PROTAC strategy.**
(A) Graphic representation of TTR-thyroxine crystal complex (PDB code 1SN0). The four monomers are colors orange(A), green (A'), violet (B') and blue (B) respectively. Both T4-BS are occupied by T4 molecules. (B) Design of protein-protein interaction PROTAC. Graphic representation of a classical PROTAC mechanism of action. Application of PROTAC strategy to TTR-Aβ 1–42 interaction. Aβ: Beta-amyloid; PROTAC: proteolysis targeting chimera.

peptide sequence, generally involved in the interaction (Pellegrino et al., 2017; Tonali et al., 2018, 2020).

These peptidomimetic foldamers will be based on the "hot spot" sequences of Aβ 1–42 peptide. Recently, four newly designed peptides have been investigated by some of us as inhibitors of the formation of toxic Aβ 1–42 oligomers by targeting the two Aβ 1–42 aggregation hot spots: KLVFF and GVVIA. In fact, tetrapeptide derivatives of the C-terminal part of Aβ (39–42) are suitable inhibitors of Aβ-induced toxicity despite they are poor inhibitors of fibril formation (Tonali et al., 2020). Furthermore, the peptide Aβ (16–20), which corresponds to the hydrophobic central region of Aβ, plays an essential role in Aβ–Aβ interactions because it binds to β-sheets and nucleates aggregation. Peptidomimetics based on this sequence and in acyclic β-hairpin conformation have been recently proved to inhibit the aggregation process of Aβ 1–42 peptide by stabilizing its monomer state (Pellegrino et al., 2017; Tonali et al., 2018).

The PROTACs molecules will be composed by peptidomimetics having good affinity for Aβ 1–42 thanks to their rationally designed sequence and their specific conformation and being able to stabilize the monomer species. In that way, the monomer of Aβ 1–42 will be more available to approach the TTR protein and their positive cross-interaction might be favored.

This new therapeutic approach, aiming to favor the protein-protein interaction of two different amyloid proteins, represents a versatile method which could be employed for other proteins than TTR, having a positive cross-interaction with Aβ, such as cystatin C and apolipoprotein AI (Ciccone et al., 2020a). In conclusion, we presented here a new promising therapeutic approach which could be used for the development of future AD therapy drugs, taking inspiration from those protein-protein cross-interactions between Aβ and other neuroprotective proteins, showing to concern potentially therapeutic interventions against AD.

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References

Chen G, Xu T, Yan Y, Zhou Y, Jiang Y, Melcher K, Xu HE (2017) Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacol Sin 38:1205-1235.
Ciccone L, Fruchart-Gaillard C, Mourier G, Savko M, Nencetti S, Orlandini E, Servent D, Stura EA, Shepard W (2018) Copper mediated amyloid-β binding to Transthyretin. Sci Rep 8:1-11.
Ciccone L, Policar C, Stura EA, Shepard W (2016) Human TTR conformation altered by rhenium tris-carbonyl derivatives. J Struct Biol 195:353-364.
Ciccone L, Shi C, di Lorenzo D, Van Baelen AC, Tonali N (2020a) The positive side of the Alzheimer's disease amyloid cross-interactions: the case of the Aβ 1-42 peptide with Tau, TTR, CysC, and ApoA1. Molecules 25:2439.
Ciccone L, Tonali N, Nencetti S, Orlandini E (2020b) Natural compounds as inhibitors of transthyretin amyloidosis and neuroprotective agents: analysis of structural data for future drug design. J Enzyme Inhib Med Chem 35:1145-1162.
Kargbo RB (2019a) Treatment of cancer and Alzheimer's disease by PROTAC degradation of EGFR. ACS Med Chem Lett 10:1098-1099.
Kargbo RB (2019b) Treatment of Alzheimer's by PROTAC-Tau protein degradation. ACS Med Chem Lett 10:699-700.
Kargbo RB (2020) PROTAC compounds targeting α-synuclein protein for treating neurogenerative disorders: Alzheimer's and Parkinson's diseases. ACS Med Chem Lett 11:1086-1087.
Laxio Arenas J, Kaffy J, Ongeri S (2019) Peptides and peptidomimetics as inhibitors of protein-protein interactions involving β-sheet secondary structures. Curr Opin Chem Biol 52:157-167.
Mutsuddi M, Mukherjee A, eds (2019) Insights into Human Neurodegeneration: Lessons Learnt from Drosophila. Singapore: Springer Singapore. Available at: http://link.springer.com/10.1007/978-981-13-2218-1. Accessed September 28, 2020.
Pellegrino S, Tonali N, Erba E, Kaffy J, Taverna M, Contini A, Taylor M, Allsop D, Gelmi ML, Ongeri S (2017) β-Hairpin mimics containing a piperidine–pyrrolidine scaffold modulate the β-amyloid aggregation process preserving the monomer species. Chem Sci 8:1295-1302.
Ribeiro CA, Saraiva MJ, Cardoso I (2012) Stability of the transthyretin molecule as a key factor in the interaction with a-beta peptide--relevance in Alzheimer's disease. PLoS One 7:e45368.
Robertson NS, Spring DR (2018) Using peptidomimetics and constrained peptides as valuable tools for inhibiting protein–protein interactions. Molecules 23:959.
Sarkar A, Irwin M, Singh A, Riccetti M, Singh A (2016) Alzheimer's disease: the silver tsunami of the 21st century. Neural Regen Res 11:693.
Tonali N, Dodero VI, Kaffy J, Hericks L, Ongeri S, Sewald N (2020) Real-Time BODIPY-binding assay to screen inhibitors of the early oligomerization process of Aβ1–42 peptide. Chembiochem 21:1129-1135.
Tonali N, Kaffy J, Soulier JL, Gelmi ML, Erba E, Taverna M, van Heijenoort C, Ha-Duong T, Ongeri S (2018) Structure-activity relationships of β-hairpin mimics as modulators of amyloid β-peptide aggregation. Structure-activity relationships of β-hairpin mimics as modulators of amyloid β-peptide aggregation. Eur J Med Chem 154:280-293.
Xi M, Chen Y, Yang H, Xu H, Du K, Wu C, Xu Y, Deng L, Luo X, Yu L, Wu Y, Gao X, Cai T, Chen B, Shen R, Sun H (2019) Small molecule PROTACs in targeted therapy: An emerging strategy to induce protein degradation. Eur J Med Chem 174:159-180.

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