Available online at www.sciencedirect.com

ScienceDirect

Biochimica et Biophysica Acta 1772 (2007) 409–412

Biochimica et Biophysica Acta BBA

www.elsevier.com/locate/bbadis

Review

The nature of the cell cycle in neurons: Focus on a "non-canonical" pathway of DNA replication causally related to death

Agata Copani [a,b], Filippo Caraci [a,g], Jeroen J.M. Hoozemans [c], Marco Calafiore [a], Maria Angela Sortino [d], Ferdinando Nicoletti [e,f,*]

[a] *Department of Pharmaceutical Sciences, University of Catania, Italy*
[b] *IBB, CNR, Catania, Italy*
[c] *Department of Neuropathology, Academic Medical Center, University of Amsterdam, The Netherlands*
[d] *Department of Experimental and Clinical Pharmacology, University of Catania, Italy*
[e] *Department of Human Physiology and Pharmacology, University of Rome "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy*
[f] *I.N.M. Neuromed, Pozzilli, Italy*
[g] *gPHD Program in Neuropharmacology, University of Catania, Italy*

Received 5 October 2006; accepted 30 October 2006
Available online 1 November 2006

Abstract

The mechanism whereby a reactivation of cell cycle in neurons causes cell death is beginning to be identified. In cellular models of Alzheimer's disease, activation of a non-canonical pathway of DNA replication contributes to neuronal death. This pathway involves the repair enzyme DNA polymerase-β, which is highly expressed in neurons of the Alzheimer's brain at early stages of the disease. Loading of DNA polymerase-β into the replication forks generates a death signal, which involves the tumor suppressor p53. The increasing knowledge of the main actors of the unscheduled DNA replication in neurons will pave the way for novel therapeutic interventions in Alzheimer's disease and other neurodegenerative disorders.

Keywords: Alzheimer's disease; β-amyloid; DNA polymerase-β; APE-1; Cell cycle; DNA Replication; Apoptosis

1. Introduction

Over a decade ago, Vincent and Davies first showed that cell cycle activation occurs in Alzheimer's disease (AD) brain [1]. Many studies since then have substantiated the hypothesis that aberrant reactivation of the cell cycle might cause neurodegeneration because postmitotic neurons that resume their cell cycle tend not to divide, but die (reviewed in ref. [2]).

So far, the so-called ectopic cell cycle activation has been investigated under pathological canditions, such as AD [2], mild cognitive impairment [3], Parkinson's disease (PD) [4], frontotemporal dementia (FTD) [5 6], and amyotrophic lateral sclerosis (ALS) [7–10]. Correlative data have begun to accumulate in animal models of pathology, including APP-transgenic mouse models of AD [11,12], aged mice expressing non-mutant human tau [13], and *Drosophila* models of tauopathies [14]. All these reports have pointed to cell cycle activation as a common effector of neurodegeneration in several different diseases. Accumulating evidence that cell cycle proteins are present in the healthy rodent and human brains (reviewed in ref. [15]) has led to the recent notion that the reawakening of the cell cycle in post-mitotic neurons represents a regulatory failure of the physiological neuronal functions of cell cycle proteins. Key components of the cell cycle machinery such as the anaphase promoting complex and the core proteins of the origin core complex have been implicated in the development of synapses [16] and in the control of dendrite and spine development [17], respectively. Thus, neurons might have found the way to use a large part of the cell cycle apparatus to control synaptic plasticity by decoupling the expression of cell cycle proteins from DNA replication [15]. However, a significant number of neurons undergo partial or full DNA replication in AD brain, and this is not seen in control brains [18]. By studying the biochemistry of the cell cycle in a tissue culture model of AD, we

* Corresponding author. Department of Human Physiology and Pharmacology, University of Rome "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy. Tel.: +39 06 49912969.
E-mail address: ferdinandonicoletti@hotmail.com (F. Nicoletti).

0925-4439/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2006.10.016

have found that cell cycle proteins resume their typical function in cycling neurons [19], and that these fail to avoid DNA replication by involving a DNA replication enzyme that normally functions outside the cell division, the DNA polymerase-beta [20,21]. These findings suggest a model of "cell cycle disregulation" that is the central hypothesis of this review: a non-canonical pathway of DNA replication is causally related to neuronal death. Molecular targets strictly related to this process might provide the key to neuronal-specific cell cycle inhibition, such targets are described below.

2. Quiescence exit, DNA replication entry and apoptosis of differentiated neurons in response to β-amyloid

Cultured neurons exposed to synthetic β-amyloid (Aβ) die by apoptosis [22], thus recapitulating the distinct feature of degenerating neurons in AD brain [23]. Interestingly, Aβ-mediated neuronal apoptosis requires cell cycle activation [19]. But, what is the nature of the cell cycle observed in a differentiated neuron? In proliferating cells, key factors are the cyclin (Cyc)/cyclin-dependent protein kinase (CDK) complexes, which control the transition through the different phases of the cycle [24]. Activation of CDK4–6 by the cyclin partner, Cyc D, is involved in the progression of the mid G1 phase, activation of CDK2 by Cyc E and CycA controls G1/S transition and S phase progression, whereas the Cyc B/CDK1 complex is the regulator of the G2/M transition [25]. This sequence of events also occurs in neurons challenged with Aβ. Following Aβ treatment of rat cortical neurons, we observed the induction of Cyc D1 and the phosphorylation of retinoblastoma (the substrate of Cyc D/CDK4–6 complexes), followed by the induction of Cyc E and A. The treated neurons started DNA replication, but then appeared to undergo apoptosis (Fig. 1). By means of FACS analysis, which allows the simultaneous evaluation of S phase and apoptosis, we found that blockade of the G1/S transition using a cyclin D1 antisense or a dominant-negative mutant of CDK-2 prevented both Aβ-induced DNA replication and apoptosis [19].

These experiments, and other studies [26–28], confirm that neurons can recruit constitutive CDKs and can also be induced to express cyclins to reactivate the cycle. Most important, the ectopic S phase is an obligatory step in the apoptotic pathway evoked by Aβ [19,29]. The question that remains is how does

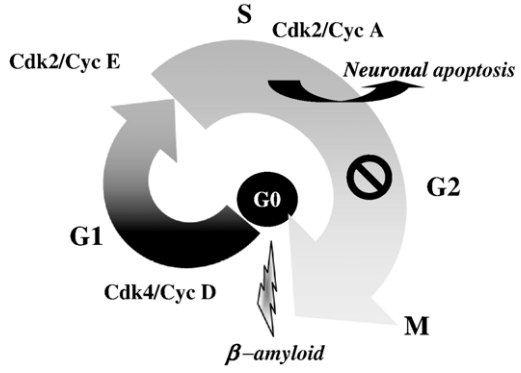

Fig. 1. Aβ acts as a proliferative signal for differentiated cortical neurons, driving them into the cell cycle. The cycle follows the typical sequence of events observed in proliferating cell, but does not progress beyond the S phase.

the ectopic S phase result in neuronal death. To answer this question, we have looked for the mechanisms that allow a differentiated neuron to replicate its DNA.

3. Activation of a non-canonical DNA replication pathway in differentiated neurons by Aβ

In proliferating cells, sequential activation of DNA polymerase (pol)-α, and -δ or -ε [30] mediates DNA replication. Polα, with its associate primase activity, is unique among DNA pols because of its ability to start *de novo* DNA replication. Polα is constituted by four distinct proteins with molecular weight of 180, 70, 58 and 49 kDa. The p49 subunit has primase activity, whereas the p180 subunit harbors the DNA polymerase activity of pol-α. By replacing polα, polδ and/or polε function as major replicative DNA pols that extend the nascent DNA primers. A fundamental characteristic of DNA replication in proliferating cells is that the different polymerases function in a way that the onset of mitosis is blocked while DNA is being synthesized or damaged [31]. While polα keeps the cell cycle in check, polδ and polε correct replication-related mismatch errors.

Other pols are able to bypass with high fidelity specific types of DNA damage occurring during replication, but they replicate undamaged DNA with high infidelity [32]. One of these, polβ, is a DNA repair enzyme expressed in postmitotic neurons [20 21]. Intriguingly, in cultured neurons, Aβ induces the over-expression of the repair enzyme polβ in a cell-cycle dependent manner [20]. This is atypical, because the expression of pol-β is expected to be regulated by DNA damage and not by the activation of the cell cycle. Moreover, Aβ induces the p49 and p58 subunits of primase, but neurons that enter the S phase in response to Aβ fail to express polα, which is essential for the canonical DNA replication [20].

It appears that DNA pol-β has a causal role in Aβ-induced DNA replication and neuronal death, as shown by knocking down the enzyme or inhibiting its activity with dideoxycytidine [20]. Thus, neurons are one of the few known cases in which pol-β carries out *de novo* DNA synthesis, others being the DNA endoreduplication of trophoblast cells [33] and bacteria replication [34].

Among the replicative pols, polδ is expressed constitutively by cultured neurons but is not further induced by Aβ. As opposed to polβ inhibition, polδ blockade reduces Aβ-induced DNA replication but not neuronal apoptosis, indicating that in neurons some DNA replication is possibly mediated by polδ but does not contribute to neuronal death [20,21]. Thereafter, we have hypothesized that the activation of a non-canonical pathway of DNA replication, which is mediated by polβ, is causally related to neuronal death.

4. A central role for DNA pol-β in AD neurodegeneration

The studies cited above leave unanswered the question of how the non-canonical enzymatic machinery activated by Aβ contributes to generate a death signal in neurons. Recently, we have found the way to study the nature of the replication forks in cultured neurons challenged with Aβ. We have discovered that

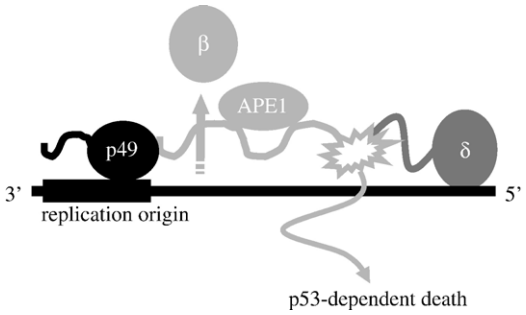

Fig. 2. Aβ activates a non-canonical pathway of DNA replication in differentiated cortical neurons. DNA primase, polβ, and polδ are sequentially loaded at neuronal replication forks. Polδ is recruited at the replication forks after polβ has been unloaded and the death signal has been triggered. The death signal is elicited by the extension of replicated DNA that depends on BER processes carried out by APE1 and polβ itself.

DNA primase, polβ, and polδ are sequentially loaded at the neuronal replication forks, where polβ possibly substitutes for the missing polα. Intriguingly, the loading of the repair enzyme polβ occurs independently of base-excision repair (BER) processes, whereas the recruitment of the replicative enzyme polδ relies in part on a process of BER carried out by the apurinic/apyrimidinic endonuclease (APE-1/Ref-1) and, likely, by polβ itself [21]. It is noteworthy that the inhibition of APE-1, and therefore of the BER process, not only prevents the loading of polδ at the replication forks, but also reduces the overall DNA synthesis and apoptotic death induced by Aβ. Since the component of DNA replication mediated by polδ does not contribute to neuronal death [20], these data suggest that an efficient BER allows polβ-mediated DNA replication to proceed up to the threshold for death before polδ loading [21] (Fig. 2).

5. Conclusions

Recent studies carried out in autoptic brain species from AD patients strengthens the hypothesis that polβ is causally involved in AD neurodegeneration. Similarly to the phosphorylated retinoblastoma, polβ is detected in neurons from cases with minor AD-related neuropathology [21]; therefore, likewise cell cycle proteins, the enzyme seems to be involved early in the pathogenesis of AD [3,35,36].

Overall data demonstrate that polβ is an essential component of the DNA-replication machinery in Aβ-treated neurons. In these neurons, the extension of DNA replication carried out by pol-β, and dependent on BER processes, is critical for the activation of a death signal [21] that is mediated by the tumour suppressor protein, p53 [20]. According to this model, joined DNA replication and repair decide whether and when neurons die and might be responsible for the slow cycling of AD neurons before death (2–3, 18).

In the attempt to put the control of the cell cycle into a therapeutic perspective, we must consider that cytostatic drugs that act as CDK inhibitors (e.g., mimosine and flavopiridol) and are protective against Aβ-induced toxicity [19,20] have adverse effects that might seriously limit their use [37]. Since polβ carries out a large component of DNA replication and apoptotic death in neurons exposed to Aβ, selective inhibitors of polβ might represent a key to neuronal-specific cell cycle inhibition as opposed to classical antineoplastic agents.

Finally, the finding that APE-1 contributes to the overall DNA replication and apoptotic death induced by Aβ points to additional targets for intervention. The evidence that the expression of APE-1 is increased in regions of neuronal injury in the AD brain [38] strengthens the suggestion that APE-1 inhibitors [39] are candidates for potential AD treatment (Box 1).

Box 1
Potential cell cycle-related neuroprotectant agents

Drugs	Targets
• CDK inhibitor (e.g., flavopiridol)	• G1/S phase transition
• DNA pol-β inhibitors (e.g., dideoxycytidine)	• Non-canonical DNA replication
• APE-1 inhibitors (e.g., methoxyamine)	• BER contributing to DNA replication

Acknowledgment

We acknowledge the support by the Ministry of Instruction, the University and Research Project of National Interest 2005 (to A.C.) that helped the conduct of the work described.

References

[1] I. Vincent, M. Rosado, P. Davies, Mitotic mechanisms in Alzheimer's disease? J. Cell Biol. 132 (1996) 413–425.
[2] K. Herrup, R. Neve, S.L. Ackerman, A. Copani, Divide and die: cell cycle events as triggers of nerve cell death, J. Neurosci. 24 (2004) 9232–9239.
[3] Y. Yang, E.J. Mufson, K. Herrup, Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease, J. Neurosci. 23 (2003) 2557–2563.
[4] S.S. Lee, Y.M. Kim, E. Junn, G. Lee, K.H. Park, M. Tanaka, R.D. Ronchetti, M.M. Quezado, M.M. Mouradian, Cell cycle aberrations by alpha-synuclein over-expression and cyclin B immunoreactivity in Lewy bodies, Neurobiol. Aging 24 (2003) 687–696.
[5] J.W. Husseman, D. Nochlin, I. Vincent, Mitotic activation: a convergent mechanism for a cohort of neurodegenerative diseases, Neurobiol. Aging 21 (2000) 815–828.
[6] A. Johansson, H. Hampel, F. Faltraco, K. Buerger, L. Minthon, N. Bogdanovic, M. Sjogren, H. Zetterberg, L. Forsell, L. Lilius, L.O. Wahlund, L. Rymo, J.A. Prince, K. Blennow, Increased frequency of a new polymorphism in the cell division cycle 2 (cdc2) gene in patients with Alzheimer's disease and frontotemporal dementia, Neurosci. Lett. 340 (2003) 69–73.
[7] S. Ranganathan, S. Scudiere, R. Bowser, Hyperphosphorylation of the retinoblastoma gene product and altered subcellular distribution of E2F-1 during Alzheimer's disease and amyotrophic lateral sclerosis, J. Alzheimer's Dis. 3 (2001) 377–385.
[8] M.D. Nguyen, M. Boudreau, J. Kriz, S. Couillard-Despres, D.R. Kaplan, J.P. Julien, Cell cycle regulators in the neuronal death pathway of amyotrophic lateral sclerosis caused by mutant superoxide dismutase, J. Neurosci. 23 (2003) 2131–2140.
[9] S. Ranganathan, R. Bowser, Alterations in G(1) to S phase cell-cycle regulators during amyotrophic lateral sclerosis, Am. J. Pathol. 162 (2003) 823–835.

[10] A. Appert-Collin, B. Hugel, R. Levy, N. Niederhoffer, G. Coupin, Y. Lombard, P. Andre, P. Poindron, J.P. Gies, Cyclin dependent kinase inhibitors prevent apoptosis of postmitotic mouse motoneurons, Life Sci. 79 (2006) 484–490.
[11] Z. Xiang, L. Ho, J. Valdellon, D. Borchelt, K. Kelley, L. Spielman, P.S. Aisen, G.M. Pasinetti, Cyclooxygenase (COX)-2 and cell cycle activity in a transgenic mouse model of Alzheimer's disease neuropathology, Neurobiol. Aging 23 (2002) 327–334.
[12] Y. Yang, N.H. Varvel, B.T. Lamb, K. Herrup, Ectopic cell cycle events link human Alzheimer's disease and amyloid precursor protein transgenic mouse models, J. Neurosci. 26 (2006) 775–784.
[13] C. Andorfer, C.M. Acker, Y. Kress, P.R. Hof, K. Duff, P. Davies, Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms, J. Neurosci. 25 (2005) 5446–5454.
[14] V. Khurana, Y. Lu, M.L. Steinhilb, S. Oldham, J.M. Shulman, M.B. Feany, TOR-mediated cell-cycle activation causes neurodegeneration in a *Drosophila* tauopathy model, Curr. Biol. 16 (2006) 230–241.
[15] U. Ueberham, T. Arendt, The expression of cell cycle proteins in neurons and its relevance for Alzheimer's disease, Curr. Drug Targets CNS Neurol. Disord. 4 (2005) 293–306.
[16] P. Juo, M. Kaplan, The anaphase-promoting complex regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of C. Elegans, Curr. Biol. 14 (2004) 2057–2062.
[17] Z. Huang, K. Zang, L.F. Reichardt, The origin recognition core complex regulates dendrite and spine development in postmitotic neurons, J. Cell Biol. 170 (2005) 527–535.
[18] Y. Yang, D.S. Geldmacher, K. Herrup, DNA replication precedes neuronal cell death in Alzheimer's disease, J. Neurosci. 21 (2001) 2661–2668.
[19] A. Copani, F. Condorelli, A. Caruso, C. Vancheri, A. Sala, A.M. Giuffrida Stella, P.L. Canonico, F. Nicoletti, M.A. Sortino, Mitotic signaling by beta-amyloid causes neuronal death, FASEB J. 13 (1999) 2225–2234.
[20] A. Copani, M.A. Sortino, A. Caricasole, S. Chiechio, M. Chisari, G. Battaglia, A.M. Giuffrida-Stella, C. Vancheri, F. Nicoletti, Erratic expression of DNA polymerases by beta-amyloid causes neuronal death, FASEB J. 16 (2002) 2006–2008.
[21] A. Copani, J.J.M. Hoozemans, F. Caraci, M. Calafiore, E.S. Van Haastert, R. Veerhuis, A.J.M. Rozemuller, E. Aronica, M.A. Sortino, F. Nicoletti, DNA polymerase-beta is early expressed in neurons of Alzheimer's disease brain and is loaded into DNA replication forks in neurons challenged with β-amyloid, J. Neurosci. 26 (2006) 10949–10957.
[22] D.T. Loo, A. Copani, C.J. Pike, E.R. Whittemore, A.J. Walencewicz, C.W. Cotman, Apoptosis is induced by beta-amyloid in cultured central nervous system neurons, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 7951–7955.
[23] J.H. Su, A.J. Anderson, B.J. Cummings, C.W. Cotman, Immunohistochemical evidence for apoptosis in Alzheimer's disease, NeuroReport 5 (1994) 2529–2533.
[24] X. Grana, E.P. Reddy, Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin dependent kinase inhibitors (CDKIs), Oncogene 11 (1995) 211–219.
[25] A.W. Murray, Recycling the cell cycle: cyclins revisited, Cell 116 (2004) 221–234.
[26] D.S. Park, B. Levine, G. Ferrari, L.A. Greene, Cyclin-dependent kinase inhibitors and dominant-negative cyclin-dependent kinase 4 and 6 promote survival of NGF-deprived sympathetic neurons, J. Neurosci. 17 (1997) 8975–8983.
[27] A. Giovanni, F. Wirtz-Brugger, E. Keramaris, R. Slack, D.S. Park, Involvement of cell cycle elements, cyclin-dependent kinases, pRB, and E2F-DP, in β-amyloid-induced neuronal death, J. Biol. Chem. 274 (1999) 19011–19016.
[28] Z. Nagy, Cell cycle regulatory failure in neurones: causes and consequences, Neurobiol. Aging 21 (2000) 761–769.
[29] A. Copani, D. Uberti, M.A. Sortino, V. Bruno, F. Nicoletti, M. Memo, Activation of cell-cycle-associated proteins in neuronal death: a mandatory or dispensable path? Trends Neurosci. 24 (2001) 25–31.
[30] U. Hubscher, H.P. Nasheuer, J.E. Syvaoja, Eukaryotic DNA polymerases, a growing family, Trends Biochem. Sci. 25 (2000) 143–147.
[31] B. Arezi, R.D. Kuchta, Eukaryotic DNA primase, Trends Biochem Sci 25 (2000) 572–576.
[32] E.C. Friedberg, R. Wagner, M. Radman, Specialized DNA polymerases, cellular survival, and the genesis of mutations, Science 296 (2002) 1627–1630.
[33] R.L. Siegel, G.F. Kalf, DNA polymerase beta involvement in DNA endoreduplication in rat giant trophoblast cells, J. Biol. Chem. 257 (1982) 1785–1790.
[34] J. Sweasy, L. Loeb, Mammalian DNA polymerase beta can substitute for DNA polymerase I during DNA replication in *Escherichia coli*, J. Biol. Chem. 267 (1992) 1407–1410.
[35] J.J. Hoozemans, M.K. Bruckner, A.J. Rozemuller, R. Veerhuis, P. Eikelenboom, T. Arendt, Cyclin D1 and cyclin E are co-localized with cyclo-oxygenase 2 (COX-2) in pyramidal neurons in Alzheimer disease temporal cortex, J. Neuropathol Exp. Neurol. 61 (2002) 678–688.
[36] J.J. Hoozemans, R. Veerhuis, A.J. Rozemuller, T. Arendt, P. Eikelenboom, Neuronal COX-2 expression and phosphorylation of pRb precede p38 MAPK activation and neurofibrillary changes in AD temporal cortex, Neurobiol Dis. 15 (2004) 492–499.
[37] M.V. Blagosklonny, Flavopiridol, an inhibitor of transcription: implications, problems and solutions, Cell Cycle 3 (2004) 1537–1542.
[38] Z. Tan, N. Sun, S.S. Schreiber, Immunohistochemical localization of redox factor-1 (Ref-1) in Alzheimer's hippocampus, NeuroReport 9 (1998) 2749–2752.
[39] S. Madhusudan, M.R. Middleton, The emerging role of DNA repair proteins as predictive, prognostic and therapeutic targets in cancer, Cancer Treat Rev. 31 (2005) 603–617.