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Cell Cycle Re-entry Mediated Neurodegeneration and Its Treatment Role in the Pathogenesis of Alzheimer's Disease

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Abstract

As one of the earliest pathologic changes, the aberrant re-expression of many cell cycle -related proteins and inappropriate cell cycle control in specific vulnerable neuronal populations in Alzheimer's disease (AD) is emerging as an important component in the pathogenesis leading to AD and other neurodegenerative diseases. These events are clearly representative of a true cell cycle, rather than epiphenomena of other processes since, in AD and other neurodegenerative diseases, there is a true mitotic alteration that leads to DNA replication. While the exact role of cell cycle re-entry is unclear, recent studies using cell culture and animal models strongly support the notion that the dysregulation of cell cycle in neurons leads to the development of AD-related pathology such as hyperphosphorylation of tau and amyloid- β deposition and ultimately causes neuronal cell death. Importantly, cell cycle re-entry is also evident in mutant amyloid- β protein precursor and tau transgenic mice and, as in human disease, occurs prior to the development of the pathological hallmarks, neurofibrillary tangles and amyloid- β plaques. Therefore, the study of aberrant cell cycle regulation in model cellular and animal systems may provide extremely important insights into the pathogenesis of AD while serving as a means to test novel therapeutic approaches.

Keywords

Alzheimer's disease; amyloid- β ; cell cycle; neurodegeneration; tau phosphorylation

CELL CYCLE ALTERATIONS IN ALZHEIMER'S DISEASE AND OTHER NEURODEGENERATIVE DISEASES

Primary neurons in the normal brain are viewed as being quiescent and in G₀ (Obrenovich et al., 2005). However, in Alzheimer's disease (AD), multiple lines of evidence suggest that neurons vulnerable to degeneration emerge from this postmitotic state -phenotypically suggestive of cells that are cycling, rather than the normal terminally differentiated non-dividing state (Arendt et al., 1995; Smith and Lippa, 1995; Arendt et al., 1996; Vincent et al.,

1996; McShea et al., 1997; Nagy et al., 1997a; Nagy et al., 1997b; Vincent et al., 1997; Arendt et al., 1998; Vincent et al., 1998; McShea et al., 1999a; McShea et al., 1999b; Raina et al., 1999a; Raina et al., 1999b; Zhu et al., 1999; Raina et al., 2000a; Raina et al., 2000b; Zhu et al., 2000a; Zhu et al., 2000b; Zhu et al., 2000c; Zhu et al., 2004b; Ogawa et al., 2003; Previll et al., 2007; Thakur et al., 2008). The successful duplication of DNA (Yang et al., 2001; Mosch et al., 2007; Spremo-Potparevic et al., 2008; Zhu et al., 2008) indicates that, at least, some neurons successfully complete S phase. This precludes the possibility that the re-expression of various cell cycle markers is merely an epiphenomena caused by reduced proteosomal activity (Bowser and Smith, 2002).

Notably, cell cycle alterations are not limited to AD. For example, nuclear accumulation of hyperphosphorylated pRb (ppRb) and altered localization of E2F-1 occurs in both lower and upper motor neurons in patients with amyotrophic lateral sclerosis (ALS), suggesting motor neurons re-enter the G₁ phase of the cell cycle (Ranganathan and Bowser, 2003). In Parkinson's disease, ppRb and E2F-1 are also increased (Jordan-Sciutto et al., 2003; Hoglinger et al., 2007) as demonstrated by the abundant ppRb and E2F-1 staining in neuronal cytoplasm of the substantia nigra, mid-frontal cortex, and hippocampus by immunocytochemistry. In controls, anti-ppRb weakly stained nucleoli of neurons in the substantia nigra and exhibited no detectable staining in mid-frontal cortex and hippocampus. In the substantia nigra, ppRb also co-localized to Lewy bodies, the pathologic hallmark of Parkinson's disease. Furthermore, injection of dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into mice, which has been widely used as a model of Parkinson's disease, activates the retinoblastoma-E2F pathway in postmitotic dopaminergic neurons (Hoglinger et al., 2007). Importantly, E2F-1-deficient mice are significantly more resistant to MPTP-induced dopaminergic cell death than their wild-type littermates proving the essential role of cell cycle re-entry in this model.

CELL CYCLE ALTERATIONS IN POST-MITOTIC NEURONS MAY LEAD TO CELL DEATH IN ALZHEIMER'S DISEASE

It is well known that the cell cycle is a highly regulated process with numerous checks and balances that ensure normal cell proliferation when appropriate environmental signals are present. The cell cycle is typically divided into four phases: G₁, S (DNA replication), G₂ and M (mitosis). The sequential expression and activation of cyclin/cyclin-dependent kinase (CDK) complexes, the main regulators of cell cycle progression, orchestrate the transition from one phase to another. Cells can exit the cell cycle to enter and rest at G₀ phase after exit from mitosis, which is the case in terminally differentiated cells such as neurons. Thereafter, the expression/activation of cyclin D/CDK4,6 complex, which is triggered by the presence of mitotic growth factors, controls the re-entry of resting (G₀) cells into the G₁ phase of cell cycle (Sherr, 1995).

Although various mitotic markers are upregulated in vulnerable neurons in AD, no evidence of actual mitosis has ever been found (Zhu et al., 2008), suggesting that these neurons are arrested at a point(s) prior to the actual event of cellular division. However, it is well known that once cyclin A is expressed, the arrested cells lack the ability to return to G₀ and therefore must either complete the cycle or die. Therefore, given the lack of evidence for successful completion of the cell cycle, it is likely that the re-activation of cell cycle machinery in post-mitotic neurons leads to their death. In support of this, when a powerful oncogene, SV40 T antigen, is expressed specifically in maturing Purkinje cells in transgenic mice, the cells replicate their DNA (i.e., initiate cell cycle) but then subsequently degenerate and die (Feddersen et al., 1992). A recent study using the mice inducibly express SV40T in neurons confirmed the degenerative effect (Park et al., 2007). Similarly, the expression of SV40T by

the rhodopsin promoter causes photoreceptor degeneration, again associated with cell cycle reactivation and DNA synthesis (al-Ubaidi et al., 1992).

It is well known that the activation of cell cycle processes is part of the mechanism by which developmental failure of trophic support leads to neuronal cell death. Additional evidence supports a role for cell cycleregulators in neuronal death evoked by various stressors (Giovanni et al., 1999; Park et al., 2000). However, transgenic mouse and cell culture studies to date do not faithfully mimic age-related neurodegenerative diseases since the data from cell culture studies used embryo-derived primary neuronal culture. Furthermore, the expression of transgenes during embryogenesis combined with neurodegeneration at an early age in the transgenic mice suggests that an error in development occurs causing neurodegeneration. Since most neurodegenerative diseases become evident in old age, the establishment of a new experimental model, which allows testing of age-specific changes and excludes effects during development, is required to delineate the role of the cell cycle in neurodegenerative diseases. Since the effects of expression of oncogene such as SV40T are not limited to cell cycle re-entry and could induce other signaling pathways, a direct and specific alteration of cell cycle re-entry in adult neurons *in vivo* would provide the ultimate answer for the role of cell cycle re-entry in neurodegeneration. In this regard, it is interesting to note that the inactivation of Rb specifically in postnatal cochlear hair cells causes cell cycle re-entry and cell death (Weber et al., 2008).

In addition, Rb deficient mice display gross neuronal defects accompanied by cell cell re-entry and mid-gestation lethality (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992). However, unlike neurons at developmental stages, it is unclear whether Rb mediated cell cycle re-entry causes neurodegeneration in post-mitotic adult neurons.

Since Rb is a direct regulator of G₁/S cell cycle transition and more specific to cell cycle than other cell cycle regulators used in the previous studies and activation of Rb is prominent in the vulnerable neurons in AD (Jordan-Sciutto et al., 2002; Thakur et al., 2008), the effect of inactivation of Rb in adult central nervous system may provide more direct evidence for the role of cell cycle re-entry in neurodegeneration and AD.

CELL CYCLE EVENTS ARE LINKED TO OXIDATIVE STRESS

There is abundant evidence that oxidative stress and free radical damage plays a key role in the pathogenesis of AD (Smith et al., 1995; Markesbery, 1997; Perry et al., 1998; Nunomura et al., 1999). Since free radicals, free-radical generators and antioxidants act as crucial controls of the cell cycle (Curcio and Ceriello, 1992; Ferrari et al., 1995), it is critical to determine the extent to which these pathways are independent or interdependent. For example, during the cell cycle, there is division and redistribution of cellular organelles such that mitochondrial proliferation is evident (Barni et al., 1996). Mitochondrial proliferation is imperative for providing the energy needed for cell division. However, in cells where the cell cycle is interrupted or dysfunctional, cells enter "phase stasis" with mitochondrial imbalance (Hirai et al., 2001; Wang et al., 2008), which serve as potent sources of free radicals and cause redox imbalance (Nunomura et al., 2001).

It is evident that both oxidative stress and cell cycle re-entry play early roles in the pathogenesis of AD (Zhu et al., 2004). Based on the studies of oxidative stress signaling and mitotic signaling pathways in vulnerable neuronal populations in AD, both oxidative stress and cell cycle re-entry could independently initiate, yet both are necessary to propagate disease pathogenesis and progression, while the temporal relationship to each other pathway is unclear (Zhu et al., 2004; Zhu et al., 2007). Therefore, the elucidation of the causal relationship between those key pathogenic mechanisms may provide an important clue for into pathogenesis of AD and the development of therapeutics (Woods et al., 2007).

ALZHEIMER'S DISEASE-ASSOCIATED PROTEINS AND THE CELL CYCLE

Tau phosphorylation

The major protein component of the neurofibrillary tangle (NFT), the major intracellular pathology of AD, is a highly phosphorylated form of the microtubule associated protein tau (Iqbal et al., 1984; Grundke-Iqbal et al., 1986; Iqbal and Grundke-Iqbal, 2006). The increased phosphorylation of tau destabilizes microtubular dynamics and, consequently, results in neuronal dysfunction (Lindwall and Cole, 1984; Alonso et al., 1996; Delacourte, 2006). However, a near identical phosphorylation of tau, driven by CDKs, also occurs when cells are mitotically active (Brion et al., 1985; Kanemaru et al., 1992; Goedert et al., 1993; Brion et al., 1994; Pope et al., 1994) suggesting an intimate link between cell cycle and tau.

Interestingly, in AD, cell cycle protein expression precedes the appearance of phosphorylated tau (Vincent et al., 1998) indicating a possible cause-effect relationship. In this regard, CDKs localized in neurons in AD are known to phosphorylate tau in *in vitro* assays in a manner similar to that found in AD *in vivo* (Arendt et al., 1995; Arendt et al., 1996; Vincent et al., 1996; Nagy et al., 1997a; Nagy et al., 1997b). Since increased phosphorylation and altered microtubule stability are coincident during progression through the cell cycle, it is highly likely that hyperphosphorylation of tau as appears in AD is intimately connected with cell cycle alterations in the vulnerable neurons in AD. While further study is required to address the causal relationship between cell cycle re-entry and tau alterations, nonetheless, it is clear that cell cycle re-entry precedes the appearance of hyperphosphorylated tau (Vincent et al., 1998) and that, in tau mutant fly models, mutations in tau cause cell cycle re-entry in the same neurons (Khurana et al., 2006). Interestingly, a recent transgenic mice study demonstrated that the overexpression of normal human tau protein induces neuronal apoptosis and cell cycle re-entry (Andorfer et al., 2005). In addition, the authors demonstrated that hyperphosphorylation of tau and NFT-containing neurons did not enter the cell cycle (Andorfer et al., 2005) suggesting that the alterations of tau such as hyperphosphorylation and aggregation are not required to induce cell cycle re-entry and neurodegeneration (Lee et al., 2005; Castellani et al., 2006). In support of the latter notion, our recent study using adenoviral-mediated expression of c-MYC and ras oncogenes to drive postmitotic primary cortical neurons into the cell cycle demonstrates that cell cycle re-entry in primary neurons leads to tau phosphorylation and conformational changes similar to that seen in AD (McShea et al., 2007). This study establishes that the cell cycle can be instigated in normally quiescent neuronal cells and results in a phenotype that shares features of degenerative neurons in AD. Importantly, supporting our study, are recent study similarly demonstrated that cell cycle re-entry, mediated by the conditional expression of the simian virus 40 large T antigen (SV40T), induced the hyperphosphorylation of tau and NFT formation (Park et al., 2007).

Amyloid- β

The major protein component of senile plaques is a 4.2 kDa polypeptide termed amyloid- β , which is derived from a larger precursor, the amyloid- β protein precursor (A β PP), encoded on chromosome 21 (Hardy, 2006; Masters and Beyreuther, 2006). Attesting to the importance of this protein, mutations in the A β PP gene are linked to the inevitable onset of familial AD (Goate, 2006). Given the role of mitotic re-entry in AD, it is notable that A β PP is upregulated secondary to mitogenic stimulation (Ledoux et al., 1993) and that A β PP metabolism is regulated by cell cycle-dependent changes (Suzuki et al., 1994). Interestingly, amyloid- β itself is mitogenic *in vitro* (McDonald et al., 1998; Pyo et al., 1998) and therefore may play a direct role in the induction and/or propagation of cell cycle-mediated events in AD. Additionally, amyloid- β -mediated cell death, at least *in vitro*, is dependent on the presence of various cell cycle-related elements (Giovanni et al., 1999). Thus, amyloid- β may only become toxic *in vivo* when the neuronal cell cycle machinery is activated.

The increase of amyloid- β and senile plaque deposition following cell cycle re-entry is evident in SV40T overexpressing mice (Park et al., 2007) supporting the causal role of cell cycle re-entry in amyloid- β in AD. However, the role of cell cycle in amyloid- β overexpressing transgenic mice is somewhat more controversial. In one study, using APP23 mice harboring the Swedish double mutation, amyloid- β deposition is associated with prominent gliosis that is characterized by an astrocytic expression of cyclins D1, E and B1 as well as the nuclear translocation of cyclin-dependent protein kinase 4 (Gartner et al., 2003). However, amyloid- β plaque formation is not accompanied by significant changes in the neuronal expression of cell cycle proteins. Therefore, the overexpression of amyloid- β in APP23 mice is not associated with cell cycle re-entry in neurons and this suggests that cell cycle re-entry in neurons is not a consequence of amyloid- β formation and deposition. In contrast to this study, a study using four different mouse models (i.e., R1.40, Tg2576, APP23 and Tg2576/*PSEN1*) apparently found neuronal cell cycle re-entry (Yang et al., 2006). The data from two groups are different, and this is especially relevant in that both analyzed the same mouse strain, i.e., APP23. In the first study, the cell cycle re-entry is associated with astrogliosis but not neurons (Gartner et al., 2003), while the other study demonstrated neuronal cell cycle re-entry (Yang et al., 2006). In the latter case, it is important to note that this neuronal cell cycle was not confirmed by double immunocytochemistry with neuronal cell markers.

In most studies, cell cycle events precede amyloid- β deposits and the appearance of active microglia suggesting that the initiation of cell cycle re-entry in neurons is an early sign of neuronal distress in these animal models, and amyloid- β deposits and gliosis are not necessary factors to induce cell cycle re-entry in A β PP transgenic mouse models.

Presenilins

Mutation in the human presenilin genes 1 and 2 found on chromosomes 14 and 1, respectively, are linked to early onset AD and, like A β PP, are implicated in cell cycle regulation. First, homologues of the human presenilins are involved in Notch-based signaling indicating that presenilins may play an important role in the determination of cell fate (Wong et al., 1997; Struhl and Greenwald, 1999; Ye et al., 1999). Second, the association of presenilins with centrosomes and centromeres suggest that they play a role in cell division and segregation of chromosomes (Li et al., 1997). Third, overexpression of presenilins leads to arrest in the G₁ phase of the cell cycle, which is potentiated by expression of the PS2(N141I) mutation (Janicki and Monteiro, 1999). In postmitotic neurons, overexpression of presenilins yields loss of calcium homeostasis, oxidative stress, and increased susceptibility to apoptotic death (Mattson et al., 1998) with AD-linked presenilins showing the greatest effects (Wolozin et al., 1996; Janicki and Monteiro, 1997). Interestingly, upregulation of Cyclin D1 in temporal cortex from familial type of AD patients expressing different PS1 mutations and experiments on neurons derived from mice carrying PS1 M146 V mutation showed that cell cycle alterations and cell death occur independently of γ -secretase activity suggesting a function for presenilins outside the γ -secretase complex (Malik et al., 2008).

We proposed that neurons in AD are attempting to re-enter the cell cycle but are blocked from progression at the G₁/S phase boundary by presenilin (and possibly A β PP) mutations (Prat et al., 2002). The block at G₁ would result in accumulation of cell cycle control proteins and cell death as is seen in AD. Thus, these mutations confer a contracted time course to the underlying pathophysiology of the disease.

CONCLUSIONS

The relevance of cell cycle and other oncogenic/mitotic markers to a bona fide mitotic process was initially thought to be confounded by the multiplicity of functions for such markers (Bowser and Smith, 2002). For example, cyclins and other proteins are involved in cell growth,

differentiation, DNA repair, and apoptotic signaling pathways (Park et al., 1998; Giovanni et al., 1999). While such processes are involved in AD, and therefore could explain the presence of some mitotic markers, it is apparent that for many proteins described in AD that a role in mitotic regulation is their only known function (e.g., MRG15) (Raina et al., 2001). Also, such neurons receive mitogenic signals furthering the notion of exit from quiescence (McShea et al., 1999b; Perry et al., 1999). More importantly, many of the neurons demarked as being mitotically active based on these markers actually show evidence of binucleation (Zhu et al., 2008) involving chromosome replication (Yang et al., 2001; Mosch et al., 2007) and/or premature chromosome separation (Spremo-Potparevic et al., 2008). Nonetheless, to date, there has not been a systematic study of how cell cycle changes relate to other neurodegenerative changes or to each other. Such studies are required not only to examine the current AD animal models for elucidating the causal relationship between cell cycle re-entry and AD related pathologies but also to develop new animal models to provide a framework for integrating all of the disparate reports of cell cycle abnormalities.

In conclusion, based on the evidence presented above, we hypothesize that terminally differentiated neurons in AD are “instructed” to enter the mitotic cycle and, thereafter, proceed (either sequentially or dysfunctionally) only through to a point(s) prior to the actual event of cellular division since they are inherently restricted in their mitotic competence. Such incomplete cell division not only leads to pathological hallmarks of disease (amyloid- β and tau phosphorylation) but also, ultimately, to cell death. As such, therapeutics targeting cell cycle entry and/or progression may be useful for the treatment of AD (Woods et al., 2007).

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