Novel therapeutic approaches to target neurodegeneration

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Abstract

Ageing is the main risk factor common to most primary neurodegenerative disorders. Indeed, age-related brain alterations have been long considered to predispose to neurodegeneration. Although protein misfolding and the accumulation of toxic protein aggregates have been contemplated as causative events in neurodegeneration, several biological pathways affected by brain ageing are also contributing to pathogenesis. Here, we discuss the evidence showing the involvement of the mechanisms controlling neuronal structure, gene expression, autophagy, cell metabolism, and neuroinflammation in the onset and progression of neurodegenerative disorders. Furthermore, we review the therapeutic strategies currently under development or as future approaches designed to normalize these pathways, which may then boost brain resilience to cope with toxic protein species. Therefore, in addition to therapies targeting the insoluble protein aggregates specifically associated with each neurodegenerative disorder, these novel pharmacological approaches may be part of combined therapies designed to rescue brain function.

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Conflict of interest All the authors have no conflict of interest.**Abstract**Ageing is the main risk factor common to most primary neurodegenerative disorders. Indeed, age-related brain alterations have been long considered to predispose to neurodegeneration. Although protein misfolding and the accumulation of toxic protein aggregates have been contemplated as causative events in neurodegeneration, several biological pathways affected by brain ageing are also contributing to pathogenesis. Here, we discuss the evidence showing the involvement of the mechanisms controlling neuronal structure, gene expression, autophagy, cell metabolism, and neuroinflammation in the onset and progression of neurodegenerative disorders. Furthermore, we review the therapeutic strategies currently under development or as future approaches designed to normalize these pathways, which may then boost brain resilience to cope with toxic protein species. Therefore, in addition to therapies targeting the insoluble protein aggregates specifically associated with each neurodegenerative disorder, these novel pharmacological approaches may be part of combined therapies designed to rescue brain function.**Keywords:** neurodegenerative disorders, synapse, dendrite, autophagy, metabolism, neuroinflammation, gene expression.**LIST OF ABBREVIATIONS**

- AD: Alzheimer's disease
- ADAM10: a disintegrin and metalloproteinase 10
- ALS: amyotrophic lateral sclerosis
- AMPK: AMP activated protein kinase
- APP: amyloid-β precursor protein
- AUTEN-67: mTOR-dependent modulator autophagy enhancer-67
- Aβ: Amyloid-β
- CNS: central nervous system
- CREB: cAMP response element-binding protein
- DDQ: methylphosphonate
- FDA: Food and Drug Administration
- FTD: frontotemporal dementia
- HAT: histone acetyltransferase
- HD: Huntington Disease
- HDACs: histone deacetylases
- iNs: induced neurons
- MCU: mitochondrial calcium uniporter
- Mdivi-1: mitochondrial division inhibitor 1
- Mdivi-1: mitochondrial division inhibitor 1
- Mfn1: mitofusin-1
- Mfn2: mitofusin 2
- MS: Multiple sclerosis
- mTOR: mammalian target of rapamycin
- mTORC1: mTOR enzymatic complex1
- NF-ϰB: nuclear factor-ϰB
- NMDAR: NMDA receptors
- OMM: outer mitochondrial membrane
- OPA1: atrophy type 1
- OPC: oligodendrocyte progenitor cells
- OPTN: optineurin
- OXPHOS: oxidative phosphorylation
- PD: Parkinson's disease
- PINK1: PTEN-induced protein kinase 1
- PKC: protein kinase C

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- PKM: pyruvate kinase M
- RNF10: RING Finger protein 10
- ROS: reactive oxygen species
- SASP: senescent associated secretory phenotype
- SQSTM/p62: sequestrosome-1
- TARDBP: (TAR)-DNA-binding protein

Ageing and neurodegenerative diseases

In the last century, advances in medical care and the creation of healthier environments contributed to an increase in life expectancy . Given that advanced age is the main risk factor for neurodegenerative diseases , elderly population growth leads to a significant increase in the number of patients affected by age-related primary neurodegenerative diseases, such as Alzheimer's disease (AD) , Parkinson's disease (PD) or amyotrophic lateral sclerosis (ALS) . Therefore, considering the current demographic changes, primary neurodegenerative diseases will have substantial socioeconomic implications for healthcare systems, due to their large costs and severe impact on the quality of life of affected individuals and caregivers, posing a critical social emergency. Hence, addressing these large burdens for the society requires an intensified research and novel approaches and solutions . In the last 20 years, substantial advances in the comprehension of neurodegenerative disorder pathogenesis have been made . Much of this progress is the result of biochemical and histochemical characterization of proteins that accumulate within various inclusions in the diseased brain and genetic linkage studies identifying mutations in genes that cause neurodegenerative diseases. The identification of specific, disease-segregating mutations in previously unknown genes directed the attention to proteins and pathways that are now considered crucial in the pathogenesis of neurodegenerative diseases. For instance, certain pathogenic mutations in the gene coding for the amyloid-β precursor protein (APP) cause AD, in the α-synuclein gene are related to PD, in huntingtin to Huntington Disease (HD), or in microtubule-associated protein tau are associated to frontotemporal dementia (FTD) with parkinsonism . The accumulation of species derived from these proteins in the brain of patients often represents a histological hallmark for each specific neurodegenerative disorder (Table 1). Despite the presence of these inherited cases, most neurodegenerative disorders develop sporadically in the absence of any known genetic etiology. The onset of these sporadic forms is significantly influenced by risk factors , ageing being the one with the highest impact . Hence, age-associated brain modifications are considered key contributors to the pathogenesis of neurodegenerative disorders . However, mechanistic interface between brain ageing and neurodegeneration have not been fully elucidated. During the 2000s the ageing research field has grown considerably ; as understanding how exactly ageing increases the risk to develop neurodegenerative diseases can provide important clues for the development of new therapeutic strategies for neurodegeneration treatment. Even though protein misfolding and the accumulation and formation of toxic species of proteins, due to inadequate folding, have been seen as causative events in neurodegenerative disorders, in this review we carefully examine the role of other, different, biological pathways that are altered during ageing and implicated in the pathogenesis and progression of neurodegenerative disorders. We focused on the mechanisms controlling neuronal structure, gene expression, autophagy system, cell metabolism, and, finally, neuroinflammation (Figure 1). Further, we summarize the therapeutic approaches developed to restore these pathways that may elevate the resilience of the brain to cope with toxic species for each neurodegenerative disorder (Table 2).

The structural disintegration: how to reshape neurons and synapses

The synapse is the biological locus responsible for the transmission of information between neurons. Neuron-to-neuron synapses are composed of a pre-synaptic and post-synaptic compartment, each with unique proteins and structures to facilitate excitatory and inhibitory neurotransmission. The majority of synapses are found on dendrites, branch-like extensions of a neuron that receive information from other neurons and carry it to the neuron soma. The excitatory postsynaptic machinery is localized in dendritic spines, small protrusions from the dendrite shaft. Dendrites can support information processing at multiple spatial scales to integrate synaptic signals finally transformed into action potentials . Neuronal synaptic structures are not static but highly dynamic. The capability of neurons to modify the efficacy of synaptic transmission

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and the synaptic structure in response to different stimuli is called synaptic plasticity . Synaptic plasticity has been proposed to play a central role in the brain's capacity to incorporate transient experiences into persistent memory traces. Astrocytes and microglia can transmit information and modulate synaptic activity . Astrocyte processes encapsulate the synaptic cleft and ensure recycling of released neurotransmitters, release co-factors important for physiological neuronal transmission, and maintain tissue ion homeostasis. Furthermore, astrocytes are connected via gap-junction-coupled networks that synchronize neuronal activity within brain regions . Microglia, the brain resident immune cells, on the other hand phagocytose inactive synapses and release co-factors that are important for the induction and maintenance of synaptic plasticity . In addition to microglia and astrocytes, myelin, a passive insulating layer formed by oligodendrocytes that ensures fast-saltatory conduction of action potentials, is also essential for neuroprotection providing physical axonal protection and trophic support. Despite being considered for long a static component of the central nervous system (CNS), it has now been demonstrated that myelin has a plastic nature, and that myelin plasticity is required for motor learning and fear memory and conditioning .

The impact of ageing and neurodegenerative disorders on the structure of brain cells

Several studies documented changes in the molecular signature, the morphology and function of brain cells with ageing. The principal age-related neuronal structural alteration involves a reduction in dendrites length and number, with a loss of various dendritic spines . On the contrary, astrocytes undergo an increased expression of cytoskeletal proteins, cell body hypertrophy, and a reduction in the number of long, slender processes with ageing . Furthermore, aged microglial cells show a gradual decrease in function, most notably in chemotactic and phagocytic capacity. In particular, mouse studies have documented impairments in the ability of ageing microglia to phagocytose Amyloid-β (Aβ) fibrils and myelin debris In addition, recent studies have revealed that myelin remodelling persists throughout the lifespan . However, white matter and thus myelin volume is shown to decline after 13 months in mice, and in humans, after the age of 44-47 with myelin alterations that contribute to age-linked functional decline being detected prior to neuronal loss. These alterations include widespread and diffuse myelin breakdown, degeneration, and reduced myelin renewal , decreased myelin stability associated with lipid peroxidation formation of splits containing cytoplasm and myelin balloons or spheroids, accumulation of myelin debris, like multilamellar myelin fragments . The age-dependent altered function of glial cells reduces their ability to homeostatically nurture, protect and regenerate neurons, generating a more inflammatory microenvironment that consequently promotes neuron and synapse loss and, thereby, neurodegeneration. Most of these alterations are mild in healthy ageing, but exaggerate in a range of neurodegenerative diseases, such as AD, PD, ALS, HD, where they contribute or accelerate neurodegeneration, facilitate protein aggregates deposition, impair cognition and motor function by disrupting connective pathways Neurodegenerative diseases are characterized by abnormalities in dendritic structure and synapse loss in different brain regions depending on the neurodegenerative diseases . In HD, for example, synapse loss is mainly detected in the striatal brain region, which is linked with progressive movement discoordination . There is growing evidence from ALS patients, FTD patients, and animal models that suggest synaptic dysfunction and alterations in dendritic branching begins very early in the disease before symptom onset and motor neuron death . In AD neurons, the dendritic tree undergoes fast decline with a decrease in the number of dendritic shafts while the few remaining show fewer and shorter branches . Furthermore, synaptic loss in the hippocampus and neocortex is known to be an early process in AD and the main structural correlate with AD cognitive dysfunction. In AD and PD, the oligomeric forms of Aβ and α-synuclein are the most toxic species for synapses. The oligomers of α-synuclein but not its fibrils contribute significantly to dopaminergic loss and neuronal cell death . Similarly, it has been shown that the Aβ oligomers cause synaptic loss and impair the mechanisms of synaptic plasticity . Interestingly, APP and the enzymes implicated in the amyloid cascade are synaptic elements located both, at pre and post synaptic side, and play a critical role in regulating synaptic function . For instance, a disintegrin and metalloproteinase 10 (ADAM10), the metalloprotease that prevents Aβ generation, is a shedding enzyme that cleaves adhesion molecules, such as N-Cadherin, and shapes spine morphology . Furthermore, ADAM10 synaptic localization and activity are finely tuned by synaptic plasticity phenomena and its synaptic abundance and activity towards APP are affected in the hippocampus of AD patients . This is the result of an impairment of ADAM10

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local forward trafficking, that depends on the PKC-regulated association with SAP97 and is also related to an increase in ADAM10 endocytosis . Notably, alterations in ADAM10 have been described also in HD, with increased levels of the mature form of ADAM10 in the brain areas that predominantly degenerate in HD have been reported in mouse models of HD and in human HD brain samples . Accumulation of active ADAM10 at the postsynaptic compartment leads to increased proteolysis of N-Cadherin, likely promoting synaptic instability in HD. These data confirm that synaptic failure is a common trait of neurodegenerative disorders but highlight the importance of investigating the molecular mechanisms underlying synaptic dysfunction in each disorder. Therefore, given that synapses are the most vulnerable regions of neurons, differences among synapse structure, metabolism and signaling mechanisms might be determinants of neuronal vulnerability in the different neurodegenerative disorders.

Therapeutic approaches to tackle synaptic and neuronal dysfunction

Although we currently lack therapeutic approaches aimed at enhancing myelination in the clinic, recent advances and high-throughput screening approaches have provided us with potential pro-myelinating compounds. Clemastine fumarate, a muscarinic receptor antagonist that was identified in a pro-remyelinating drug screening, has met clinically defined efficacy endpoints in a clinical trial in patients with Multiple sclerosis (MS) . Recent evidence has also shown that Clemastine prevents age-related myelin loss, neurodegeneration, and cognitive decline in healthy ageing as well as in a mouse model of AD . Additionally, other drugs that have been shown to promote myelin repair in the context of MS, such as metformin or LY294002 , could also be beneficial to prevent myelin breakdown and degeneration with age or in other neurodegenerative diseases, but their efficacy in this context is yet to be investigated. As neuronal connections represent the hardware for proper cognitive abilities, therapeutic strategies aimed at preserving dendritic and synaptic connections could conceivably be useful in neurodegenerations. The process of neurite repair to replenish the degenerated dendrites would involve regrowth and rewiring of the new connections within the network. However, the local molecular and cellular milieu in the CNS opposes neurite growth and thus renders this approach particularly challenging . A better option could be to use strategies aimed at upholding neuronal dendritic integrity rather than promoting its regrowth. In this view, promising findings showed that specifically preserving dendritic architecture in mouse models of acute neurodegeneration (i.e. stroke) counteracted the loss of neurons which is typically accelerated by disrupted connectivity and, ultimately, resulted beneficial also at a functional level . Regarding synaptic dysfunction, the 2022 drug pipeline for AD showed that synaptic plasticity/ neuroprotective agents for which phase 2 and phase 3 clinical trials are currently ongoing are 17% and 19% of all disease-modifying therapies respectively, indicating that significant efforts are being made for targeting these mechanisms . A systematic review analysed the efficacy profile of 12 published results of clinical trials investigating the safety and efficacy of disease-modifying drugs targeting synaptic plasticity in dementia . However, only three molecules (Levetiracetam, Bryostatin 1 and Masitinib) show promising results. Levetiracetam is a second-generation antiepileptic drug approved as an adjunct therapy for partial seizures. The mechanism of action seems to involve neuronal binding to the synaptic vesicle protein 2 A, inhibiting the release of calcium from intraneuronal deposits, opposing the activity of negative modulators of GABA and glycine-dependent currents, and inhibiting excessive synchronized activity between neurons . An important role of this molecule also appears to be linked to synaptic plasticity. The administration of Levetiracetam to AD mice was reported to reverse synaptic dysfunction . The initial clinical trials with Levetiracetam showed limited results because of the high clinical heterogeneity of the enrolled cohort. On the other hand, a recent clinical trial found that Levetiracetam was able to significantly improve cognitive status only in patients with cortical hyperexcitability. This suggests that preselection of AD patients presenting symptoms ranging from subclinical epileptiform activity to seizures and network hyperexcitability could improve the capacity to identify therapeutic effects of Levetiracetam. Bryostatin 1 is a macrocyclic lactone that activates protein kinase C (PKC) and, thereby, regulates neurogenesis, axonal transport, and synaptic plasticity . The administration of Bryostatin 1 in AD mice restored the number of dendritic spines in the hippocampal CA1 area . The results of phase 1 and 2 trials were conflicting, while a recent pooled analyses from 2 randomized clinical trials confirmed a significant cognitive restoration elicited by Bryostatin 1 in the absence of a Memantine treatment . Masitinib, a tyrosine kinase inhibitor, is usually used in the

treatment of mast cell tumours in animals. Studies in animal models showed that Masitinib administration has a protective effect on synapses because of mast cell inhibition and reduction of the secretion of specific mediators potentially toxic to synapses . Initial studies in patients reported that participants treated with Masitinib showed significant improvement in cognitive function . Thus, Masitinib is being currently investigated in a multicentre phase 3 trial for patients with mild-to-moderate AD (NCT01872598) and showed some beneficial effects in ALS . Pharmacological treatments of ALS patients aim at counteracting glutamate excitotoxicity. Riluzole, an inhibitor of glutamate release, was approved by the United States Food and Drug Administration (FDA) in 1995. Despite being associated with a short survival benefit of 2–3 months , the subsequent adoption of riluzole as a treatment for ALS was perhaps reflective of the need for therapeutic options in the face of this devastatingly progressive disease . Remarkably, findings from several open-label non-randomised trials have suggested that the greatest benefit occurs at earlier disease stages . Therefore, is possible that riluzole's therapeutic benefit is likely to affect or activate different cellular pathways depending on the disease stage . Another molecule investigated to counteract excitoxicity is the antibiotic Ceftriaxone that causes the upregulation of glutamate transporter and decreases glutamate-induced toxicity . Phase-3 trial of ceftriaxone indicated an overall increase in survival of patients with ALS. Overall, these strategies tackle synaptic failure and dysfunction as common molecular mechanism across neurodegenerative disorders. Next challenge for drug discovery research will be to design tools and molecules specifically targeting the mechanisms underlying synaptic dysfunction or dendrite degeneration in each of the different neurodegenerative disorders. An example is the control of the trafficking of ADAM10. As mentioned above, alterations in ADAM10 synaptic localization and activity are implicated both in AD and in HD. In AD however, ADAM10 levels are reduced in hippocampal synapses while active ADAM10 is overexpressed in HD striatal synapses. Therefore, the strategy to counteract synaptic failure in AD takes advantage of the administration of a cell permeable peptide that blocks ADAM10 endocytosis, upregulates its activity, restores synaptic function without affecting plaque deposition and rescues cognitive defects in AD mice . On the other hand, the use of a peptide designed to inhibit SAP97-mediated ADAM10 trafficking to the synapse , normalizes ADAM10 activity and rescues cognitive deficits in HD mice . Therefore, understanding the mechanisms implicated in synaptic failure in the different neurodegenerative disorders is required to design disease-tailored therapeutic strategies.

The main players controlling gene expression during ageing and in neurodegenerative disorders

A key aspect sustaining and enabling various forms of plasticity is the regulation of gene expression. Basal transcription ensures the mere survival of neurons as cells; to be functional computational units, neurons further need to adapt their transcriptional responses to physiological and pathological cues. To do so, neurons employ an array or regulatory elements, epigenetic mechanisms, and transcription factors, all of which are modulated by diverse stimuli. One of the most prominent stimuli that neurons are specialized to adapt to is synaptic activity and calcium signals. Among the many epigenetic mechanisms employed by neurons, the ones influencing chromatin accessibility -DNA methylation and post translational modifications of histone proteins - received the most attention and are the best characterized. However, more recently, non-coding RNAs have received increasing attention. Given its key role in ensuring neural functions, it comes as no surprise that a long list of alterations in the transcriptional landscape or dysfunctions of molecular players have been associated with neurodegeneration. Mechanistically, the role of transcription factors or epigenetic regulators in the pathogenesis of neurodegenerative conditions is explained by their capability to affect genes directly involved in the pathology or in mediating co-morbidities. Here, we highlight some of the better studied transcription factors and epigenetic regulators and their involvement in neurodegeneration. cAMP response element-binding protein (CREB) is a ubiquitous transcription factor playing multiple roles in the CNS. Several signaling cascade converge on CREB which acts in cooperation with cofactors such as CBP/p300. CREB is fundamental for memory and learning but also essential to neuronal survival and protection . Due to its broad expression, being downstream of different signaling events and driving expression of a myriad of critical neuronal genes, alterations of CREB activity and/or expression CREB has been indeed reported for neurodegenerative conditions such as AD or PD both in animal models and humans . Not all transcription factors are always localized to the nucleus, a considerable number of them is instead regulate

their expression by subcellular localization. Thus, failures in the nucleo-cytoplasmic shuttling of transcription factors represent another possibility leading to transcriptional dysfunction in neurodegenerative conditions. The first transcription factor for which movement between the cytosol and the nucleus was described was nuclear factor-ϰB (NF-ϰB). Activity of NF-ϰB is prevented when sequestered in the cytosol. Alterations of NF-ϰB localization have been observed in the proximity of plaques in post-mortem samples from AD patients but also in dopaminergic neurons of PD patients and are tightly linked to inflammatory states. Furthermore, synapse to nucleus shuttling of proteins with the capacity to modulate gene transcription is a prominent way in which inputs received at the synapse are transferred to the nucleus to implement long-term changes. Synapses contain several nuclear localization signals-containing cargo proteins and different components of the nuclear import machinery, like importin-α and importin-β, which have been shown to translocate to the nucleus in an activity-dependent manner . In the last decade, few synaptonuclear protein messengers (i.e. Abi- 1, AIDA-1D, Jacob, RNF10) have been identified, and shown to play key roles in plasticity and synapse function . Notably the activation of NMDA receptors (NMDAR) can regulate gene transcription , thus affecting global protein synthesis and, thereby memory formation. Such effect of NMDAR activation requires the long-distance trafficking of synaptonuclear proteins. The synaptonuclear messengers can associate with different classes of receptors and specifically translate their activation in gene expression changes. For instance, the RING Finger protein 10 (RNF10) operates as a mobile hub that docks GluN2A-containing NMDAR derived signalosomes to nuclear target sites , while protein messenger such as Jacob can encode the synaptic and extrasynaptic origin of NMDAR signals, following long-distance transport and nuclear import . Remarkably, alterations in synaptonuclear messengers have been reported in neurodegenerative disorders such as AD . From the epigenetic perspective, reduced global methylation levels have been found in the blood of AD and PD patients in comparison to healthy controls, probably due to the decrease in expression of DNA methyltransferase 3a , a key mediator of this epigenetic mark in the context of signal-regulated neuroepigenetics . The scenario is however unclear as studies derived from post-mortem samples of AD patients in some cases reported a reduction of global methylation levels in cortex and hippocampus , while in other studies lack changes or even an increase in global DNA methylation have been observed. Studies focusing on DNA methylation changes at specific genomic regions, functional elements, and individual gene loci have yielded a more comprehensive view in the context of AD, but also revealed that epigenetic changes are complex, and often contradictory results are reported from different models (reviewed in). Similarly, studies have indeed reported an association between PD disease progression and global methylation levels detected in brain or blood or changes in the methylation pattern of specific genes . Given the prominent role that histone deacetylases (HDACs) play in the modulation of several neuronal functions, it comes as no surprise that they have also been implicated in neurodegeneration. These heterogenous group of proteins, which are classified based on their activity, structure and co-factors, are responsible for the removal of the acetyl group from histone as well as non-histone proteins. An example of their involvement in neuropathologies comes from HDAC2, HDAC6 or HDAC5, whose levels are elevated in brain areas of post-mortem AD patients . Interestingly, an increased association between HDAC1 and CREB, possibly facilitating CREB pathological dephosphorylation, was observed in neuronal samples of PD patients . Expression levels of HDAC4, which has been implicated in many forms of pathologies of the nervous systems in animal models , was shown to be strongly associated with rapid progression of ALS in patients . Alteration in the expression level of HDAC11 and HDAC2 were also reported in in post-mortem brain and spinal cord tissue of ALS patients . Beside their action as transcriptional regulators, HDACs act also on cytosolic proteins of which the most prominent is tubulin. For example, HDAC6 acts on tubulin and has been associated to deficits in axonal transport in ALS patients-derived motor neurons.

Therapeutic approaches to modify gene expression in neurodegenerative disorders

Targeting transcriptional regulators might be beneficial against neurodegenerative conditions as this would result in a widespread modulation of many affected downstream processes. However, this aspect also represents a potential caveat as affecting the transcription of several genes might be detrimental as not all of them necessarily participate in the pathogenesis. Nevertheless, efforts have been made in exploring, designing, and testing of therapeutical approaches aiming at modulation of transcription-related processes. One of the most

sought-after targets in the treatment of neurodegenerative disorders is CREB due to the copious evidence showing a reduction in its functionality or expression in many diseases. At present, however, there are no available drugs specifically boosting CREB-dependent signaling. Multiple strategies could be followed, from acting on its upstream signaling regulators to using molecular biology or genetic approaches to restore its expression levels. The complexity and multifactorial nature of many neurodegenerative disorders is likely to push the development of drugs towards targets different than the prototypical players associated with a certain disorder. A case for this is NF-κB in the treatment of AD. Memantine, an FDA-approved molecule for the treatment of AD seems to contrast the pro-inflammatory activity of NF-κB. Additional anti-inflammatory drugs are under preclinical or clinical evaluation for their capacity to interfere with NF-κB (see for review . Great efforts have been spent in the exploration of HDACs inhibitors as useful drugs in neurodegeneration. HDACs inhibitors may broadly facilitate gene expression via rendering the chromatin more permissive for transcription. In animal models, non-selective HDAC inhibitors successfully restored memory function and neuronal structural aberrations . However, care should be taken to follow the path of non-selective inhibition due to its potential side effects . The design and development of specific HDAC inhibitors has been hindered by different problems including the fact that all HDACs share considerable structural similarities and are expressed across different organs and cell types. Nevertheless, with the advent of better technological opportunities, is it quite likely that specific HDAC inhibition may be a viable therapeutic avenue to pursue . An encouraging example comes from inhibition of HDAC6 via Tubastatin A, ACY-1215, MPT0G211, or 5-Aroylindoles which showed promising results in AD animal models and are currently under clinical trial evaluation for diseases other than neurodegeneration.

Autophagy: when the clearance system fails and drives neurodegeneration

Cellular homeostasis is the process that controls different cellular activities. It is a fundamental condition that allow the cell to maintain the physiological balance in term of cellular identity, resilience, and survival. Defects in protein homeostasis contribute to the lack of intracellular proteins and organelles, which progressively accumulate in the cytoplasm. Therefore, such alterations can be implicated in the onset and progression of neurodegenerative disorders . Cellular component degradation within the cell can follow two different pathways: autophagy and the ubiquitin system, specific for protein degradation. Both processes require a concerted action of different proteins that are recruited and specifically recognize the damaged material, driving protein degradation as the last step to maintain cellular homeostasis. Under stress conditions, such as ageing or disease, this tuned process fails, resulting in an engulfment in cell physiology. In mammalian cells there are three types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy, simply known as autophagy, plays the major role in maintaining the cellular homeostasis because it helps in the removal of bulky protein aggregates and bigger cytoplasmic bodies. It begins with the phagophore formation that entraps the misfolded proteins and, after different steps, finally fuses with lysosomes to generate the autophagolysosome . Autophagy is tightly controlled by mammalian target of rapamycin (mTOR) and AMP activated protein kinase (AMPK). Autophagy is promoted by AMPK, which is a key energy sensor and regulates cellular metabolism to maintain energy homeostasis . Conversely, autophagy is inhibited by mTOR, a central cell-growth regulator that integrates growth factor and nutrient signals . Given its crucial role in cell homeostasis, a defect in autophagy is associated with neuronal loss and cognitive decline, both in physiological conditions, such as ageing, but also in neurodegenerative disease . Interestingly, defect in the autophagy machinery have also been linked to axonal and dendritic degeneration and might thereby further promote the dysfunction of neural network dysfunctions . Several neurodegenerative diseases are characterized by defects in the degradation process of misfolded proteins and thus, abnormal protein aggregation. Autophagy failure can be attributed to the impairment in the clearance of pathological protein such as α-synuclein, Aβ and tau . For instance, Beclin1 is a protein involved in the regulation of autophagy and has been shown to be reduced in AD patients . Furthermore, it has been shown that the downregulation of Beclin-1 in mice resulted in reduced neuronal autophagy and Aβ accumulation . Moreover, the most common autosomal-dominant form of PD and a familial variant that closely resembles sporadic PD is associated to LRRK2 mutation . The altered function of mutated LRRK2 has been linked to defects in endosomal-lysosomal trafficking and chaperone-mediated autophagy .

In dopaminergic neurons, the lysosome number has been reported to be depleted in a mouse model of PD . In addition to autophagy, cells take advantage of mitophagy, a specific process responsible for the selective degradation of dysfunctional mitochondria in PD . The PTEN-induced protein kinase 1 (PINK1), localized on the external mitochondrial membrane, phosphorylates mitofusin 2 (Mfn2) and ubiquitin triggering the recruitment of the Parkin protein. This event activates several ubiquitin-binding proteins such as optineurin (OPTN) and sequestrosome-1 (SQSTM/p62), that initiate mitochondria to the pathway of mitophagy. Defects in the mitophagy machinery are also a pathological pathway in PD and the accumulation of damaged mitochondria represents one of the main pathogenetic alterations . The PD-associated familial autosomal recessive mutations in the genes PINK1 and Parkin have been discovered to have a key role in mitochondrial quality control . The phosphorylation of PINK1 at the outer mitochondrial membrane (OMM) leads to the recruitment of parkin. Once active, parkin allows the synthesis of ubiquitin chains on OMM proteins leading to ubiquitin chain assembly. The mutations in the PINK1 (PARK6) and parkin (PARK2) genes are linked to autosomal recessive early-onset PD and a pathological accumulation on the OMM that triggers an abnormal mitophagy . In PD α-synuclein, the main component of those pathological Lewy bodies, has been found to bind mitochondria components , inhibiting the protein import and leading to an impaired cellular respiration. In particular, α-synuclein interacts with and disrupts mitochondrial proteins such as TOM20, VDAC, and F_1Fo-ATP synthase, causing mitochondrial metabolic impairment .

Targeting autophagy: how to promote protein and organelle clearance

In the last two decades, several studies have reported the neuroprotective activity of rapamycin, one of the most powerful pro-autophagy agents, in both cellular and animal models of neurodegenerative diseases and, with some limitations, in human . Rapamycin acts blocking the kinase activity of mTOR enzymatic complex1 (mTORC1), removing its autophagy suppressor activity observed under physiological conditions. The strategy of the rapamycin treatment is to activate the autophagic flux negatively controlled by mTORC1. Interestingly, rapamycin has been proposed as an anti-ageing drug in mice, since it was reported to increase the lifespan of animal models treated . However, rapamycin treatment has been also tested in animal models of AD. It has been shown that rapamycin administration reduced the accumulation of Aβ aggregates and prevented tau phosphorylation in the brain of AD transgenic mice, showing a global impact in maintaining cognition . Regarding the use of rapamycin in humans, some encouraging results have been reported in the ALS field where a recent, randomized, placebo-controlled, phase II clinical trial was started to evaluate the efficacy of rapamycin in patients affected by ALS . Another approach to modulate the mTOR signaling level is possible using mTOR-dependent modulator autophagy enhancer-67 (AUTEN-67), a small molecule identified as a potent candidate with anti-ageing and neuroprotective effects, by significantly increasing autophagic flux in neurons and protecting them from undergoing stress-induced cell death . Other agents that can indirectly trigger AMPK-dependent mTOR inactivation are metformin and resveratrol. Metformin activates AMPK which in turn promotes autophagy blocking mTORC1 activity through direct inhibitions of regulatory associated protein of mTOR. Moreover, the activation of AMPK directly promotes the activation of phagophore-forming enzymatic complex unc-51-like kinase (Ulk1)1/2, which is considered the initiator of the autophagic cascade. Furthermore, PROteolysis TArgeting Chimeras (PROTAC) technology has emerged as one of the most promising approaches to remove specific disease-associated proteins using the autophagy machinery of the cells. In the last few years, the PROTAC approach has been extensively used, with several PROTACs molecules currently in clinical trials in the cancer field . A PROTAC molecule targeting tau protein has also been developed. It is a chimera construct made of a tau-binding peptide, a linker, a VHL-binding peptide, and a cell-penetrating peptide. Interestingly, this molecule leads to a significant degradation of tau and reduced neurotoxicity of Aβ, highlighting the therapeutic potential of this approach .

Metabolic failure and energy crisis of brain cells

Among the different brain resident cell types, neurons are extremely energy demanding. Neurons rely almost exclusively on the mitochondrial oxidative phosphorylation (OXPHOS) system to fulfill their energy needs through ATP. The OXPHOS-mediated mitochondrial functions are diverse, ranging from the cell-intrinsic energy production to the regulation of intracellular calcium homeostasis, synaptic plasticity, and

neurotransmitter synthesis . On the other hand, this important energy production is accompanied by the formation of reactive oxygen species (ROS), which in excess are detrimental for cells. ROS derive mainly from the process of the OXPHOS, which reduces O_2 into H_2O using the electrons flux deriving from the respiratory chain and leading to the formation of superoxide anion radicals of the oxygen. Even if cell-intrinsic antioxidant defense systems can buffer ROS, when this buffering systems is overloaded and cell-homeostasis altered, ROS become toxic. Given the fundamental role of mitochondria in neuronal energy supply, their dysfunction leads to an impairment of basal neuronal energy source, impacting on several aspects of brain physiology. In addition to their metabolic activity, mitochondria play a key role in cellular calcium homeostasis. Cellular calcium concentration is strictly regulated as it sustains vital aspects of the neurons such as secretion, motility, metabolic regulation, synaptic plasticity, proliferation, gene expression, and apoptosis . Therefore, mitochondrial calcium dysregulation contributes to neurodegeneration since it is the major mechanism by which increased excitatory neurotransmission triggers mitochondrial depletion from and retraction of dendritic structures . The function of mitochondria is strictly related to their structure and dynamics. Thus, the mitochondrial efficiency is measured through their capacity to undergo continue fusion and fission cycles. Hence, mitochondrial dynamism is important for their morphology and function, and therefore, relevant for neuronal viability and synaptic activity. The equilibrium between fission and fusion is key for adequate mitochondrial function and is compromised in different neurodegenerative disorders. Drp1, the GTPase that controls the process of fission, is altered in AD, leading to an excessive mitochondria fragmentation and, thereby, altering their function . This peculiar phenotype has been shown in neuronal cultures upon Aβ exposure as well as in several neurodegenerative disease animal models . Defects in the dynamin-related GTPase proteins mitofusin-1 (Mfn1) and Mfn2, and atrophy type 1 (OPA1) protein cause mitochondrial fusion alterations and have been reported in various neurodegenerative disorders, including AD . Failure in mitochondria trafficking, function and positioning in dendrites and synapses have been also observed in ALS and FTD and may contribute to early synaptic loss in disease . In FTD, several pathways controlling mitochondrial trafficking, dynamics, and consequently activity are altered . In the genetic FTD caused by MAPT mutations, a decrease in mitochondria-Tau interactions in iPSC-derived neurons has been observed. The authors reported that neurons expressing the Tau protein carrying the V337M mutation were characterized by alterations in mitochondria bioenergetics affecting efficiency in maintaining ATP levels under prolonged energetic stress. Also, Tau P301L mutation, which is known to cause Tau hyperphosphorylation, decreases mitochondrial respiration and ATP production, leading to a global mitochondrial and oxidative impairment . Mutations in transactive response (TAR)-DNA-binding protein (TARDBP), coding for the TDP-43 protein, are also associated with FTD. Interestingly, TDP-43 inclusions aggregate outside of the nuclear compartment and can directly affect the dynamics and trafficking of mitochondria, both at the axonal and dendritic compartment, leading to functional impairment. The overexpression or reduction of TDP-43, in different animal model systems, leads to a mitochondria dysfunction In general, an altered cellular metabolism is considered a hallmark of ageing . During cellular senescence, which is distinct from, but associated with biological aging, the mitochondria exhibit numerous changes in their structure, dynamics, and function. Therefore, in senescent cells a decrease in mitochondrial membrane potential, an increase in proton leakage and a lowered oxidative capacity has been observed. The consequence of modified processes resides in an altered aged metabolic homeostasis, with a significant increase in ROS generation, diminished antioxidant defense and a decrease in ATP production. All these phenomena have a great impact on neurons, post-mitotic cells that are particularly sensitive to stress and to the accumulation of a senescent profile typical of ageing . In addition, the pool of healthy mitochondria tends to decrease with ageing. In addition to the role played by mitochondria in neurons themselves, the demanding energy request by neurons is also sustained by glial cells, which are extremely flexible and respond to environmental changes providing neurons the required energy . Every time that neurons need energy to perform the highly energy consuming neuronal synaptic burst, the so-called astrocyte-neuron lactate shuttle responds to this energy demand by creating an energy bridge by which astrocyte produced lactate is received by the neuronal part . The astrocytic-neuronal metabolic bridge is supported by the capacity of astrocytes to convert GABA and glutamate, removed from the synaptic cleft, into glutamine, which is used as a precursor for refill synaptic vesicles or for phosphorylation via the TCA cycle Neuronal metabolism pathways are comparatively inflexible and believed to be strictly regulated.

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However, metabolic changes including state shifts and alteration is individual metabolites can have great impacts on the neuronal epigenome. This enables the cells to adapt to environmental changes, but also poses a risk for, as energetic challenges may lead to highly consequential epigenetic alterations . Indeed, the cell fate specification and the consequent cell identity are established by a highly specific epigenetic control which must be also plastic to allow the cell to adapt to the environment. For instance, high glucose levels produce high acetylCoA:CoA ratio, which regulates histone acetyltransferase (HAT) activity, and contributes to increased chromatin accessibility and gene activation . Both the early TCA cycle intermediate α-ketoglutarate and oxygen are co-substrates for demethylases, affecting DNA and histone methylation and, thereby, changing transcription scenario . Moreover, metabolic enzymes can translocate directly to the nucleus in a splicing- and signaling-dependent manner, and act directly on histones triggering changes in transcription. This is the case of pyruvate kinase M (PKM) which translocates to the nucleus where it phosphorylates histone 3 and leads to a de-repression of cell-cycle and glycolytic genes . In directly converted induced neurons (iNs) from AD-patient-derived fibroblasts, a cancer-like metabolic switch from neuronal OXPHOS to aerobic glycolysis in AD iNs is associated with a higher level of the PKM2 nuclear isoform compared to the physiological PKM1. PKM2 prevalence is associated with metabolic and transcriptional changes in AD iNs, contributing to AD-related-neuronal defects. Overall, all these new findings are suggestive of the presence of a metabolic reprogramming towards an aerobic glycolytic profile in AD, throughout a Warburg-effect . The epigenetic modulation of metabolism, influenced by the combination of pathology and ageing, could be primary or secondary to the mitochondrial impairment . In general, in the neurodegenerative context, there is an accumulation of macromolecular damage and metabolic reprogramming that leads to the damage of organelles, including mitochondria, and eventually to tissue dysfunction .

Targeting mitochondria as therapeutic strategy for treating neurodegeneration

To reduce and buffer mitochondrial dysfunction the most used indirect therapies rely on the use of antioxidants that mitigate the mitochondrial ROS production. Some of those compounds include the lipophilic MitoQ, CoQ10, MitoVitE, MitoTEMPOL , and resveratrol, which indirectly activates PGC-1α and induces mitochondrial biogenesis . There are also compounds that modify mitochondrial dynamics, such as mitochondrial division inhibitor 1 (Mdivi-1), and methylphosphonate (DDQ) . In particular, Mdivi-1, an inhibitor of Dpr1, showed activity against Aβ-induced excessive mitochondrial fragmentation . A novel approach, recently proposed, suggest combining the antioxidant effect with epigenetic modulation. Shikonin, an anticancer PKM2 modulator, would act on the metabolic shift caused by neuronal PKM2 in AD, acting as an apoptotic brake on mature . Given the pathogenic role for excitatory mitochondrial calcium dysregulation in mediating sublethal dendritic atrophy observed in chronic neurodegenerative diseases, inhibiting calcium uptake has been reported to be neuroprotective. The major protein complex involved in mitochondrial calcium uptake is the mitochondrial calcium uniporter (MCU) and, thereby, MCU inhibitors are neuroprotective in different genetic models of chronic neurodegenerative diseases

Neuroinflammation

In recent years, it has become clear that despite having many different primary causes, all neurodegenerative diseases share a common hallmark: neuroinflammation. Inflammation is the first line in host pathogen defence and essential to the body's healing processes. However, chronic or prolonged inflammation, like that observed in ageing and further exacerbated in neurological diseases, is detrimental for tissue homeostasis. Neuroinflammation can be triggered by CNS resident immune and glial cells (e.g. microglia, astrocytes, oligodendrocyte lineage cells), cells from the peripheral innate or adaptive immune system (e.g. T cells, B cells, macrophages), meningeal inflammation, or autoantibodies directed to the CNS.

Neuroinflammation in ageing and neurodegeneration

There is growing evidence showing that both, innate and adaptive immune cells are present in the healthy CNS, where they have key roles in maintaining homeostasis and immunosurveillance, being associated with neurogenesis , learning and memory and synaptic pruning , among other functions. However, this tightly regulated immune-CNS interaction is distorted with ageing, and even more abruptly in neurodegenerative

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disorders, leading to pathological neuroinflammation and subsequent neurodegeneration . Even though present at low levels in the healthy young CNS , an increase in adaptive immune cell infiltration, mainly CD8$^+$ T cells and to a lesser extent CD4$^+$ T cells, has been observed in the neurogenic niches, the white matter, and the optic nerve with age. Enhanced T cell infiltration alters CNS resident cell function increasing the expression of interferon responsive genes in CNS stem and glial cells (neural stem cells, microglia, and oligodendrocytes) and contributes to age-related myelin degeneration, impaired neurogenesis, and axonal degeneration . Increased CNS infiltration of peripheral immune cells with ageing, may result from blood-brain-barrier alterations, increased permeability and a decreased CNS perfusion and lymphatic drainage . Similar changes in T cell infiltration have been also observed in a range of neurodegenerative disorders not considered primary autoimmune disorders such as AD, PD and ALS. Evidence has shown increased in T cell numbers, especially CD8$^+$ T cells, in the post-mortem CNS tissue of AD, ALS and PD patients , and altered T cell levels or subsets in the cerebrospinal fluid and peripheral blood AD, PD and ALS patients . The role of adaptive immune cell-mediated inflammation in AD remains controversial. Even if T cell depletion has rendered beneficial roles reversing cognitive decline, increasing Aβ clearance and promoting neuronal survival , other studies have described detrimental roles for T cells in AD pathology . In PD models on the other hand, mice lacking mature lymphocytes show attenuated dopaminergic cell , while in an ALS mouse model CD8$^+$ T cells infiltrate in the CNS are associated with motor neuron loss . Thus, the role of T cell-mediated neuroinflammation in neurodegenerative diseases may be subset and context specific, highlighting the complexity of the CNS-immune crosstalk and the role of neuroinflammation in neurodegeneration. Prolonged CNS immune infiltration together with the enhanced production of pro-inflammatory cytokines (e.g: IFN-γ, TNF-α, IL-6 or IL-1β) described in both, ageing and neurodegenerative diseases, also contribute to neurodegeneration indirectly, by perpetuating inflammation through the priming of CNS glial cells. Single cell sequencing analysis of CNS resident cells such as microglia, astrocytes and oligodendrocyte lineage cells in different neurodegenerative diseases contexts and ageing has unveiled disease-specific phenotypes characterized by the expression of inflammatory and neurotoxic markers such as *Clec7a,C3, Lgals3, Trem2* in microglia , *Serpina3n, Lcn2, Ifitm3, Timp1, Chi3l*1 in astrocytes and Serpina3n, C4b, or Klk6 in oligodendrocyte lineage cells . Beyond this disease-specific phenotype, an elevated expression of interferon responsive/stimulatory genes (e.g:*Irf1, Irf7, Irf8, Isg15, Ifit3*) has also been described across glial cells in ageing and neuroinflammation . Additionally, neuroinflammation enhances the expression of antigen presenting genes (e.g: *Cd74, B2m, Cd9, H2-K1, H2-D1*) as well as immune cell chemoattractant cues such as *Icam-1, Ccl2, Cxcl12 or Ccl3* by all the main glial cells, which in turn further activate CNS infiltrating T cells, contributing to a positive feedback loop that perpetuates neuroinflammation and thus, neurodegeneration In addition to immune cell infiltration and CNS resident cell-driven pro-inflammatory reactions, another source of neuroinflammation is linked to the accumulation of senescent cells with ageing and in CNS pathology. Mounting evidence has demonstrated the accumulation of senescence markers such as P16, P21, YH2A.X, lipofuscin, GATA4 and high-mobility group box protein 1 in microglia, oligodendrocyte progenitor cells, oligodendrocytes, astrocytes, and neurons with ageing and in pathology such as AD . Senescent cells accumulate in aged tissues and contribute to the pathogenesis of a range of neurodegenerative diseases, at least in part, through their pro-inflammatory senescent associated secretory phenotype (SASP) , which can propagate senescence to neighbouring cells in a paracrine manner and contributes to immune cell recruitment to eliminate senescent cells . Hence, neuroinflammation and senescent cells establish an additional positive feedback loop exacerbating ageing and disease pathogenesis: chronic inflammation like that observed in ageing and neurodegenerative disorders enhances the appearance of senescent cells, which in turn further contribute to neuroinflammation by the secretion of pro-inflammatory molecules. Therefore, it is plausible that either by eliminating senescent cells or by modulating SASP, we can limit neuroinflammation and prevent neurodegeneration.

Therapeutic approaches to counteract neuroinflammation

Several drugs that limit immune cell infiltration in the CNS have been developed in the context of MS, like Siponimod, Fingolimod or Natalizumab However, whether these drugs are beneficial in other primary neurodegenerative diseases such as AD or ALS is still under debate. Natalizumab is a monoclonal antibody

that blocks the extravasation of immune cells in the CNS and has been proven successful in mouse models of ALS such as SOD1^{G93A} and TDP43^{A315T,} where it has diminished astrocyte and microglia priming, increasing motor neuron number and survival . Natalizumab has also shown beneficial effects in pre-clinical models of AD such as APP/PS1 and 3xTg mice. In APP/PS1 mice Natalizumab reduced of proinflammatory cytokines in the spleen, CD4 immunoreactivity and general inflammation in the CNS . Similarly, in 3xTg-AD mice, Natalizumab has improved memory and reduced microgliosis, Aβ load and tau hyperphosphorylation . Fingolimod on the other hand, is a structural sphingosine analogue and sphingosine-1p-phosphate (S1P) receptor modulator which blocks immune cell migration outside primary lymphoid organs and thus, reduces T and B cell number in circulation. Fingolimod has shown to promote survival and improve the phenotype in SOD1^{G93A} mice and is well tolerated by patients with ALS, although its efficacy in disease progression is yet to be evaluated . In AD on the other hand, fingolimod has shown to ameliorate Aβ neurotoxicity in neuronal cultures while reducing Aβ and neuronal loss and astrocyte and microglial activation and improving memory and learning deficits in 5xFAD mice . Furthermore, in PD mouse models such as the MPTP mouse model and a model performed by intracerebral injections of 6-hydroxydopamine, fingolimod attenuated neuroinflammation, neuronal loss and motor deficits . Moreover, low-dose of Fingolimod improved motor function and reduced brain atrophy, leading to the extended survival of R6/2 mice, a mouse model of HD . Thus, even though drugs that limit immune cell trafficking to the CNS appear to have a beneficial effect and help preventing neurodegeneration in different mouse models, further pre-clinical investigations followed up by clinical trials are needed before clearly establishing their benefits for patients in other neurodegenerative diseases beyond MS. An alternative approach to limit not only immune cell mediated inflammation, but also neuroinflammation mediated by CNS resident cells, involves the use of other less specific anti-inflammatory drugs such as minocycline or non-steroidal anti-inflammatory compounds. Minocycline is a broad-spectrum antibiotic with important anti-inflammatory properties and as such, it has been studied for several years now in mouse models of neurodegeneration. Even if minocycline has been proven successful in limiting neuroinflammation and in some cases neurodegeneration in mouse models of AD , PD and ALS , its beneficial effect in subsequent clinical trials has been less robust, with no clear neurocognitive improvement observed in AD or HD, and disease worsening was detected in ALS trials . Therefore, although most of the pre-clinical data using minocycline reported positive results, its current negative outcomes or even the symptom worsening observed in some clinical trials, questions its effectiveness as a therapy for neurodegenerative diseases . Other anti-inflammatory therapies considered for neurodegeneration include non-steroidal anti-inflammatory drugs (NSAIDs), however, the potential beneficial effects observed in some animal models, have not been reproduced in clinical trials and thus their use as potential therapy for neurodegeneration dropped . Considering the negative impact of sustained inflammation, mammals have developed their own endogenous anti-inflammatory break, which is mediated by regulatory T cells (Tregs), a subset of immune cells with high immune suppressive capacity. Tregs are known to be either depleted or functionally impaired in multiple neurodegenerative disorders . Thus, systemic Treg expansion or Treg adoptive transfer have been considered as a potential therapeutic approach to tackle neuroinflammation and prevent neurodegeneration. Treg expansion through peripheral interleukin-2/interleukin-2 monoclonal antibody complexes or adoptive transfer upon *ex vivo* activation has rendered positive results in mouse models of ALS and AD, such as SOD1^{G93A} mice , 5xFAD -Rag2Ko mice , 3-Tg-AD mice and APP/PS1 mice . Tregs have been shown to protect motor neurons, supress astrocytic and microglial immunoreactivity, reduce amyloid burden and restore cognitive dysfunction. Moreover, an inverse correlation was observed between Treg numbers and disease progression upon Treg expansion in ALS patients, suggesting a neuroprotective effect also in humans . Despite the ample evidence of a beneficial role for Treg having in neurodegenerative diseases, systemic Treg expansion has not been widely considered for clinical trials, as it can lead to systemic immune suppression in patients of advanced aged and already vulnerable to infections, limiting its therapeutic use. Recent investigations have developed a gene delivery approach to locally expand Treg in the CNS by overexpressing interleukin-2 in astrocytes, and thus, avoiding systemic immune suppression. This adenoviral based gene delivery approach has rendered positive results in mouse models of MS, stroke, and traumatic brain injury . Even though still to be tested in primary neurodegenerative disorders, such as ALS, PD or AD, this approach opens novel therapeutic venue to harness Treg immunosuppressive capacity to limit CNS neuroinflammation and

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neurodegeneration. Last, we review the potential use of senolytics to limit neuroinflammation and prevent neurodegeneration. The fact that mice genetically engineered to remove p16^{INK4a+} senescent cells show a decrease in age-related pathologies in several tissues, together with an extended lifespan and health span , boosted the interest in the development of senolytics, such as dasatinib, digoxin or quercetin, as potential therapeutic approaches for neurodegeneration . Senescent oligodendrocyte progenitor cells (OPC) have been found around Aβ plaques in post-mortem tissue of patients with mild-cognitive impairment or AD and APP/PS1 mice. The elimination of senescent OPCs by the administration of the senolytic cocktail formed by dasatinib and quercetin, decreased microglial activation, Aβ load, and the concentration of inflammatory cytokines IL-6, IL-1β and TNF-α and improved cognitive performance . Additionally, elimination of senescent astrocyte and microglia via the administration of senolytic AP20187 in MAPT^{P301S}PS19 tau-pathology mouse model prevented gliosis, hyperphosphorylation of tau and neurodegeneration and preserved cognitive function . Similarly, removal of senescent microglia via AP20187 or dasatinib and quercetin administration prevented age-related cognitive decline and neuroinflammation . Thus, senolytics prevent neurodegeneration in ageing and pre-clinical models of AD, supporting their use in clinical trials with older adults suffering from MCI or early-stage AD (NCT04685590. SToMP-AD and NCT04785300, ALSENLITE) , However, their beneficial effect in other neurodegenerative diseases such as ALS or PD is yet to be investigated. Even though the pre-clinical results in AD and ageing look promising, the use of senolytic approaches to eliminate senescent cells should be considered cautiously, due to the lack of knowledge regarding the role of senescent cells in neurodegeneration. One alternative to avoid the potential negative effects of eliminating senescent cells is to focus on developing therapies aiming at reducing or eliminating SASP to limit neuroinflammation . Senomorphics or SASP inhibitors can limit senescent cell SASP production by inhibiting NF-kB, JAK-STAT, mTOR or mitochondrial complex I and IV related targets. However, since senomorphics do not eliminate senescent cells, continuous treatment with SASP inhibitor would be required to obtain long-lasting effects, which could also increase off-target effects associated with the suppression of cytokine secretion by other cells. Thus, a better understanding of the role of SASP and senescent cells in the CNS diseases and ageing is essential to successfully develop senotherapeutic interventions to limit neuroinflammation and target neurodegeneration .
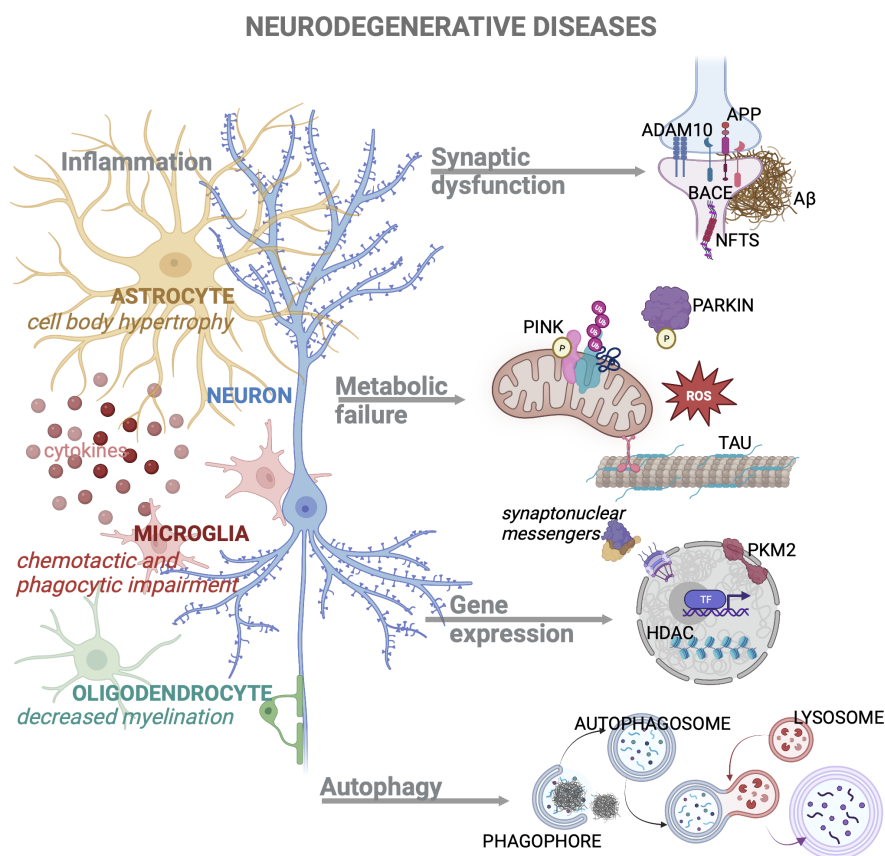
Conclusions and future perspectives

We have attempted to briefly review the enormous field of the biological pathways that are affected during ageing and implicated in the pathogenesis of neurodegenerative disorders. Even though a common feature of neurodegenerative diseases is the abnormal deposition and mis-localization of insoluble protein aggregates, different cellular pathways contribute to neuronal loss. Furthermore, these pathways are all affected by ageing, which represents the main common risk factor for most neurodegenerative disorders. Cells in all regions of the CNS are affected by ageing, as indicated by the decline of sensory, motor, and cognitive functions with time . However, how cellular and molecular changes that occur during normal ageing render neurons vulnerable to degeneration has not yet been fully elucidated. Currently, most efforts to treat neurodegenerative disorders focus on strategies that target the insoluble aggregates of proteins specifically associated with each neurodegenerative disorder. So far, most of the clinical trial results have been disappointing because cognitive function is not restored even when protein aggregates are removed. These results point towards the importance of studying the cellular pathways that contribute to neuronal dysfunction, in order to provide combined therapies to the patients. Neuronal function requires an efficient network of pathways that are strictly connected and interdependent. For instance, synaptic function is affected by the inflammatory microenvironment generated by the ageing glial cells. Furthermore, synaptic transmission requires energy and perturbed mitochondrial function has been associated with ageing and neurodegeneration as the quality control of the cellular components is regulated by energy sensors. Additionally, the lysosomal-dependent self-digestive process of damaged proteins and organelles, called autophagy, is important to generate nutrients and energy to maintain essential cellular activities. Defects in autophagy results in intracellular protein accumulation, contributing to the formation of the insoluble aggregates of protein specifically associated to neurodegenerative disorders. Finally, gene expression translates synaptic activity and alterations in metabolic function into changes in gene expression that can profoundly modify neuronal structure and

function. Different therapeutic strategies have been developed to target these cellular pathways and the drugs have been evaluated for the treatment of different neurodegenerative disorders. However, the future challenges in drug discovery for neurodegenerative disorders are (i) the detection of the earliest events in the neurodegenerative cascade and (ii) the identification of the pathways responsible for the specific vulnerability of cellular populations in each neurodegenerative disease. The understanding of these mechanism is critical to develop disease-modifying therapies and to design tailored therapies that can be administered to specific patients' populations.**ReferencesFigure legendFigure. 1** Scheme of the biological pathways contributing to neurodegeneration

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NEURODEGENERATIVE DISEASES

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Table 2.docx available at https://authorea.com/users/568586/articles/614389-novel-therapeutic-approaches-to-target-neurodegeneration