Introduction

Alzheimer’s disease (AD) and related dementias together constitute an enormous social and economic burden not only for Australia, but most societies worldwide. Age is the most important risk factor for dementia, and with an increasing life expectancy, the number of patients suffering from dementia will increase. What makes things worse is that the current treatment is mainly symptomatic and does not target the underlying biology. What causes the sporadic forms of AD is not completely understood, and in this short review article, it is impossible to cover comprehensively. Because both AD and also frontotemporal dementia (FTD), another prevalent dementia studied by us, develop very slowly over many decades, it is impossible to identify with certainty a single triggering event or condition. An exception are the rare familial cases caused by autosomal dominant mutations, but even for those it is not completely understood how disease is actually initiated and why it remains dormant for so long. We will restrict our discussion to one crucial organelle, the mitochondrion, and how its function is impaired in AD. This impairment encompasses several aspects of mitochondrial function: fission (biogenesis) and fusion, bioenergetics, motility and transport, as well as turnover by mitophagy (1). These processes are highly interdependent and therefore cannot be discussed in isolation. Any of these features are amenable to therapeutic intervention. Oxidative stress has been implicated in AD pathogenesis, and mitochondria are the major site of production of reactive oxygen species (ROS); these have a role in signal transduction, but under conditions of stress, their levels increase dramatically and thereby damage a host of cellular constituents. The challenge presented by anti-oxidant strategies to reduce ROS levels asks for novel approaches to restore mitochondrial function and combat ROS activity in human neurodegenerative conditions such as AD and FTD.

Mitochondria – Maternally Inherited Organelles

According to the endosymbiotic theory, mitochondria are derived from free-living $α$-proteobacteria that were engulfed by eukaryotic host cells. Mitochondria proliferate on their own during the cell cycle, in a manner similar to bacterial division (2). They also contain a small amount of their own DNA, known as mitochondrial DNA (mtDNA), that in most animals is inherited maternally. Mitochondria have a complex, dynamic structure that is compartmentalised: they are bounded by two membranes, an outer one that contacts the cytoplasm and an inner one, which is organised into folded structures called cristae, that contacts the mitochondrial matrix.

Mitochondria are the powerhouses of all cells except erythrocytes, producing adenosine triphosphate (ATP) via the combined efforts of the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation (OxPhos) system of the electron transport chain (ETC) (Fig. 1). The respiratory chain, which is under the control of both nuclear DNA and mtDNA, comprises four multisubunit complexes (I, II, III and IV), and two small mobile electron carriers, ubiquinone/coenzyme Q and cytochrome c, that are both localised at the inner membrane (3). By using the energy stored in nutritional sources, the respiratory chain generates a proton gradient across the inner membrane, thereby driving ATP synthesis via complex V, a rotary motor protein that converts ADP into ATP. In addition to providing energy, mitochondria buffer calcium ions, and failure to regulate calcium levels can trigger apoptosis and cell death (4).

The mitochondrial ETC is the major producer of ROS, which react with a host of substrates causing lipid peroxidation, protein modifications and DNA damage (5) (Fig. 1). Because neuronal mitochondria have a particularly long half-life compared to those of other cell types, the likelihood of accumulating damage is significantly increased. Mitochondria also need to be transported over long distances into the neuronal processes, away from the cell body, where most of their biosynthesis occurs. Together these two features can result in the demanding requirement for neurons to remove dysfunctional mitochondria by first transporting them back to the cell body, and then eliminating them via the autophagy–lysosomal pathway in a process called mitophagy (6).

Alzheimer’s Disease

AD is characterised by early memory deficits, which are followed by gradual erosion of other cognitive functions. Histopathologically, the AD brain is characterised by massive neuronal cell and synapse loss at specific sites, as well as two hallmark lesions, known as amyloid-β ($Aβ$) plaques and neurofibrillary tangles (NFTs). The major proteinaceous component of the plaques is the polypeptide $Aβ$ that is derived from the larger amyloid precursor protein (APP) by proteolytic cleavage. The NFTs contain aggregates of the microtubule-associated protein tau in a hyperphosphorylated form. NFTs are also abundant in a subset of FTD, in which there is an absence of overt plaques.
A quarter of all people aged 55 years and above have a family history of dementia. For most, the family history is due to genetically complex disease, where many variations of small effect interact to increase the risk. The lifetime risk of dementia is about 20% for these families, compared with 10% in the general population. A small proportion of families have an autosomal dominant family history of early-onset dementia (7). In AD, the corresponding mutations account for less than 1% of the total number of cases. Mutations have been identified in three genes: APP, presenilin 1 (PSEN1) and PSEN2. The presenilins are components of the proteolytic γ-secretase complex that, together with β-secretase, generates Aβ. Most familial AD cases are caused by mutations in PSEN1 and PSEN2, of which over 130 have been identified. Of the more than 20 pathogenic mutations that have been identified in APP, several have been expressed in transgenic mice (8). Among the risk or susceptibility genes for sporadic forms of AD are common variants of the gene encoding apolipoprotein E (APOE). Although no AD mutations have been identified in the MAPT gene encoding tau, these have been identified in a subset of FTD, establishing that dysfunction of tau in itself can cause neurodegeneration and lead to dementia. Of the 42 known mutations in MAPT, several have been expressed in transgenic mice (8).

A number of hypotheses have been put forward to explain the pathogenesis of AD, and slowly, the dots between Aβ, tau and neurodegeneration are being connected (9,10). Prominent among these hypotheses is the ‘amyloid cascade hypothesis’ that presents a pathocascade in which Aβ is placed upstream of tau (11). There is, however, also a role for tau, because when tau is genetically removed (such as in tau knockout mice), the toxic effects of Aβ are abrogated (12). Research into mitochondrial functions has revealed that Aβ and tau cause impairments both separately and synergistically as we outline below.

Mitochondrial Impairment in Alzheimer’s Disease

Broadly speaking, the study of mitochondrial functions and their impairment in AD is divided into two general areas. The first is concerned with the imbalance in the generation and elimination of ROS, and the effects of this imbalance on downstream substrates. ROS are also generated under normal conditions, however, their levels are kept relatively low because the rates of production and clearance are tightly balanced. Thus, either enhanced ROS production or an impaired antioxidant system will tip the cellular redox balance (13). The second area is concerned with the dynamic behaviour of mitochondria in terms of morphological changes, their transport and distribution in different cellular compartments, and how efficiently they are removed from the system when they have accumulated damage.

Mitochondria are the major source of oxidative stress because of the unavoidable electron leakage that occurs during electron transfer, leading to a constant
production of superoxide anions responsible for 90% of all endogenous ROS (13). In the AD brain, there is increased lipid peroxidation, protein oxidation and oxidative damage of RNA and DNA, with the latter presenting with double-strand breaks, cross-linking and base modifications. Furthermore, plasma levels of antioxidants such as albumin and specific vitamins are decreased in AD patients (14). Similarly, significant reductions have been reported for the activity of antioxidant enzymes such as superoxide dismutase, catalase or glutathione peroxidase (15). Oxidative stress is an early event in AD, as revealed by reduced levels of antioxidants and antioxidant enzymes in mild cognitive impairment (MCI), a stage conferring a 10–15% annual risk of converting to probable AD (16,17). In fact, almost all aspects of mitochondrial function have been reported to be impaired in AD compared with age-matched controls (Fig. 1) (1).

Modelling Mitochondrial Dysfunction in Mice

Key aspects of the human AD pathology have been modelled in animals, in particular mice, mostly by expressing gene mutations that are found in familial cases of AD and FTD (8). These models were instrumental in unravelling how Aβ and tau impair neuronal, and to some degree, glial functions. With regards to mitochondrial dysfunction, the analysis was assisted by proteomic and transcriptomic methods that together with functional validation, collectively demonstrated that mitochondrial dysfunction and oxidative stress constitute central pathomechanisms in animal models of AD, and by extension, in human AD.

One of the earliest studies found reduced respiratory complex activities, impaired mitochondrial respiration and ATP synthesis, higher levels of ROS, modified lipid peroxidation levels, and an upregulation of antioxidant enzymes in response to oxidative stress in FTD tau mutant mice (18). Subsequent work analysed the effect of elevated levels of both mutant tau and Aβ and found that, whereas Aβ mainly impaired complex IV, tau mainly impaired complex I, at both the protein and the activity level (Fig. 1) (19). Interestingly, human amylin, which forms insoluble aggregates in the pancreas of diabetic patients and is similar in structure to Aβ, also impairs complex IV activity (20). Exposing primary neuronal cultures to oligomeric Aβ preparations was found to elicit a significantly reduced mitochondrial length in neurites, suggestive of enhanced mitochondrial fragmentation (21). Many studies have analysed the key molecules involved in mitochondrial fission and fusion, a process termed mitochondrial dynamics. The fission protein DRP1, for example, is localised to mitochondria in an actin-dependent process. Because tau can induce stabilisation and bundling of filamentous actin, this has direct consequences for mitochondrial dynamics (22). In fact, increased filamentous actin in transgenic mice and flies that overexpress wildtype and FTD mutant forms of human tau disrupts the physical association of mitochondria and DRP1, leading to mitochondrial elongation (23). Given that AD is characterised by tau and Aβ pathology, mitochondrial dynamics therefore needs to take both molecules into consideration. Although there are many studies that address oxidative phosphorylation, as well as mitochondrial dynamics and transport in transgenic mouse models of AD, little work has been done to determine how mitophagy is affected by AD pathology (24).

Therapeutic Interventions Targeting Mitochondrial Function

The road to translation for AD has not been easy. The Alzforum website (www.alzforum.org) at the time of writing this review article lists eight antioxidant-based therapeutics that have entered clinical trials, three of which have been discontinued, one of which is inactive, and the others are currently at stage 2, 3 or 4. Interestingly, a recent study found that 2-year supplementation with folic acid and vitamin B12 in a stratified group of elderly people did not affect cognitive performance (25), whereas a randomised placebo-controlled pilot trial of omega-3 fatty acids and α-lipoic acid was found to slow cognitive and functional decline in AD over the course of a 12 month-treatment (26). The final verdict on the benefit of an antioxidant approach in AD is still awaited.

More recently, several new strategies for targeting mitochondrial functions have been pursued in animal models (1). These include antioxidants such as the lipophilic cation-based MitoQ and the aromatic-cationic SS31, both of which target and accumulate in mitochondria, in order to reduce the toxic insult of ROS by scavenging H2O2. Both compounds have been shown to reverse Aβ-induced mitochondrial disruptions and toxicity in cell culture and in mouse models. Because increased levels of tau as found in AD impair complex I, the use of pectin-functionalised platinum nanoparticles has been explored as a means to oxidise NADH to NAD+ and reduce ubiquinone (CoQ) to ubiquinol (CoQH2), thereby compensating for the loss of complex I function in AD. An interesting target is the enzyme ABAD (amyloid-binding alcohol dehydrogenase) that localises to the mitochondrial matrix and is critical for neuronal survival. Aβ has been shown to bind to and inhibit ABAD, with an ABAD-specific small molecule inhibitor showing efficacy by blocking the interaction of ABAD with Aβ, thereby reversing Aβ-induced toxicity and mitochondrial dysfunction. All of these approaches are yet to enter clinical trials and the field awaits innovative approaches to improve brain mitochondrial respiration based on pharmacology, nutritional interventions and innovative approaches such as irradiation therapy with near infrared light using LED (light-emitting diode) arrays (27).

Outlook

In summary, there is convincing evidence from both human and transgenic animal studies that impaired mitochondrial functions and increased oxidative stress play a role in AD and related neurodegenerative diseases. Therefore, supporting and improving the mitochondrial respiration of neurons constitutes a promising therapeutic principle. The situation is more complex when one contemplates how to manipulate mitochondrial transport, dynamics and turnover. For example, even within a single neuron the requirements for having longer tubular mitochondria versus shorter fragmented mitochondria
differ depending on the subcellular compartment. The homeostasis of the mitochondrial network in any given cell at any point of time is only incompletely understood, making it difficult to design an effective therapeutic strategy. Therefore, much needs to be learned about how mitochondria are regulated under both physiological and pathological conditions. Novel approaches are also needed to develop methods to repair or replace mitochondria when the damage burden is too high (such as when mutations have accumulated in mtDNA) or to assist in their removal when they have accumulated damage. However the hope remains that improving the activity of mitochondria can be used as a strategy to increase memory functions and delay neuronal degeneration in AD.

References