Mitogen-activated protein kinases and the evolution of Alzheimer’s: a revolutionary neurogenetic axis for therapeutic intervention?

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Abstract

Alzheimer’s disease (AD) is a neurogenetic condition that affects the processes via which the brain functions. Major observable hallmarks of AD are accumulated clusters of proteins in the brain. These clusters, termed neurofibrillary tangles (NFT), resemble pairs of threads wound around each other in a helix fashion accumulating within neurons. These tangles consist of a protein called Tau, which binds to tubulin, thus forming microtubules. Unlike NFTs, deposits of amyloid precursor protein (β-APP) gather in the spaces between nerve cells. The nearby neurons often look swollen and deformed, and the clusters of protein are usually accompanied by reactive inflammatory cells, microglia, which are part of the brain’s immune system responsible for degrading and removing damaged neurons or plaques. Since phosphorylation/dephosphorylation mechanisms are crucial in the regulation of Tau and β-APP, a superfamily of mitogen-activated protein kinases (MAPKs) has recently emerged as key regulators of the formation of plagues, eventually leading to dementia and AD. The complex molecular interactions between MAPKs and proteins (plaques) associated with the evolution of AD form a cornerstone in the knowledge of a still burgeoning field of neurodegenerative diseases and ageing. This review overviews current understanding of the molecular pathways related to MAPKs and their role in the development of AD and, possibly, dementia. MAPKs, therefore, may constitute a neurogenetic, therapeutic target for the diagnosis and evolution of a preventative medical strategy for early detection, and likely treatment, of Alzheimer’s.

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Abbreviations:
AD, Alzheimer’s disease; β-APP, β-amyloid precursor protein; CaM, calmodulin; CAL, calyculin; CREB, cAMP-response element binding protein; cdk, cyclin-dependent kinase; EOFAD, early-onset familial AD; EGF, epidermal growth factor; ERT kinase, EGF receptor threonine kinase; ERK, extracellular receptor kinase; FRET, fluorescence resonance energy transfer; GSTp, glutathione S-transferase Pi; GSK, glycogen synthase kinase; GAP, GTPase activating protein; GEF, guanine nuclear exchange factor; HO, heme oxygenase; HEK, human embryonic kidney; IL, interleukin; JNK, Jun N-terminal kinase; JIP, JNK-interacting protein; NMDA, N-methyl-D-aspartate; MAP, microtubule associated protein; MKP, MAP kinase phosphatase; MAPK, mitogen-activated protein kinase; MAPKKK, MAP kinase kinase kinase; MAPKK, MAP kinase kinase; MLK, mixed lineage kinase; MBP, myelin basic protein; NGF, nerve growth factor; NFT, neurofibrillary tangles; nAChR, nicotinic acetylcholine receptors; NO, nitric oxide; OKA, okadaic acid; PHF, paired helical filament; PMA, phorbol 12-myristate 13-acetate; Pi3-K, phosphatidylinositol 3 kinase; PDGF, platelet-derived growth factor; PS, presenilin; PKA, protein kinase A; PKC, protein kinase C; PP, protein phosphatase; PTK, protein tyrosine kinase; PTP, protein-tyrosine phosphatase; ROS, reactive oxygen species; RAGE, receptor for advanced glycated end products; RTK, receptor tyrosine kinase; RSK, ribosomal S6 protein kinase; SRF, serum response factor; SAPK, stress-activated protein kinase; TPK, Tau protein kinase; TGF, transforming growth factor; TNF, tumor necrosis factor.

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1. Introduction

The syndrome of dementia, which represents a progressive deterioration in intellectual abilities, can be so severe to an extent it may interfere with the person’s usual social and occupational functioning (Burnham, 1988; Floyd, 1999; Harper and LoGrasso, 2001; Peel, 2004; Wurtman, 1985). An estimated 5–10% of the US adult population (ages 65 and older) is affected by a dementing disorder, and the incidence doubles almost every 5 years among people falling within this age group (Erickson, 1991; Selkoe, 1991; Runden et al., 1998).

Alzheimer’s disease (AD) is the most common form of dementia, especially in the US; it and related dementias affect at least two million, and possibly as many as four million, residents. Despite its prevalence, dementia often goes unrecognized or is misdiagnosed in its early stages (Beardsley, 1993; Beardsley, 1995; Zhu et al., 2001a). Some disorders that result in dementia are ‘reversible’ or ‘potentially reversible’, which means that they can be treated effectively to restore normal or nearly normal intellectual function (St. George-Hyslop, 2000).

Among the most frequent reversible causes of dementia are depression, alcohol abuse and drug toxicity. In elderly persons, drug use — particularly drug interactions caused by ‘poly-pharmacy’ (simultaneous use of multiple drugs) — is a common cause of cognitive decline. Depression also is an under-diagnosed condition in this population (Erickson, 1991). However, the majority of dementias, including AD, are considered non-reversible. Even for these conditions, correct diagnosis of the problem in its early stages can be beneficial (Martindale, 2002). In addition, many of the non-reversible dementias include symptoms such as incontinence, wandering and depression that can be treated effectively (Wurtman, 1985).

The underlying factors contributing to the evolution and perpetuation of AD remain largely obscure (Martindale, 2002; St. George-Hyslop, 2000). Biomedical researchers have devised psychological screening tests to try to identify people in the early stages of AD. Apart from memory loss, there are nine other warning signs — among them difficulty performing familiar tasks, problems with language, such as remembering words, disorientation in time and space, and changes in personality. Susceptible people appear to have a greater sensitivity to the drug tropicamide, which is ordinarily used to dilate the pupils in a routine eye exam (Martindale, 2002; Wurtman, 1985). But for the most part, diagnosis has been a process of elimination. Microscopic views, for instance, have revealed a loss of nerve cells in certain regions of the brain, such as the hippocampus, a center for memory, and the cerebral cortex, which is involved in reasoning, memory, language and other important thought processes (St. George-Hyslop, 2000).

The other more directly observable hallmarks of AD are clusters of proteins that accumulate in the brain. These accumulations usually occur in two forms: those found inside nerve cells and those found between cells. The clusters in the interior are called neurofibrillary tangles (NFTs) and they resemble pairs of threads wound around each other in a form of a helix. Biochemical analyses showed that these tangles consist of a protein called Tau (Anderton et al., 2001). Tau is significant because it binds to a protein named tubulin, which in turn forms structures known as microtubules (microtubules are crucially important in the structural framework of living cells). Like the girders and pillars of buildings, they run through cells, thereby imparting support and shape. Microtubules also provide routes along which nutrients, other molecules and cellular components such as vesicles and mitochondria move through cells (Martindale, 2002; St. George-Hyslop, 2000).

Unlike NFTs, deposits of amyloid protein gather in the spaces between nerve cells. The nearby neurons often look swollen and deformed, and the clusters of protein — sometimes called senile or amyloid plaques — are usually accompanied by reactive inflammatory cells called microglia, which are part of the brain’s natural immune system that help degrade and remove damaged neurons or perhaps the plaques themselves (Haddad, 2002e; Martindale, 2002). It is unclear, however, whether the neurons in or near these plaques function normally, because the density of plaques is only weakly correlated with the severity of dementia. Further, such plaques are present in most elderly people. Nevertheless, their extensive presence in the hippocampus and the cerebral cortex is specific to AD and they appear long before NFTs do.

Intensive efforts to isolate the ingredients of these plaques culminated with the discovery that a principal component
was a peptide made up of 40–42 amino acids (St. George-Hyslop, 2000). The sequencing of the gene for the longer protein from which this peptide originates quickly followed this identification of what is now termed the β-amyloid peptide: the β-amyloid precursor protein (β-APP). Although the precise biological role of normal β-APP molecules remains obscure, it is currently known that many kinds of cells and tissues produce β-APP and that it can be between 695 and 770 amino acids long (Martindale, 2002). The protein runs through the outer cell membrane, with a short piece jutting into the cell and a longer piece sticking into the extracellular space. The β-amyloid peptide, for its part, is snipped out of the section of β-APP that spans the cell membrane. β-APP is cut in one of two ways: in one process, the protein is first cleaved by an enzyme called α-secretase; it is then cut by another putative enzyme, γ-secretase. Together these cuts produce a harmless peptide fragment called p3 (St. George-Hyslop, 2000). The second way in which β-APP is cleaved is another two-step process, one that is not always so harmless. First, an enzyme called β-secretase clips the protein; one of the resulting pieces, called C99-β-APP fragment, is then snipped by γ-secretase and the β-amyloid peptide is born.

At the molecular level, the biochemical, molecular, genetic, epidemiological and clinical discoveries have significantly advanced our understanding of the mechanisms underlying the evolution of AD and made it increasingly likely that, in the years to come, useful therapeutic treatments will be generated/proposed. Some of these will probably come from the recent insights into the mis-processing or phosphorylation of Tau, a key mechanism in the regulation of Alzheimer’s (St. George-Hyslop, 2000). Indeed, the insights into β-APP and β-amyloid peptide are already fueling treatment research. For instance, some investigators are designing compounds that will block the ability of either β- or γ-secretase enzymes to cut β-APP, thus preventing the creation of the damaging β-amyloid peptide. Others are seeking to alleviate the peptide’s effects once it has been created. Clinical trials are under way to investigate whether antioxidants, such as vitamin E, or non-steroidal anti-inflammatory drugs (NSAIDS), such as ibuprofen, could alleviate some of the toxic effects of β-amyloid (Kanaan et al., 1997; St. George-Hyslop, 2000).

Since phosphorylation/dephosphorylation mechanisms are crucial in the regulation of Tau and β-APP (Hwang et al., 2004), a superfamily of mitogen-activated protein kinases (MAPKs) has recently emerged as key regulators of the formation of plagues, eventually leading to dementia and AD (Wang et al., 2004) (Fig. 1). To this end, this review will focus on the mechanisms mediated by MAPKs, which may modulate AD-related proteins and their functions and thereby can potentially determine the development of NFTs and, perhaps, the evolution of AD.

2. MAPK signaling modules and pathways: a network overview

2.1. MAPK signaling pathways as viewed through their identification and bifurcations

Signal transduction at the cellular level refers to the movement of signals (messages) from outside (extracellular) the cell to inside (intracellular) (Chattopadhyay and Brown,
The movement of signals can be simple, like that associated with receptor molecules of the acetylcholine class receptors that constitute channels which, upon ligand interaction, allow signals to be passed in the form of small ion movement, either into or out of the cell (Chattopadhyay and Brown, 2000; Dhanasekaran and Dermott, 1996; Guha and Mackman, 2001; Haddad, 2001, 2002b; Haddad et al., 2003; Pursiheimo et al., 2002; Sontag, 2001). These ion movements result in changes in the electrical potential of the cells that, in turn, propagates the signal spatially along the cell. More complex signal transduction, furthermore, involves the coupling of ligand-receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and/or serine/threonine kinases (Haddad, 2001, 2002b; Haddad et al., 2003; Holmes-McNary, 2002; Levin, 2002; Lowes et al., 2002). The phosphorylation of proteins may change enzyme activities and protein conformations. The eventual outcome is an alteration in cellular activity and changes in the programming of genes expressed within the responding cells. Phosphorylation and dephosphorylation mechanisms schematically involved in the regulation of gene transcription are shown in Fig. 1.

MAPKs were identified by virtue of their activation in response to growth factor stimulation of cells in culture, hence the name mitogen-activated protein kinases (Fig. 2) (Avruch et al., 2001; Cano and Mahadevan, 1995; Chakraborty, 2001; Haddad, 2002a; Haddad et al., 2003; Kennedy et al., 1999; Lee et al., 2000; Mordret, 1993; Pierce et al., 2001). MAPKs are also called extracellular receptor kinases (ERKs) for extracellular-signal regulated kinases. On the basis of in vitro substrates, the MAPKs have been variously called microtubule associated protein-2 kinase (MAP-2 kinase), myelin basic protein kinase (MBP kinase), ribosomal S6 protein kinase (RSK-kinase); i.e., a kinase that phosphorylates a kinase) and epidermal growth factor (EGF) receptor threonine kinase (ERT kinase) (Avruch et al., 2001; Cano and Mahadevan, 1995; Chakraborty, 2001; Haddad, 2001; Haddad et al., 2003; Kennedy et al., 1999; Lee et al., 2000; Pierce et al., 2001). All of these

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**Fig. 2.** The modules and various components of the MAPK signaling pathways. The cellular response to growth factors, inflammatory cytokines and other mitogens is often mediated by receptors that either are G-protein-linked or are intrinsic protein tyrosine kinases. The binding of the ligand to receptor tyrosine kinases induces dimerization and auto-phosphorylation (activation) of the kinase. The activated tyrosine kinase binds to and phosphorylates an adaptor protein, such as Grb2, that, in turn, activates a guanine nucleotide exchange factor, such as mSOS, that, in turn, activates a small GTP-binding protein, such as Ras or Rac. The GTP-binding proteins then transmit the signal to one of several cascades of protein Ser/Thr kinases that utilize the sequential phosphorylation of kinases to transmit and amplify the signal. These kinase cascades are collectively known as mitogen-activated protein kinase (MAPK) signaling cascades. The best studied of these kinase cascades is the MAPK^ERK^ (MAPK^p44/p42_) signaling cascade. Downstream targets of MAPK^ERK_ include p90rsk (p90 ribosomal S6 protein kinase) and the Elk-1 and Stat3 transcription factors. The Jun kinase (MAPK^JNK/SAPK_) and MAPK^p38_ kinase (HOG) pathways are stress-activated MAP kinase cascades. The MAPK^JNK_ cascade is activated by inflammatory cytokines as well as by heat shock and UV irradiation. Downstream targets of MAPK^JNK_ include the transcription factors c-Jun and ATF-2. The MAPK^p38_ pathway is activated by bacterial endotoxins, inflammatory cytokines and osmotic stress. Downstream targets of MAPK^p38_ include the transcription factors ATF-2, Max and CREB. MAPK^p38_ is also involved in the phosphorylation and activation of heat shock proteins.
proteins have similar biochemical properties, immunocross-reactivities, amino acid sequence and ability to in vitro phosphorylate similar substrates.

Maximal MAP kinase activity requires that both tyrosine and threonine residues are phosphorylated. This indicates that MAP kinases act as switch kinase that transmits information of increased intracellular tyrosine phosphorylation to that of serine/threonine phosphorylation (Guan, 1994; Had-
dad, 2001, 2002d; Ono and Han, 2000; Sugden and Clerk, 1997). Although MAP kinase activation was first observed in response to activation of EGF, platelet-derived growth factor (PDGF), nerve growth factor (NGF) and insulin and insulin-like receptors, other cellular stimuli such as T-cell activation (which signals through the Lck tyrosine kinase), phorbol esters (that function through activation of protein insu-
lin-like receptors, other cellular stimuli such as T-cell

The best-characterized vertebrate MAPKs fall into three major subgroups (Fig. 2). The first subgroup includes the founding members of the MAPK family, extracellular signal-regulated kinase-1 (ERK1; MAPK\textsuperscript{ERK1/p44}) and ERK2 (MAPK\textsuperscript{ERK2/p42}), and their closest relatives (Belcheva and Coscia, 2002; Daulhac et al., 1997; Denhardt, 1996; Frye, 1992; Howe et al., 2002; Peyssonnaux and Eychene, 2001; Zhang et al., 2000). This subgroup is often referred to as ERKs, although some ERK proteins are not in fact members of this subgroup family.

The second subgroup is the Jun N-terminal kinases (JNKs), so called because they can activate the Jun tran-
scription factor by phosphorylating two residues near its N-
terminus (Barr and Bogoyevitch, 2001; Davis, 2000; Dick-
ens et al., 1997; Dong et al., 2001; Fleming et al., 2000; Harper and LoGrasso, 2001; Ip and Davis, 1998; Leppa and Bohmann, 1999; Mielke and Herdegen, 2000; Noselli, 1998; Noselli and Agnes, 1999; Okazawa and Estus, 2002; Rincon et al., 2000; Weston and Davis, 2002; Yao et al., 1997).

The third subgroup is the p38 MAPKs, so named because of the molecular weight (38 kDa) of the first representative of the subgroup to be discovered (Bulavin et al., 2002; English et al., 1999; Haddad, 2001; Ichijo, 1999; Lee and Young, 1996; Lopez-Illasaca, 1998; Obata et al., 2000; Rincon, 2001). Members of both the MAPK\textsuperscript{JNK} and MAPK\textsuperscript{p38} pathways are also classified as stress-activated protein kinases (SAPKs), because they are activated in response to osmotic shock, UV irradiation, inflammatory cytokines and other stressful conditions (Haddad et al., 2003).

In all three subgroups, a large number of MAKKKs feed into the activation of a smaller number of MAPKKs and MAPKs. The diversity of the MAPKKKs thus allows a wide variety of upstream receptors to couple to MAPK cascades (Fig. 2) (Haddad, 2002a, 2002f; Haddad and Land, 2002; Lewis et al., 1998; Marshall, 1994; Pearson et al., 2001). MAPKs and their corresponding substrates are given in Table 1.

2.2. MAPK signaling as viewed through receptor and non-receptor coupled cofactors

The MAPKs are a grouping of closely related families of Ser/Thr kinases involved in regulating growth, differen-
Table 1
MAPKs and downstream transcription factors and substrates

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPK\textsuperscript{ERK}</td>
<td>Elk-1; SAP-1; Mnk-1/2; MAPKAP-K1/p90\textsuperscript{Raf}; MSK-1</td>
</tr>
<tr>
<td>MAPK\textsuperscript{JNK/SAPK}</td>
<td>c-Jun; ATF-2; Elk-1</td>
</tr>
<tr>
<td>MAPK\textsuperscript{p38}</td>
<td>ATF-2; Elk-1; SAP-1; CHOP; MEF2C; MAPKAP-K2/K3; Mnk-1/2; MSK-1; PRAK</td>
</tr>
<tr>
<td>MAPKAP-K1/p90\textsuperscript{Raf}</td>
<td>c-Fos; SRF</td>
</tr>
<tr>
<td>MSK-1</td>
<td>CREB; Histone H3 and HMG-14</td>
</tr>
</tbody>
</table>

ATF: activating transcription factor; CHOP: C/EBP homologous protein; CREB: cAMP response element binding protein; HMG-14: high mobility group-14; MAPK\textsuperscript{ERK}: mitogen-activated protein kinase/extracellular signal-regulated kinase; MAPK\textsuperscript{JNK/SAPK}: MAPK-activated protein kinase; MEF2C: myocyte enhancer factor 2C; Mnk: MAPK interacting protein kinase; MSK: mitogen- and stress-activated protein kinase; PRAK: p38-related/activated protein kinase; Rsk: ribosomal S6 kinase; SAP-1: SRE accessory protein-1; SRF: serum response factor.

3. Alzheimer’s disease: an overwhelming etiological condition or a maneuverable syndrome?

AD is the most common cause of dementia in older people (Li et al., 2001; Marx, 2001). Simply put, it’s a medical condition that disrupts the way the brain works. AD most notably affects the parts of the brain that control thought, memory and language (Martindale, 2002). Although the risk of getting the disease increases with age, it is not a normal part of aging (St. George-Hyslop, 2000). AD is characterized by adult-onset slowly progressive dementia associated with diffuse cerebral atrophy on neuro-imaging studies. AD is named after Dr. Alois Alzheimer, a German psychiatrist (Wurtman, 1985).

In 1906, Dr. Alzheimer described changes in the brain tissue of a woman who had died of an unusual mental illness. He found abnormal deposits (called senile or neuritic plaques) and tangled bundles of nerve fibers (called NFTs). These plaques and tangles in the brain have come to be characteristic brain changes due to AD. The diagnosis of AD is based on the histological findings of β-amyloid plaques and intra-neuronal neurofibrillary tangles. A significant association with the e4 allele of apo-lipoprotein E supports the diagnosis of AD in patients with dementia and increases the risk that asymptomatic individuals will eventually...
develop AD. ApoE genotyping, however, is neither fully specific nor sensitive.

Early and careful evaluation is important because many conditions can cause dementia, some of which are treatable or ‘reversible’. Potentially reversible conditions include depression, adverse drug reactions, metabolic changes, and nutritional deficiencies (St. George-Hyslop, 2000). A comprehensive patient evaluation includes a complete health history, physical examination, neurological and mental status assessments, and other tests including analysis of blood and urine, electrocardiogram, and an imaging exam, such as CT or MRI. While this type of evaluation may provide a diagnosis of possible or probable AD, confirmation requires examination of brain tissue at autopsy.

Although no cure for AD is yet available, medical and social management of the disease can ease the burdens on the patient, and his or her caregiver and family (St. George-Hyslop, 2000). To date there are four FDA-approved drugs for the treatment of AD — tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Reminyl®) — and several others in clinical trials. In addition to treating the symptoms, advising the patient and his or her caregiver to initiate health care directives and decisions while the patient still has the capacity to do so can ease the burden for the family as the disease progresses (Beardsley, 1995; St. George-Hyslop, 2000; Martindale, 2002).

4. The role of MAPKs in Alzheimer’s: mechanisms and possible therapeutic targets

4.1. Paired helical filaments and Tau phosphorylation

Paired helical filaments (PHFs) are a characteristic pathological feature of AD; their principal component is the microtubule-associated protein Tau (Fig. 4). The Tau in PHFs (PHF-Tau) in AD is hyper-phosphorylated, but the cellular mechanisms responsible for this hyper-phosphorylation are still being elucidated (Ganju et al., 1998; St. George-Hyslop, 2000; Zhao et al., 2003). MAPKs are a family of Ser/Thr kinases that link cell surface signals to changes in enzyme activity and gene expression (Shanavas and Papasozomenos, 2000; Haddad, 2002d; Haddad et al., 2003).

Moreover, MAPKs have been shown to phosphorylate Tau in vitro at Ser/Thr proline sites, thereby generating a multiply phosphorylated Tau protein that is similar to the hyper-phosphorylated Tau found in AD neurofibrillary tangles (NFT) (Dalrymple, 2002; Johnson and Bailey, 2003; St. J.J. Haddad / Progress in Neurobiology 73 (2004) 359–377

Fig. 3. An overview of the complex network of MAPK signaling pathways and their interactions and bifurcations. The sign denotes inhibition; the solid arrows indicate activation (stimulation); P, phosphorylation.
4.2. Tau phosphorylation and MAPK inhibition

Mobility shift, phospho-epitope analysis and direct measurement of kinase activity indicated that the MEK-1 inhibitor (PD-098059) dose-dependently blocked basal and OKA-induced MAPK activation (Ekinci and Shea, 1999; Ho et al., 1997; Rapoport and Ferreira, 1999, 2000; Schneider et al., 1999). Despite a block of MAPK activation by this inhibitor, robust Tau hyper-phosphorylation was observed in response to OKA. In addition, activation of MAPK by phorbol 12-myristate 13-acetate (PMA) did not result in Tau phosphorylation, indicating that in primary cultures of cortical neurons elevated MAPK activity was not sufficient to induce Tau hyper-phosphorylation.

Similarly, using immuno-histochemistry, Yamaguchi et al. (1996) examined the localization of four types of proline-directed kinases in the brains of control rats and in the brains of non-demented aged human subjects, subjects with AD and those with Down’s syndrome. The four kinases were: cyclin-dependent kinase (cdk)-5, a component of Tau protein kinase (TPK)-II, TPK-I/GSK-3β, GSK-3α and MAPK-ERK2. Antiserum for cdk-5 showed the most preferential and consistent labeling of intra-neuronal NFT. Antiserum for TPK I/GSK-3β also labeled intra-neuronal NFT. Double immuno-labeling for TPK I/GSK-3β and Tau-1 showed that TPK I/GSK-3β was closely associated with NFT. Antiserum for GSK-3α labeled neurons weakly and the intensity of labeling did not differ between neurons with and without NFT. In addition, antiserum for MAPK labeled neurons in superficial cortical layers, but NFT appeared in both superficial and deep cortical layers (Reynolds et al., 2000; Yamaguchi et al. 1996). These findings suggested that cdk-5 and TPK I/GSK-3β are the critically important kinases for the generation in vivo of hyper-phosphorylated Tau, the main component of the paired helical filaments in NFT.

In concert with these observations, the novel brain protein kinase PK-40 was characterized by its ability to phosphor-
ylate Lys-Ser-Pro sites in neuro-filament and Tau proteins (Mandelkow et al., 1993). PK-40 is recognized as a member of the family of MAPK\(^{ERK2}\) by its reactivity with MAPK\(^{ERK1}\), specific antibodies (PK-40\(^{ERK}\)). Particularly, protein sequence analysis suggested that PK-40 is a form of MAPK\(^{ERK2}\). Bovine Tau or recombinant human Tau proteins can be hyper-phosphorylated by PK-40\(^{ERK}\) to produce the electrophoretic mobility shifts and certain immunoreactional properties characteristic of PHF-Tau isolated from AD brain tissue (Trojanowski et al., 1993).

On the mechanisms related to PK-40\(^{ERK}\), a Ca\(^{2+}\)-dependent protein kinase A (PKA)-mediated pathway is involved. It was observed that PKA phosphorylates Tau to a lesser extent; however, the product is not like the hyper-phosphorylated Tau of AD in several important respects. Subsequently, it was reported that in vitro PK-40\(^{ERK}\) further phosphorylates Tau that was previously saturated by PKA, provided that the concentrations of free un-complexed ATP are low (Blanchard et al., 1994; Raghunandan and Ingram, 1995). Interestingly, the actions of different kinases on Tau are not independent, but may depend on the order in which they work on Tau; i.e., prior phosphorylation by PKA partially inhibits the action of PK-40\(^{ERK}\). Furthermore, a subpopulation of MAPK\(^{ERK2}\) species in soluble brain fractions can be efficiently phosphorylated and activated in cell-free systems, simply by adding Mg\(^{2+}\)-ATP. Two phosphorylated species of PK-40\(^{ERK2}\) are formed in this process, which have reduced gel mobility, very much like the MAPK\(^{ERK2}\) form obtained in cell culture by stimulation with growth factors (Roder et al., 1993, 1995). One of these low-mobility forms cannot be inactivated with PP-2A or with tyrosine phosphatases; the second form can be slowly inactivated by PP-2A. In this case two Ser/Thr phosphorylates are removed at different rates during inactivation: One phosphate is very quickly removed to result in the formation of a high-mobility 39-kDa MAPK\(^{ERK2}\) species without consequence for activity; the other, slowly removed Ser/Thr phosphate controls the activity but has no effect on the gel mobility of MAPK\(^{ERK2}\). These results unequivocally showed that forms of MAPK\(^{ERK2}\) exist with properties different from the previously characterized MAPK\(^{ERK2}\) (MAPK\(^{p2}\)) from stimulated cell cultures. The active MAPK\(^{ERK2}\) forms produced in the presence of Mg\(^{2+}\)-ATP alone could provide an explanation for the existence of constitutive MAPK\(^{ERK2}\)-like NFT phosphorylation in vivo. Excessive formation of an MAPK\(^{ERK2}\) species resistant to inactivation by PP-2A might be relevant to the persistent pathological Tau hyper-phosphorylation in AD. It was subsequently postulated that PK-40\(^{ERK}\) might play a crucial role in the etiology of this disease.

Furthermore, incubation of rat, human and rhesus monkey temporal neo-cortex slices with the phosphatase inhibitor OKA induced epitopes of Tau similar to those found in PHFs. OKA induced variant forms of Tau at 60–68 kDa, which were recognized by the monoclonal antibodies Alz-50 (in humans only) and 5E2 and two polyclonal anti-peptide antisera, OK-1 and OK-2 (Garver et al., 1995).

The phosphorylation-sensitive monoclonal antibody Tau-1 failed to recognize the slowest mobility forms of Tau after okadaic acid treatment. Moreover, FK-520 (1–10 \(\mu\)M), a potent inhibitor of calcineurin activity, was tested in brain slices and found not to alter Tau mobility. However, combinations of FK-520 and OKA caused Tau mobility shifts similar to those seen after OKA treatment; similar results were seen using the calcineurin-selective inhibitor cyperme-thrin (Garver et al., 1995). Treatment of human slices with OKA decreased both PP-2A and calcineurin activity; FK-520 inhibited only PP-2B activity. A proposed Tau-directed kinase, 42-kDa MAPK\(^{ERK2}\), was activated by OKA but not FK-520. Of note, NGF activated MAPK\(^{ERK2}\), particularly when used in combination with OKA; changes in Tau mobility were seen when this kinase was activated. Forskolin, in addition, antagonized the effects of NGF on both MAPK\(^{ERK2}\) activity and Tau phosphorylation and forskolin alone had little effect on PHF-like Tau formation induced by phosphatase inhibitors, outlining complex interactions between Tau-directed protein kinases and protein phosphatases and suggesting potential sites for therapeutic targeting.

### 4.3 Tau structural analysis and MAPK-related protein phosphorylation

The interactions of Tau protein with microtubules could be visualized from two points of view, phosphorylation and domain structure (Anderton et al., 2001; Angulo et al., 2003; Floyd, 1999; James et al., 1996; Jenkins et al., 2000; Mandelkow et al., 1995; Stein and Johnson, 2003). Tau can be phosphorylated at many sites and by several kinases, notably by proline-directed kinases (MAPK, GSK-3, cdk-5), which generate Alzheimer-like antibody epitopes. Other kinases phosphorylate Ser 262, a site that has a particularly pronounced influence on the affinity of Tau for microtubules (Fig. 4). All of these sites can be cleared by phosphatases PP-2A and CAL. The site Ser-262 lies within the repeat domain structure (Anderton et al., 2001; Angulo et al., 2003; Floyd, 1999; James et al., 1996; Jenkins et al., 2000; Mandelkow et al., 1995; Stein and Johnson, 2003). Tau can be phosphorylated at many sites and by several kinases, notably by proline-directed kinases (MAPK, GSK-3, cdk-5), which generate Alzheimer-like antibody epitopes. Other kinases phosphorylate Ser 262, a site that has a particularly pronounced influence on the affinity of Tau for microtubules (Fig. 4).

All of these sites can be cleared by phosphatases PP-2A and CAL. The site Ser-262 lies within the repeat domain of Tau. However, when probing the domains of Tau for their effects on microtubule binding, nucleation, assembly, or bundling, the repeat domain has only a weak influence. Whereas the repeat domain of Tau binds to microtubules with low affinity, repeat-less Tau binds strongly yet unproductively in terms of microtubule assembly.

Productive binding of Tau to microtubules depends on the combination of (some) repeats with the flanking regions, as if the flanking regions acted as "jaws" for the proper positioning of Tau on the microtubule surface. Analysis of phosphorylation of Tau is often performed using phosphorylation-sensitive monoclonal antibodies thought to report the presence or absence of one or two specific phosphorylations (cognate sites). Using several such antibodies, it was found that a much more complicated relationship existed between phosphorylation at specific sites, as monitored by two-dimensional phospho-peptide mapping, and antibody recognition of these sites (Roder et al., 1997). Multiple phosphorylation of Tau in several stages by PK-40, for
instance, suggested that phosphorylation at cognate sites is sometimes necessary, but not sufficient, to induce a change of antibody reactivity and in some cases is not even necessary in the background of multiple phosphorylation at other sites. No single phosphorylation site was found to be responsible for any level of gel mobility shift associated with phosphorylation. Moreover, Tau acquired its maximal gel mobility retardation and final immunocytochemical profile at sub-stoichiometric phosphorylation of most sites. This suggested that many alternate phosphorylation patterns could produce the same conformational and immunocytochemical presentation on SDS-PAGE. Although PK-40ERK2 prefers to produce the same conformational and immunochemical sub-stoichiometric phosphorylation of most sites. This suggested that many alternate phosphorylation patterns could produce the same conformational and immunocytochemical presentation on SDS-PAGE. Although PK-40ERK2 prefers some phosphorylation sites, most notably Ser-235, followed by Ser-199 or Ser-202 and Thr-205, the phosphorylation of multiple Ser/Thr-Pro sites is not highly sequential (Fig. 4). Ser-396 is one of the least preferred sites and seems to require prior phosphorylation at Ser-404.

Moreover, MAPK immunoreactivity and in situ hybridization patterns of the two major genes that comprise MAPK activity, MAPK$^{ERK1}$ and MAPK$^{ERK2}$, were reported in the human hippocampal formation (Hyman et al., 1994). The goal was to determine whether the pattern of MAPK$^{ERK}$ expression was consistent with the hypothesis that MAPKs directly contribute to NFT formation. MAPK$^{ERK1}$ mRNA was detected in small amounts and confined primarily to dentate gyrus granule cells (Hyman et al., 1994). MAPK$^{ERK2}$ mRNA, by contrast, gave a much stronger hybridization signal and was present in dentate gyrus granule cells and pyramidal cells throughout all hippocampal sub-fields and adjacent temporal neo-cortex. Quantitative measures of MAPK$^{ERK2}$ mRNA revealed that neuro-filament bearing neurons contained approximately 15% less MAPK$^{ERK2}$ mRNA than nearest normal neighbors (Hyman et al., 1994). NFT-bearing neurons contained approximately 25% less poly-A mRNA, suggesting a relative preservation of MAPK$^{ERK2}$ mRNA even in metabolically compromised cells.

MAPK immunoreactivity (which represented both MAPK$^{ERK1}$ and MAPK$^{ERK2}$) was reported in neuronal soma, dendrites, axons and in reactive astrocytes (Hyman et al., 1994). In AD, neurons that contain NFTs are also MAPK immunoreactive, but neurons that contain the highest amounts of MAPK immunoreactivity are not necessarily vulnerable for NFTs. MAPK immunoreactivity was present in the same neurons as NFTs and in the same subcellular compartments as Tau, supporting a role for MAPKs in Tau phosphorylation in AD. However, the presence of MAPK$^{ERK}$ immunoreactivity was not sufficient to predispose neurons to NFT formation (Korneyev, 1998).

4.4. Tau/β-APP-mediated phosphorylation mechanisms and MAPK regulation

Biological effects related to cell growth, as well as a role in the pathogenesis of AD, have been ascribed to the β-amyloid precursor protein (β-APP). The aberrant expression or processing of β-APP is the only known genetic basis for presenile familial AD, and the molecular connection between β-APP and Tau has always been perplexing (Grant et al., 1999; Jones et al., 1999). Transgenic experiments, for instance, have revealed that long-term memory is dependent on cAMP-response element binding protein, CREB (Sato et al., 1997). CREB phosphorylation at Ser-133 is essential for its transcriptional activity. In this respect, it was demonstrated that β-APP induced CREB phosphorylation at Ser-133 in rat pheochromocytoma PC-12 cells. β-APP, furthermore, induced the phosphorylation of MAPK$^{ERK1}$ and MAPK$^{ERK2}$ at Tyr-204, and PD-98059, a MEK1 inhibitor, inhibited β-APP-induced CREB phosphorylation at serine-133 (Sato et al., 1997). It was concluded that elevated β-APP level induces CREB phosphorylation at Ser-133 via MAPK$^{ERK1,2}$-dependent pathway.

In addition, it was independently reported that the secreted form of β-APP potently stimulates MAPKs. For instance, brief exposure of PC-12 pheochromocytoma cells to β-APP secreted by transfected Chinese hamster ovary cells stimulated the 43-kDa form of MAPK (Greenberg et al., 1994). Induction of a dominant inhibitory form of Ras in a PC-12-derived cell line prevented the stimulation of MAPK by secreted β-APP, demonstrating the dependence of the effect upon p21-Ras. Moreover, it was found that enhancement in Tau phosphorylation was associated with the β-APP-induced MAPK stimulation (Greenberg et al., 1994). In the Ras dominant inhibitory cell line, β-APP failed to enhance phosphorylation of Tau, thereby providing a link between secreted β-APP and the phosphorylation state of Tau. Furthermore, β-APP has been shown to serve as a G$i$-coupled receptor in cell-free systems (Okamoto et al., 1995). It was shown that stimulation of β-APP by anti-APP antibody as well as by a mutation found in familial AD resulted in the activation of a specific set of MAPK in multiple vertebrate cells (Murayama et al., 1996), thereby concluding that β-APP might act as a cell surface receptor of biological relevance that turns on specific Ser/Thr kinases, and suggested that the signaling function of β-APP is a potential target of familial AD mutations.

On the mechanisms reported, β-APP, which accumulates extracellularly in AD brain, induces Ca$^{2+}$ influx in culture via the $i$-gated voltage-sensitive Ca$^{2+}$ channel. Since this channel is normally activated by PKA-mediated phosphorylation, kinase activities recruited following β-APP treatment of cortical neurons and SH-SY-5Y neuroblastoma were examined. β-APP increased channel phosphorylation; this increase was unaffected by the PKA inhibitor, H89, but was reduced by PD-98059 (Abé and Saito, 2000; Ekinci et al., 1999; Guise et al., 2001).

Pharmacological and antisense oligonucleotide-mediated reduction of MAPK activity also reduced β-APP-induced accumulation of Ca$^{2+}$, reactive oxygen species (ROS), phospho-Tau immunoreactivity and apoptosis, indicating that MAPK mediates multiple aspects of β-APP-induced neuro-toxicity and suggesting that Ca$^{2+}$ influx initiates...
and GSK-3

extensive phosphorylation in vitro by several candidate
in AD, using nano-electrospray mass spectrometry, an
addition, neuro-degeneration in AD (Zhang and Jope, 1999). In

Another mechanism known involves phosphorylidyinositol
3-kinase (PI3-K) (Shaw et al., 2001). Because of the
physiological role of PI3-K in the translocation of glucose
transporter-containing vesicles, it was speculated that PI3-K
involvement in β-APP metabolism might act at the level of
vesicular trafficking (Solano et al., 2000). In accord with the
aforementioned observations, exposure of primary rat
microglia and human THP-1 monocytes to β-APP resulted in
the tyrosine kinase-dependent activation of two parallel
signal transduction cascades involving members of the
MAPK superfamily. β-APP stimulated the rapid, transient
activation of MAPK[^ERK1/2] in microglia and only MAPK[^ERK2]
in THP-1 monocytes (McDonald et al., 1998).

A second superfamily member, MAPK^[p38], was also
activated with similar kinetics. Scavenger receptor and
receptor for advanced glycated end products (RAGE)
ligands failed to activate MAPK[^ERK] and MAPK^[p38] in the
absence of significant increases in protein tyrosine
phosphorylation, demonstrating that scavenger receptors and
RAGE are not linked to these pathways (McDonald et al.,
1998; Sun et al., 2003). Importantly, MAPK[^JNK] SAPKs were
not significantly activated in response to β-APP. Moreover,
exposure of microglia and THP-1 monocytes to β-APP
resulted in the activation of RSK-1 and RSK-2 and phos-
phorylation of CREB at Ser-133, providing a mechanism for
β-APP-induced changes in gene expression (McDonald et al.,
1998).

In reinforcement of these reports, Shin et al. (1999)
investigated the role of presenilin-1 (PS-1) in the secretion of
α-secretase derived sAPP-α and associated intracellular
signaling pathways. Human embryonic kidney (HEK) 293
cells, in this respect, were transfected with exon-9 deletion
(ΔE9) mutant PS-1 cDNA in an edcyson-inducible system.
sAPP-α secretion was lower in the mutant PS-1 expressing
cells compared with non-expressing cells (Shin et al., 1999).

When activated by PDBu, secretion of sAPP-α and the level
of phosphorylated MAPK were enhanced in ΔE9 PS-1 un-
induced cells, but not in the mutant PS-1 induced cells. PD-
98059, in addition, blocked PDBu induced sAPP-α secretion
from ΔE9 PS-1 un-induced cells, but had no effect on the
mutant PS-1 induced cells, indicating that PS-1 mediates
PDBu-induced sAPP-α secretion and MAPK activation.

In contrast to the neutral role of MAPK[^JNK] and MAPK^[p38]
in AD, using nano-electrospray mass spectrometry, an
extensive phosphorylation in vitro by several candidate
Tau kinases, namely, MAPK[^JNK], MAPK^[p38], MAPK[^ERK2]
and GSK-3β was observed (between 10 and 15 sites were
identified for each kinase). The three MAPKs phosphory-
lated Ser-202 and Thr-205 but not detectably Ser-199,
whereas conversely GSK-3β phosphorylated Ser-199 but
not detectably Ser-202 or Thr-205 (Oth et al., 2003; Rey-
nolds et al., 2000; Troy et al., 2001). In addition, phos-
phorylated Ser-404 was found with all of these kinases
except MAPK[^JNK]. To recapitulate, the MAP kinases may
not be strictly proline specific: MAPK[^p38] phosphorylated
the non-proline sites Ser-185, Thr-245, Ser-305 and Ser-356,
whereas MAPK[^ERK2] was the strictest. Furthermore, all
of the sites detected except Thr-245 and Ser-305 are known
or suspected phosphorylation sites in paired helical filament-
Tau extracted from AD brains, suggesting that the three
MAPKs are importantly all strong candidates as Tau kinases
that may be involved in the pathogenic hyper-phosphoryla-
tion of Tau in AD (Reynolds et al., 2000; Zhu et al., 2000).
In addition, Thr-668 within the carboxy-terminus of the AD β-
APP is a known in vivo phosphorylation site. Phosphoryla-
tion of β-APP/Thr-668 is believed to regulate β-APP func-
tion and metabolism (Standen et al., 2001). Thr-668
precedes a proline, which suggests that it is targeted for
phosphorylation by proline-directed kinase(s). The ability
of four major neuronally active proline-directed kinases, cdk-5,
GSK-3β, MAPK[^ERK2] and MAPK[^JNK1] to phosphorylate β-
APP/Thr-668 indicated a robust phosphorylation of this site
both in vitro and in vivo (Fig. 4) (Matsuda et al., 2001; Troy
et al., 2001).

Another interesting mechanism reported for the action of
β-APP in AD indicated a major role for glutamate, the
principal excitatory neurotransmitter in the mammalian
brain. Several lines of evidence suggested that glutamatergic
hyp-o-activity exists in the AD brain, where it may contribute
to both brain amyloid burden and cognitive dysfunction
(Mills and Reiner, 1999). Although metabotropic glutamate
receptors have been shown to alter cleavage of β-APP, little
attention has been paid to the role of NMDA receptors in this
process. It was reported that the activation of NMDA
receptors in transiently transfected human embryonic kidney
293 cells increased the production of sAPP (Mills and
Reiner, 1999). Moreover, using both pharmacological and
gene transfer techniques, it was shown that this effect was
largely due to activation of the MAPK, specifically MAPK[^ERK].
These observations further our understanding of the
pathways that regulate APP cleavage and buttress the notion
that regulation of β-APP is critically dependent upon
MAPKs (Bi et al., 2000).

Conversely, β-APP has been reported to induce the
phosphorylation of MAPKs. For instance, using acute and
organotypic hippocampal slice preparations, Dineley et al.
(2001) demonstrated that β-APP peptide 1-42 (β-APP42)
couples to the MAPK cascade via α7 nicotinic acetylcholine
receptors (nAChR). In vivo elevation of β-APP, such as that
exhibited in animal models for AD, leads to the upregulation
of α7-nAChR protein. This upregulation occurred concomi-
tantly with the down-regulation of MAPK[^ERK2] in hippocampi
of aged animals (Dineley et al., 2001). The phosphorylation
state of a transcriptional mediator of long-term potentiation
and a downstream target of the MAPK<sup>ERK</sup> cascade, CREB protein, were affected also, supporting the model that derangement of hippocampus signal transduction cascades in AD arises as a consequence of increased β-APP burden and chronic activation of MAPK<sup>ERK</sup> in an α7-nAChR-dependent manner that eventually leads to the down-regulation of MAPK<sup>ERK</sup>2 and decreased phosphorylation of CREB protein. Potential pathways involving cell death-related cofactors and AD are schematized in Fig. 5.

4.5. Redox/oxidative mechanisms and MAPK-mediated Tau phosphorylation

Oxidative stress play a major role on the pathogenesis of AD (Chen et al., 2003; de la Monte et al., 2000; Floyd, 1999; Tamagno et al., 2003). For example, increased expression of heme oxygenase-1 (HO-1) is a common feature in a number of neuro-degenerative diseases, including AD (Floyd, 1999; Haddad, 2002d, 2003; Haddad and Land, 2000a, 2000b; Haddad et al., 2000; Takeda et al., 2000; Zhang and Jope, 1999). Interestingly, the spatial distribution of HO-1 expression in diseased brain is essentially identical to that of pathological expression of Tau.

The relationship between HO-1 and Tau was explored, using neuroblastoma cells stably transfected with sense and antisense HO-1 constructs, as well as with the vector alone. In transfected cells over-expressing HO-1, the activity of heme oxygenase was increased, and conversely, the level of Tau protein was dramatically decreased, when compared with antisense HO-1 or CEP transfected cells (Haddad, 2002c, 2002d; Takeda et al., 2000). The suppression of Tau protein expression was almost completely reversed by zinc-deuteroporphyrin, a specific inhibitor of HO activity. The activated forms of MAPK<sup>ERK</sup> were also decreased in cells over-expressing HO-1, although no changes in the expression of total MAPK<sup>ERK</sup><sub>1/2</sub> proteins were observed (Takeda et al., 2000).

These data are in agreement with the finding that the expression of Tau is regulated through signal cascades including the MAPK<sup>ERK</sup>, whose activities are modulated by oxidative stresses (Fig. 6). The expression of Tau and HO-1, then, may be regulated by oxidative stresses (Haddad, 2002b, 2002c) in a coordinated manner and play a pivotal role in the cytoprotection of neuronal cells (Pei et al., 2003).

4.6. Inflammatory mediators and MAPK-mediated Tau phosphorylation

Inflammatory mediators (Haddad, 2002d, 2002e) have been implicated in the pathophysiology of neuro-degenerative diseases, including AD (Paris et al., 2003; Xia and Hyman, 2002). For instance, the chemokine receptor CXCR3 and its ligand, IP-10, were detected in AD brains (Xia et al., 2000). CXCR3 was detected constitutively on neurons and neuronal processes in various cortical and subcortical regions; IP-10 was observed in a subpopulation of astrocytes in normal brain and was markedly elevated in astrocytes in AD brains. Many IP-10<sup>+</sup> astrocytes were also associated with senile plaques and had an apparently coordinated upregulation of MIP-1β (Xia et al., 2000). Moreover, it was shown that CXCR3 ligands, IP-10 and Mig, were able to activate MAPK<sup>ERK1/2</sup> pathway in mouse cortical neurons, suggesting a novel mechanism of neuronal-glial interaction.
Similarly, one of the CXCR2 ligands GRO-α/KC can be a potent trigger for the MAPK<sub>ERK1/2</sub> and PI-3 kinase pathways, as well as Tau hyper-phosphorylation in the mouse primary cortical neurons (Xia and Hyman, 2002). GRO-α immunoreactivity can be also detected in a subpopulation of neurons in normal and AD. Therefore, the CXCR2-ligand pair may have a potent pathophysiological role in neurodegenerative diseases.

In concert with this, reactive microglia have been suggested to play a role in AD process, and previous studies have shown that expression of CD45, a membrane-bound protein-tyrosine phosphatase (PTP), is elevated in microglia in AD brains (Tan et al., 2000). To investigate the possible role of CD45 in microglial responsiveness to β-APP, primary cultured microglia were co-treated with a tyrosine phosphatase inhibitor [potassium bisperoxo (1,10-phenanthroline) oxovanadate (phen), 5 micrometer] and freshly solubilized β-APP peptides (1000 nm). Data showed synergistic induction of microglial activation as evidenced by TNF-α production and nitric oxide (NO) release, both of which were dependent on the activation of MAPK<sub>ERK1/2</sub>. Furthermore, co-treatment with phen and β-APP peptides resulted in microglia-induced neuronal cell injury (Fig. 5). Stimulation of microglial CD45 by anti-CD45 antibody markedly inhibited these effects via inhibition of MAPK<sub>ERK1/2</sub>, suggesting that CD45 is a negative regulator of microglial activation (Tan et al., 2000). Accordingly, primary cultured microglia from CD45-deficient mice demonstrated hyper-responsiveness to β-APP, as evidenced by TNF-α release, NO production and neuronal injury after stimulation with β-APP peptides.

As a validation of these findings in vivo, brains from a transgenic mouse model of AD [transgenic Swedish APP-over-expressing (Tg APP(Sw)) mice] deficient for CD45 demonstrated markedly increased production of TNF-α, compared with Tg APP(Sw) mice (Chong et al., 2001; Smits et al., 2001; Tan et al., 2000; Tomidokoro et al., 2001; Del Villar and Miller, 2004). Taken together, these results suggested that therapeutic agents that stimulate the CD45/PTP signaling pathway might be effective in suppressing microglial activation associated with AD.

Moreover, a similar mechanism has been proposed for IL-1. For instance, activated MAPK<sub>p38</sub> and IL-1 have both been implicated in the hyper-phosphorylation of Tau (Sheng et al., 2001). This, together with findings showing that IL-1 activates MAPK<sub>p38</sub> in vitro and is markedly over-expressed in AD brain, suggested a role for IL-1-induced MAPK<sub>p38</sub> activation in the genesis of NFT in AD. Frequent co-localization of hyper-phosphorylated Tau protein (AT8 antibody) and activated MAPK<sub>p38</sub> in neurons and in dystrophic neurites in AD and frequent association of these structures with activated microglia over-expressing IL-1 were reported (Sheng et al., 2001). Tissue levels of IL-1 mRNA as well as of both phosphorylated and non-phosphorylated isoforms of Tau were elevated in those brains. Furthermore, significant correlations were found between the numbers of AT8- and MAPK<sub>p38</sub>-immunoreactive neurons, and between the numbers of activated microglia over-expressing IL-1 and the numbers of both AT8- and MAPK<sub>p38</sub>-immunoreactive neurons. In addition, rats bearing IL-1-impregnated pellets showed a six- to seven-fold increase in the levels of MAPK<sub>p38</sub> mRNA, compared with rats with vehicle-only pellets (Sheng et al., 2001). These results suggested that microglial activation and IL-1 overexpression are part of a feedback cascade in which MAPK<sub>p38</sub> overexpression and activation leads to Tau hyper-phosphorylation and NFT pathology in AD (Atzori et al., 2001; Daniels et al., 2001; Ferrer et al., 2001; Williamson et al., 2002; Hooze-
mans et al., 2004). More recently, IL-6 was also reported to induce Alzheimer-type phosphorylation of Tau protein by deregulating the cdk5/p35 pathway (Quintanilla et al., 2004).

5. Summary, conclusions and future prospects

There are multiple lines of evidence showing that oxidative stress (Haddad, 2004a, 2004b) and aberrant mitogenic signaling (Zhu et al., 2002) play an important role in the pathogenesis of AD (Fig. 6) (Haddad and Land, 2000a, 2000b; Haddad et al., 2000; Zhu et al., 2001a, 2001b). However, the chronological relationship between these and other events associated with disease pathogenesis is not strictly elucidated. Given the crucial role that MAPK pathways play in mitogenic signaling (MAPK\textsuperscript{ERK}) and cellular stress signaling (MAPK\textsuperscript{JNK/SAPK} and MAPK\textsuperscript{\textgamma\textalpha\textbeta}), the chronological and spatial relationship between activated MAPKs during disease progression is warranted (Savage et al., 2002; Taru et al., 2002). While all three kinases are activated in the same susceptible neurons in mild and severe cases, in non-demented cases with limited pathology, both MAPK\textsuperscript{ERK} and MAPK\textsuperscript{JNK} are activated but MAPK\textsuperscript{\textgamma\textalpha\textbeta} is not. However, in non-demented cases lacking any sign of pathology, either MAPK\textsuperscript{ERK} alone or MAPK\textsuperscript{JNK} alone can be activated, indicating that MAPK pathways are differentially activated during the course of AD and, by inference, suggesting that both oxidative stress/inflammatory and abnormalities in mitotic signaling can independently serve to initiate, but both are necessary to propagate, disease pathogenesis (Daniels et al., 2001; Koistinaho et al., 2002). The complex molecular interactions between MAPKs and proteins (plagues) associated with the evolution of AD form a cornerstone in the knowledge of a burgeoning field of neurodegenerative diseases and ageing. MAPKs, therefore, may constitute a neurogenetic, therapeutic target for the diagnosis of a preventative medical strategy for early detection, and possible treatment, of Alzheimer’s.

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References


