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ALZHEIMER S DISEASE AND BRAIN DEVELOPMENT: COMMON MOLECULAR PATHWAYS

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1. ABSTRACT

Research on the causes and treatments of Alzheimer's disease (AD) has led investigators down numerous avenues. Although many models have been proposed, no single model of AD satisfactorily accounts for all neuropathologic findings as well as the requirement of aging for disease onset. The mechanisms of disease progression are equally unclear. We hypothesize that alternative gene expression during AD plays a critical role in disease progression. Numerous developmentally regulated genes and cell cycle proteins have been shown to be re-expressed or activated during AD. These proteins include transcription factors, members of the cell cycle regulatory machinery, and programmed cell death genes. Such proteins play an important role during brain development and would likely exert powerful effects if re-expressed in the adult brain. We propose that the re-expression or activation of developmentally regulated genes define molecular mechanisms active both during brain development and in AD.

2. INTRODUCTION

Alzheimer's Disease (AD) has been the subject of intense investigation in recent years. Yet, in spite of many advances, the mechanisms leading to neurodegeneration remain elusive. As in other diseases of aging, it has proven to be a multifaceted process involving many gene products and cellular pathways only recently the subject of serious investigation. This review will examine the role of molecular mechanisms implicated in neural development that are re-expressed during neurodegeneration in AD.

AD is characterized by several neuropathologic features. These include beta-amyloid (A-beta) containing neuritic plaques, neurofibrillary tangles (NFTs), dystrophic neurites, and glial activation. Although the number of NFTs correlates best with disease progression and are used in staging AD progression (1), there is strong genetic evidence supporting a role for A-beta in disease progression (Reviewed in 2, 3). Yet, one must remember that aging is a prerequisite of AD, even in familial forms of AD. This suggests that AD is a disease that results from an accumulation of insults over a lifetime that can be accelerated by genetic defects in the amyloid precursor protein (APP), Presenilin 1, Presenilin 2 (PS1 and PS2), the presence of specific ApoE alleles, or additional currently unknown genes (2, 4, 5). Such insults have been reported to include accumulation of oxidative damage, mitochondrial defects, and activation of immunologic mechanisms (6-9).

In this review, we will discuss gene products that function during brain development and are re-expressed or re-activated in AD brain. Such proteins and the molecular pathways in which they function will hold important clues as to the regenerative and degenerative events that regulate neurodegeneration not only in AD but potentially in other neurologic diseases. A brief discussion of current models for AD will first be provided. Although neurotrophic factors, neurotransmitters and their respective receptors exhibit altered gene expression in AD, these will not be discussed in the present review. Instead, we will focus our discussion to transcription factors, cell cycle proteins and developmentally regulated genes and how these proteins may participate to control the formation and progression of AD.

2.1. The Amyloid Hypothesis

A-beta plaques consist of fibrils formed from a proteolytic product of the amyloid precursor protein (APP). APP has one transmembrane domain, a short cytoplasmic tail and a larger extracellular domain (For review see (10)). It is normally cleaved to form a 90 kD secreted protein that stimulates cell proliferation and mediate cell substratum adhesion or neurite outgrowth. In AD patients the APP protein is processed to a 40-42 amino acid fragment that includes part of the transmembrane and extracellular domains. It is this fragment that is found in A-beta containing plaques. Other studies also suggest A-beta plays an active role in AD neurodegeneration. Not only is A-beta localized to these pathologically affected regions of the AD brain, it has been demonstrated that aggregated A-beta is neurotoxic to cultured neurons (11-15) and upregulates a number of genes involved in apoptosis (Bax, Caspase 3, etc.) (16, 17). This is suggestive of a mechanism by which A-beta deposition may lead to AD.

The amyloid hypothesis is further supported by genetic studies that show a mutation on chromosome 21 in the APP gene which results in increased production the highly agregable A-beta1-42, develop premature AD (age of onset 40 - 65 years; For review see (2)). Finally, Down's syndrome patients which have trisomy of chromosome 21 also develop AD at an accelerated rate (40 years of age onset). Although there is much evidence to establish a role for A-beta in AD progression, current studies fail to explain how A-beta is produced, what is the connection with other existing pathology, and why it takes a lifetime to develop the disease.

2.2. The Presenilins, Support of the Amyloid Hypothesis?

Recent advances in understanding AD etiology have come from studying familial AD (FAD). Using affected families, two additional genetic defects have been identified on chromosomes 1 and 14. These genes, named presenilin 1 and 2 (PS1 and PS2), are highly homologous (67%) (For review see (2, 4, 5). The presenilins contain six to eight transmembrane domains and have been localized to the endoplasmic reticulum and golgi of neurons, suggesting a role in protein processing or trafficking (18, 19). Mutations in the presenilins may increase intracellular levels of calcium and increase oxidative stress, contributing to the neurodegenerative processes in AD (20). Both proteins are proteolytically cleaved between transmembrane domains 6 and 7 to form amino and carboxy terminal fragments which appear to exhibit different subcellular localization (21). For example, using antibodies generated against amino acids 331-360 (C-terminal PS1 fragment), it has been localized to amyloid containing plaques (22), but using an antibody to amino acids 263-280 (N-terminal PS1 fragment), PS1 has been localized to NFTs (23). However, the interpretation of these antibody results has become somewhat complicated by other disparate results. Using antibodies generated to different epitopes within either proteolytic fragment, different pathologic markers or even no lesions at all have been recognized as presenilin immunoreactive (24-26). Regardless of their subcellular localization, it is clear that PS1 and PS2 affect APP processing. Patients carrying mutations in PS1 or PS2 express higher levels of the amyloidgenic A-beta1-42 (27). Transgenic mice that express human mutant PS1 and humans carrying PS1 or PS2 mutations have increased levels of APP processed to A-beta1-42 (28). Similar results have been observed in cells transfected with mutant presenilins (29). These data suggest that PS1 and/or PS2 may regulate APP processing. This is further supported by recent reports of direct interaction between presenilins and APP demonstrated by co-immunoprecipitation and the yeast dihybrid screen (30-32). Overall, these data further support A-beta as a major factor in regulating FAD neurodegeneration. However, many questions remain, including the formation and significance of NFTs, and how disease is initiated in sporadic AD where the A-beta1-42 burden is significantly lower than in FAD.

2.3. Cytoskeletal Abnormalities

The other major neuropathologic feature of AD, NFTs, consist of paired helical filaments (PHF-tau) composed of polymerized tau protein (For review see (33-35). Tau plays an important role in microtubule organization, an activity that is regulated by phosphorylation on specific serine and threonine residues. PHF-tau is aberrantly phosphorylated at additional sites. Hyperphosphorylated tau is protease resistant and able to form PHF *in vitro*. Tau is detected in both NFTs and dystrophic neurites in AD brain. The quantitation of NFTs in specific brain regions is currently the best correlate to dementia. NFTs are present in early stages of disease within the entorhinal cortex and the CA1 region of the hippocampus, regions that show early and severe neuronal abnormalities. The presence of extracellular "ghost tangles" in later stages of AD suggests that NFT formation directly leads to neuronal cell loss (36). Thus, the priming event for AD may include activation of a kinase that inappropriately phosphorylates tau, or inactivation of a phosphatase. Although a variety of kinases have been found to phosphorylate tau *in vitro* (glycogen synthase kinase b, cyclin dependent kinase 5; reviewed in (37)), the promiscuity of kinases within *in vitro* assays precludes the confirmation of the kinase responsible for aberrant tau phosphorylation *in vivo*. Since the nature of tau phosphorylation is likely an early and necessary step, it suggests that some earlier event, such as activation of a signal transduction mechanism, exists to activate the tau kinase. Again, as with the amyloid hypothesis, we

are left not only with intriguing results and hypotheses regarding AD formation but equally important unanswered questions.

2.4. Alternate Pathways

Elucidating the causes of AD must include reconciliation of the two existing models, both with ample scientific evidence supporting their importance. Recently, other models of AD progression have gained validation that may begin to link these two seemingly disparate events. Mounting evidence suggests that early events leading to AD include accumulation of oxidative stress and glial activation. The role of oxidative damage in AD is supported by the presence of glycated proteins, lipid peroxidation, and the expression in AD brain of proteins that reduce oxidative damage (7, 38-43). This model of increased oxidative stress and protein glycation is interesting because it accounts for the prerequisite aging prior to disease onset. Interestingly, the receptor for advanced glycation end products (RAGE) appears to bind A-beta (44, 45), further integrating this model into the current paradigms.

The role of inflammation in AD has recently received considerable support. Numerous reports indicate that patients who take non-steroidal anti-inflammatory drugs (NSAIDs) have reduced risk of developing AD (46-49). There is also evidence for microglial and astrocytic activation surrounding neuritic plaques, along with elevated levels of cytokine activity (50). However, these results do not prove that inflammatory mechanisms directly cause neurodegeneration, as opposed to being a secondary event that stimulates a response to the primary neuronal injury. Thus, additional studies are required to demonstrate that molecular cascades initiated by factors released by activated glia play a role in AD formation and progression.

One common theme that emerges from each model is the reliance on a change in the molecular constituents present in the cells. Whether it is a kinase, a receptor, oxidative stress, or inflammatory cascade, there is a common thread of alternative gene expression and activity in AD. Many of the genes alternatively expressed in AD function during brain development. Further investigation of alternative gene expression in AD will yield pathways, both novel and pre-existing, that underlie the mechanisms of AD and may unite the current hypotheses into a cohesive model of disease formation and progression.

3. GENES ALTERNATIVELY EXPRESSED IN AD

3.1. Transcription Factors

Changes in gene expression are usually mediated by transcription factors. Several members of this class of proteins have been examined in AD and found to be expressed at increased levels. Among these are c-fos, c-jun, NF-kappa B, and Krox24 (51-55). These proteins regulate gene expression in a number of cellular pathways. The possible role of their re-expression in AD will be discussed in terms of their known function.

c-jun and c-fos were among the first transcription factors studied. The cellular form of fos, c-fos, was isolated through its identity with a viral oncoprotein and used to co-purify c-jun (56). Both induce tumorogenesis when overexpressed, suggesting that their target genes are involved in cellular proliferation. Later studies revealed that these proteins were not only involved in mitogenesis but in other processes as well, including programmed cell death or apoptosis (57-59). Their role in multiple cellular processes from apoptosis to development to proliferation is regulated at several levels including post-translational modification, homo- and heterodimer formation with each other and other members of this gene family (60-63). The various homo- and heterodimers have different transactivation capacities and it is likely that additional transcription factors play an important role in regulating overall activity.

Initial immunocytochemical reports indicated that c-jun is upregulated in the CA1 region of the hippocampus in AD (52, 53). However, a recent study has cast doubt on the past observations (55). Dragunow and colleagues demonstrated that the antibodies used in prior studies do not specifically recognize c-jun on a western blot from post-mortem hippocampus. Instead, they recognize several bands of apparent molecular weights distinct from the c-jun protein family. This raises questions as to the identity of the protein visualized in the AD brain by immunocytochemistry. In addition, this recent study demonstrated by in situ hybridization that the mRNA levels of c-fos and c-jun are not altered in AD brain. Additional studies using antibodies specific to c-jun and c-fos are required to clarify differences observed in the literature and to make direct comparisons to the in situ hybridization data. An intriguing observation is the upregulation of c-jun and its activating kinase, JNK, during neuronal regeneration (60, 61). One proposed model for AD states that neurodegeneration is a failed regenerative response. Such a model may explain the long term nature of the disease onset and progression and the re-expression of genes that function in neuronal regeneration.

Krox24 is a zinc finger containing transcription factor that induces apoptosis upon overexpression (64). This protein also participates in the persistence and stabilization of long term potentiation in animal models, indicating a role in memory (65, 66). Krox24 has been localized to A-beta plaques and NFTs in AD hippocampus by immunocytochemistry (55). It has also been demonstrated to exhibit increased gene expression in AD brain by in situ hybridization. This demonstration of increased mRNA provides strong evidence for transcriptional activity during AD progression. Although the role of Krox24 in apoptosis, memory, learning, and AD is unclear, identification of promoters with Krox24 binding elements will lead to

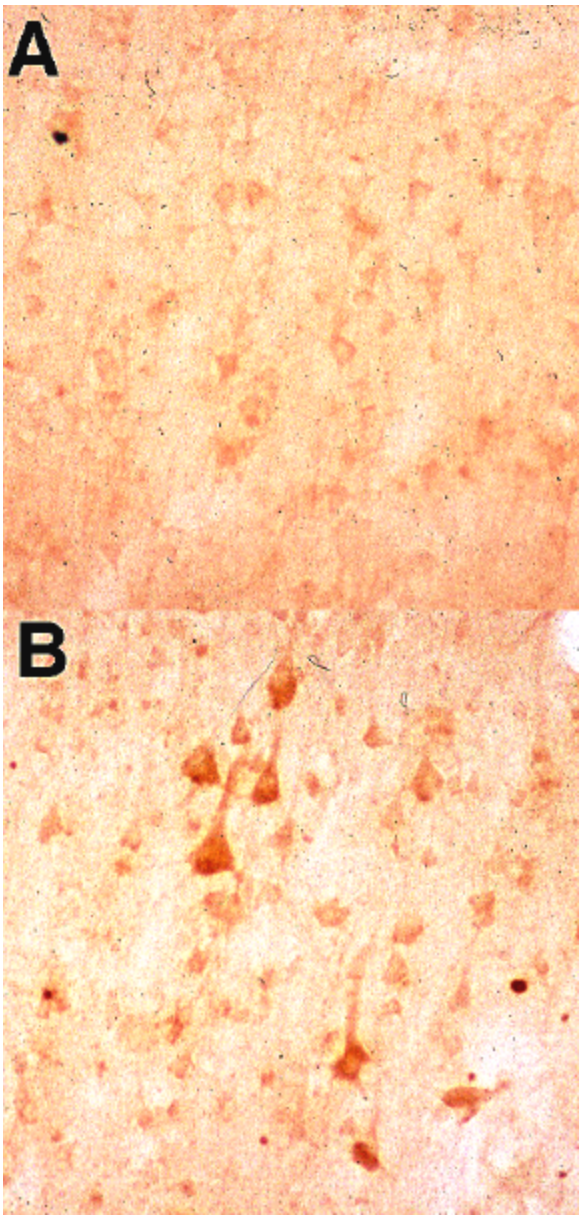

Figure 1. MAZi expression is increased in temporal cortex of Alzheimer's patients. Immunohistochemical staining of temporal cortex from A) control and B) Alzheimer's disease patients with the MAZi polyclonal antibody. The antibody was kindly provided by Dr. David J. Hall from Thomas Jefferson University, Philadelphia, PA. Magnification of both panels is 20X.

discovery of additional cellular pathways altered in AD.

Another transcription factor determined to be upregulated in expression in AD is Nuclear Factor kappa B (NF-kappa B) (54). This protein is well known for its activation during inflammation. It is activated by cytokines and growth factors and upregulates target promoter of genes required for wound healing and ablation of infection (67), 68). NF-kappa B activity is regulated by association with another protein called I-kappa B (inhibitor of kappa B) (67). This protein binds NF-kappa B and inhibits translocation into the nucleus. Activation of NF-kappa B involves phosphorylation of I-kappa B by a member of the IKK family (I-kappa B kinase), resulting in ubiquitination and subsequent proteosome mediated degradation (69). Liberated NF-kappa B is then translocated into the nucleus where it acts as a strong transcriptional activator. Because of this post-translational regulation of activity, NF-kappa B activity must be assessed in normal and AD brain and is a more critical measurement than its expression level.

Recent studies convincingly demonstrate that active NF-kappa B is present in early stages of plaque formation, but not in mature senile plaques (54). Using an antibody that recognizes an NF-kappa B epitope obscured by I-kappa B binding, the center of primitive plaques as well as neuronal nuclei and microglia adjacent to the plaque are immunoreactive for free NF-kappa B. These findings support the role of an inflammatory response in early stages of AD. NF-kappa B also upregulates APP expression, suggesting a dual role in AD pathogenesis (54). NF-kappa B regulation of the APP gene is interesting since it was found that aggregated A-beta 1-40 stimulates NF-kappa B activation at low concentration *in vitro* (54). This activation is dependent on H2O2 production, suggesting NF-kappa B is a mediator of A-beta toxicity and neurodegeneration.

However, there is also evidence that NF-kappa B provides a neuroprotective role. Several proteins such as TNF alpha, TNF beta, and NGF that have been shown to exhibit neuroprotection against A-beta toxicity induce NF-kappa B activation (70). NF-kappa B is also upregulated during nerve regeneration in response to cytoskeletal disruption (71). In neurons NF-kappa B is localized to synapses and axons (possibly accounting for its presence in neuritic plaques) (72). Cytoskeletal changes that occur due to neurotoxicity may induce translocation of NF-kappa B to the nucleus. These results suggest that cellular responses to active NF-kappa B depend upon concurrent pathways activated in the cell and its local environment. NF-kappa B may induce regeneration in particular neurons or degeneration in others. Identification of the precise mechanism controlling NF-kappa B activity and IKK activation will likely contribute greatly to our understanding of AD pathogenesis.

We have recently identified another transcription factor that appears to be upregulated in AD, the Myc associated zinc finger protein (MAZi/ZF87). This protein was first identified by its ability to bind the purine rich element in the myc promoter (73, 74). MAZi/ZF87 enhances the transcriptional activation capacity of proximal transcription factors (73, 75). We have determined that MAZi/ZF87 is re-expressed in pyramidal neurons in AD brain (figure 1). These results indicate that overall gene expression is altered during AD and suggests that altered gene expression plays an important role in AD pathogenesis.

Each transcription factor affects the expression of numerous genes and therefore it is difficult to identify particular genes or molecular mechanisms important for AD. However, multiple transcription factors often are utilized to regulate a specific molecular pathway. Therefore, as the list of active transcription factors upregulated in AD expands, along with the knowledge of what genes they regulate, specific molecular mechanisms activated or repressed in AD will likely emerge. Thus, molecular mechanisms involved in AD formation and progression will likely be identified. Many of these transcription factors, such as Krox24, are important regulators of gene expression during brain development and are re-expressed in AD. This suggests that molecular mechanisms important for normal brain development are re-activated in AD.

3.2. Cell Cycle Genes in AD

Another group of proteins re-expressed during AD are those regulating cell cycle progression. Cell cycle proteins are expressed or activated during cell proliferation in the developing brain and are subsequently inactivated or repressed in post-mitotic neurons of the adult brain (Reviewed in (76). However, select members of cell cycle proteins are found to be expressed at increased levels or localized near pathologic markers in AD patients. Among these proteins are tumor suppressors, cyclin dependent kinases (CDK), cyclin dependent kinase inhibitors (CDKI), and proliferation antigens (37, 77-82). Increased expression of cell cycle regulatory genes has one of two effects on post-mitotic neurons, either entry into apoptosis or induction of a regenerative, proliferative response.

Among the first cell cycle proteins characterized in AD pathology were the CDKs and their regulatory subunits the cyclins and CDKIs (37, 78, 80, 82). CDKs were assessed for their role in tau and APP phosphorylation (78, 83). Both proteins are subject to phosphorylation by CDKs *in vitro* and APP becomes phosphorylated in proliferating cells prior to entry into mitosis, implicating a specific CDK in its phosphorylation. CDKs require interactions with a cyclin protein and specific phosphorylation to become active (For review see (76, 84)). Each cyclin:CDK pair regulates progression through specific points in the cell cycle. For example, cyclin E:CDK2 regulates exit from G1 and entry into S phase, and cyclin D:CDK4 regulates exit from G0 into G1. The cyclin subunits regulate the substrates phosphorylated by the CDK. Each cyclin:CDK pair is recognized by a specific member of the CDKI family (76, 84, 85). These proteins inactivate the kinase by dissociating the cyclin:CDK complex. Thus, the activities of the CDKs are regulated by a variety of mechanisms.

Although numerous CDKs have been shown to phosphorylate tau *in vitro*, only a few have actually been found to be alternatively expressed in AD (86, 87). CDK4 was localized to NFTs and in neuronal populations of AD patients (82). However, its normal cyclin partner, cyclin D, is not detected in NFTs but instead the CDKI P16 (CDK4 specific inhibitor) is upregulated and localized to NFTs. Upregulation of antagonistic proteins raises several questions. What is stimulating the change in gene expression? Is CDK4 produced at a higher level resulting in active kinase formation with a different cyclin subunit or is p16 produced in sufficient quantities to inhibit this effect? Is it CDK activation or inhibition that plays a role in AD pathogenesis? These questions must be addressed before the results can be properly interpreted.

CDK5 is another cell cycle protein expressed during brain development and at increased levels in AD (88). In normal adult brain CDK5 is expressed at high levels, and when coupled to a non-cyclin regulatory subunit, functions as a neurofilament kinase (89-91). In AD brain CDK5 is expressed at increased levels, though its function in AD brain is not known and expression levels of the non-cyclin regulatory subunit have not been determined. However, CDK5 has been shown to phosphorylate tau and may contribute to NFT formation. The function of CDK5 in normal adult brain suggests that CDKs may provide additional, non-cell cycle functions in post-mitotic neurons that participate in AD pathogenesis.

In addition to the upregulation of CDK proteins, cyclins are also upregulated in AD brain. These include cyclin B and cyclin E (79). Cyclin B binds cdc2 and regulates the G2/M boundary (84). Increased expression of cyclin B in areas of AD pathology correlate well with the observation that both tau and APP are phosphorylated at the G2/M boundary in dividing cells. This suggests that a kinase, perhaps a cdc2-like activity, can associate with cyclin B and phosphorylate APP and tau in AD (37, 78). The presence of cyclin B also suggests that these cells have been stimulated to re-enter the cell cycle and the nuclear localization indicates a population of cells are in late G2. The presence of cyclin E in cells indicates a subset of cells are preparing to enter S phase. Upregulation of cyclins B and E in the absence of other cell cycle checkpoints such as cyclins A and D is more difficult to interpret. It is possible that aberrant expression of these two cyclins results in altered phosphorylation states of specific proteins without inducing cell cycle progression. However, due to the known instability of cyclins, cyclins D and A may be undetectable in AD brain because they are degraded in post-mortem tissue or interact with other proteins that obscure antibody epitopes. To gain insight into how these proteins influence AD progression, their associated proteins and kinase activity must be assayed in AD brain extracts.

The function of the cyclin:CDK complexes in AD is uncertain due to the increase in CDKIs. The CDKI for cyclin E and cyclin B:CDK complexes is the p21 cip1/waf1/kip1 protein, which also exhibits increased expression in AD (76, 82, 84, 85). This protein, in addition to stopping cells in the G1/S and G2/M boundaries, is also inducible by DNA damage (92, 93). Its expression is upregulated by activation of p53 tumor suppressor protein, yet another protein upregulated in AD (94, 95). These two proteins are also involved in the decision to enter apoptosis or enter S phase (93). p21 inhibits cyclin A, B, and E:CDK complexes, halting the cell in G1 or G2. The exact effect of CDKIs on neurons in which they are expressed requires

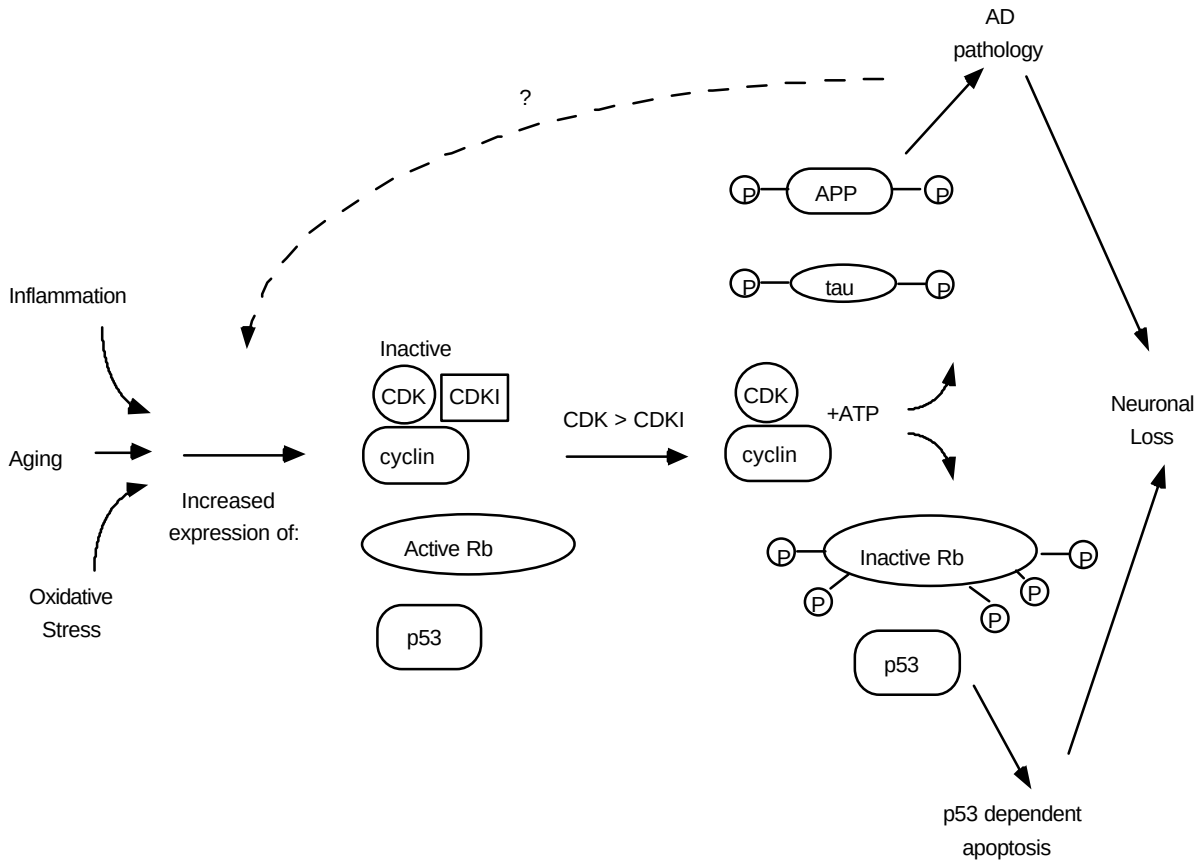

Figure 2. A model for the role of cell cycle proteins in Alzheimer's disease progression. See text for details.

quantitation of the amount of p21 vs cyclin:CDK complexes, because they interact in stoichiometric amounts. The most abundant protein will dictate cell fate. Therefore, these proteins must be quantitated and functionally assayed for kinase activity, in addition to being immunolocalized to cells under pathologic stress. However, it is not known if the conflicting molecular pathways described above are activated in the same neuron.

Another tumor suppressor protein required for normal nervous system development and re-expressed during AD is the retinoblastoma (Rb) susceptibility gene product (96, 97). This protein will halt the cell in G0 when overexpressed (Reviewed in (98). Active Rb prevents apoptosis caused by p53 induction (99, 100). However, if inactivated by phosphorylation (usually by CDK4 or CDK6), it will be unable to prevent apoptosis. In order to evaluate the role of Rb in AD its functional status must be established both in aged matched non-demented controls and AD cases.

Additional evidence that a number of neurons in AD have re-entered the cell cycle comes from detection of p105 and ki67, mitotic antigens present in proliferating cells (77, 81, 101). In AD brain p105 and ki67 were associated with NFTs and neuritic plaques. These data confirm a correlation between re-entry into cell cycle progression and formation of pathologic features of AD.

From the current data the following model can be proposed (figure 2). In response to stimulatory factors such as inflammatory molecules or oxidative stress, neuronal expression of CDKs, CDKIs, and cyclins is increased and p53 is activated. Cells are held in G0 until the amount of cyclin:CDKs accumulate above the level of CDKIs. At this point Rb, tau, and APP become phosphorylated and additional neuronal insult and AD pathology occurs. It is possible that A-beta deposition may feedback to alter expression of CDKs, CDKIs, etc. through interaction with p75 neurotrophin receptor further propagating neuronal loss (102). Cell death may ultimately occur by multiple mechanisms. AD pathology (cytoskeletal and A-beta deposition) may directly induce neurodegeneration by unclear mechanisms. Alternatively, inactivation of Rb or other repressors of apoptosis induces re-entry into the cell cycle to repair DNA or other cellular damage. p53 recognizes the cell is not fit to divide and initiates an apoptotic cascade. Additional studies are required to determine if the functional data will support such a model, and if there are previously uncharacterized functions for the cell cycle proteins in post-mitotic neurons. This model suggests that neurodegeneration may result from parallel and overlapping processes of NFT and A-beta induced cell death and p53 induced apoptosis. In support of this model, previous studies have shown that DNA

fragmentation and increased expression of apoptotic proteins occurs both in neurons containing AD pathology and cells lacking neurofibrillary changes (16, 103).

3.3. Developmentally Regulated Genes

The microtubule binding protein tau is alternatively spliced to generate six isoforms expressed during neurodevelopment (104, 105). The expression of these isoforms is developmentally regulated, and both differential expression and phosphorylation are important regulators of tau function. Tau is the major protein component of neurofibrillary tangles (PHF-tau) that form during Alzheimer's disease (106). Fetal isoforms of tau are transiently phosphorylated in a manner similar to tau found in NFT (107, 108). Therefore kinases activated in AD brain may be identical to kinases active in developing brain and result in similar patterns of tau phosphorylation (109). One kinase that phosphorylates both fetal and adult tau at sites found in tangles is CDK5 (87, 88). The cyclin dependent kinase cdc2 also phosphorylates tau *in vitro* in many sites identical to those in PHF-tau. Fetal tau is phosphorylated at a higher level than normal adult tau and to a similar extent as PHF-tau (88). These studies suggest that cell-cycle regulated kinases active in developing brain may function to contribute to the formation of PHF-tau in adult brain.

The presence of phosphorylated forms of tau similar in both AD and fetal brain suggests that disease progression involves a recapitulation of early developmental processes. This hypothesis is supported by the presence of embryonic forms of other cytoskeletal components including alpha-tubulin and beta-tubulin (110, 111). At present several genes re-expressed in AD are known to be developmentally regulated. Although understanding of the molecular mechanisms underlying brain development is incomplete, several interesting genes have been identified.

Most prominent of these genes are the presenilins (PS1 and PS2) which share significant homology with the C. elegans protein sel-12 (50% identity) (112). sel-12 functions in cell fate determination mediated by lin12/notch signaling (112). The ability of PS1, but not mutant PS1, to functionally replace sel-12 in a sel-12 knockout suggests PS1 and PS2 are likely involved in the notch pathway (113). Also, PS1 knockout mice exhibit a variety of developmental defects and disruption of the notch signaling pathway (114, 115).

Another protein that functions in development and is re-expressed in AD is the FAC1 protein (116, 117). This protein is expressed at high levels in fetal brain, at low levels in adult brain and re-expressed in AD (116). FAC1 co-localizes with a subset of diffuse and neuritic plaques, swollen dendrites and Hirano bodies in AD patients (117). FAC1 has no overall homology to known proteins but contains two zinc fingers and exhibits specific binding to nucleic acids ((118), Jordan-Sciutto and Bowser, unpublished results). The exact function of FAC1 remains unknown, but its expression pattern and subcellular distribution in developing cortex and AD are intriguing. During cortical development FAC1 is found in the nucleus and dendrites of migrating neurons (116). However, in more fully differentiated neurons FAC1 exhibits a predominantly nuclear localization. In normal adult brain FAC1 is localized predominantly to neuronal nuclei and in AD brain FAC1 is localized to a subset of A-beta containing plaques (117). The level of FAC1 protein expression also changes during AD progression. FAC1 is expressed at high levels during Braak stages III and IV, and diminished levels in stages V and VI (Bowser, unpublished results). This suggests that FAC1 may play a role in the early stages of AD. It has been proposed that during early stages of AD neurons attempt to compensate for injured neighboring cells by sprouting (119). Re-expression of FAC1 in AD may contribute to a sprouting or regenerative mechanism. In agreement with this hypothesis, FAC1 re-expression has been observed in an animal model for regeneration (120).

Neuronal development involves not only cell growth, but programmed cell death (PCD). This review would be incomplete if it did not mention members of the PCD pathway implicated in development that are also expressed during AD. The bcl-xl gene is a bcl-2 homologue expressed at high levels in embryonic and fetal brain, but at undetectable levels in young adult brain (121, 122). The importance of Bcl-xl protein expression in CNS development is illustrated by severe neuronal death in bcl-xl knockout mice (123). Bcl-xl protein is also expressed at high levels in normal aged brains, possibly due to oxidative damage (124). However, in AD Bcl-xl expression is low except for a population of activated microglia surrounding A-beta plaques (124). Specific expression only in microglia may explain the reduced resistance of neurons to injury in the aged brain. A similar result is observed for Bcl-2, in which a three fold increase of Bcl-2 protein occurs in AD brain in comparison to control adult brain (125). In AD brain Bcl-2 protein is predominantly localized to activated astrocytes, not neurons. Decreased expression of two anti-apoptotic genes within neurons of the aged brain suggests that these cells lose the ability to activate expression of PCD protective molecules. Loss of Bcl-xl and Bcl-2 expression in neurons may also be indicative of aging and result in increased susceptibility to degenerative stimuli.

Another facet to this story is Bax and Caspase 3, apoptosis inducing proteins. Both have been found to be expressed at increased levels in AD hippocampi (16, 17). Bax also shows localization to both senile plaques and NFTs (16). Upregulation of PCD inducing genes in regions of pathology also suggests a pathway for neuronal loss in AD. The presence of these proteins in AD pathology suggests PCD pathways play a role in AD progression.

4. PERSPECTIVE

Although progress has been gained into the possible genetic causes and treatments of AD, the mechanisms underlying a majority of sporadic AD cases and the cellular mechanisms that propagate the neurodegenerative process remain elusive. The developmentally regulated proteins described in this review are unlikely to be the primary pathologic events. However, the analysis of genes alternatively expressed during AD progression and their function in this process will lead to the discovery of cellular pathways that impact on a variety of processes such as regeneration, proliferation, neural inflammation, and neurodegeneration. Such pathways may help define the necessity of aging for onset of AD and identify cell death mechanisms that are activated in AD. It will be important to understand such mechanisms in order to form effective treatments for AD and other neurodegenerative diseases.

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