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Cell cycle inhibition as a strategy for treatment of central nervous system diseases which must not block normal neurogenesis

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Abstract

Classically, the cell cycle is regarded as the central process leading to cellular proliferation. However, increasing evidence over the last decade supports the notion that neuronal cell cycle re-entry results in post-mitotic death. A mature neuron that re-enters the cell cycle can neither advance to a new G0 quiescent state nor revert to its earlier G0 state. This presents a critical dilemma to the neuron from which death may be an unavoidable, but necessary, outcome for adult neurons attempting to complete the cell cycle. In contrast, tumor cells that undergo aberrant cell cycle re-entry divide and can survive. Thus, cell cycle inhibition strategies are of interest in cancer treatment, but may also represent an important means of protecting neurons. In this review, we put forth the concept of the “expanded cell cycle” and summarize the cell cycle proteins, signal transduction events and mitogenic molecules that can drive a neuron into the cell cycle in various CNS diseases. We also discuss the pharmacological approaches that interfere with the mitogenic pathways and prevent mature neurons from attempting cell cycle re-entry, protecting them from cell death. Lastly, future attempts at blocking the cell cycle to rescue mature neurons from injury should be designed so as to not block normal neurogenesis.

Keywords

neuron; cell cycle; mitogen; mitogenic pathway; cell cycle re-entry; cell stress; CNS diseases; neurogenesis

Introduction

Postmortem studies over the last decade have revealed pathological evidence of aberrant expression of cell cycle related molecules in the neurons of the hippocampus, subiculum, locus coeruleus and dorsal raphe nuclei. Direct proof of DNA replication was also identified in brains of patients with Alzheimer's disease (AD) (McShea et al., 1997; Nagy et al., 1997; Vincent et al., 1997; Busser et al., 1998; Yang et al., 2001), epilepsy (Nagy and Esiri, 1998), Parkinson's disease (PD) (Jordan-Sciutto et al., 2003) and amyotrophic lateral sclerosis (ALS) (Ranganathan and Bowser, 2003). These important discoveries stimulated new hypotheses and studies challenging the traditional concept that post-mitotic neurons are terminally differentiated and maintained in the G0 quiescent phase. Although still controversial, evidence

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of cell cycle re-entry in neurons related to pathological conditions has been further confirmed in a number of reports, including experiments in primary neuron cultures (Copani et al., 1999; Copani et al., 2006; Zhang et al., 2006; Rao et al., 2007; Liu et al., 2008) and animal models of disease including AD (Herrup et al., 2004; Khurana et al., 2006; Yang et al., 2006), ALS (Nguyen et al., 2003), stroke (Imai et al., 2002; O'Hare et al., 2002), traumatic brain injury (TBI) (Di Giovanni et al., 2005) and cerebral hypoxia-ischemia (Kuan et al., 2004). Furthermore, our recent genomic and bioinformatic studies have consistently placed “cell cycle” as among the top ranked functional categories for gene transcripts that are altered in rats one day following any of several different types of insults to the central nervous system (CNS) (e.g., cerebral ischemia, intracerebral hemorrhage and kainate-induced seizures) (Tang et al., 2001; Tang et al., 2002; Liu et al., 2009b).

These examples demonstrate aberrant cell cycle re-entry as a hallmark of CNS diseases with dying neurons, and while cell cycle re-entry is more commonly associated with tumor cells, there are very different consequences of this event between these tissues. In contrast to neurons, when tumor cells re-enter the cell cycle, they survive and may continue to proliferate in the presence of an oncogene. For reasons not completely understood, a mature neuron that re-enters the cell cycle is neither able to advance to a new G0 quiescent state nor revert to its earlier G0 state. This presents a critical dilemma to the neuron from which death may be an unavoidable, but necessary, outcome for these mature neurons attempting to complete the cell cycle. Since re-entry into the cell cycle by neurons has been associated with many diseases and linked inextricably to death, the cell cycle represents a viable target for treatments and therapies, so long as the consequences on other cell types, such as neuroprogenitor cells, are considered.

As a way to describe potential therapeutic targets, we propose the “expanded cell cycle” — one which includes not only the traditional cell cycle proteins, but also the mitogenic molecules and the signaling pathways that interact with them. The “expanded cell cycle” includes some of the current targets for treating CNS diseases. It provides a composite perspective encompassing a broad range of molecules representing potential targets and thus approaches that can serve as treatments for CNS diseases by sharing a common outcome — cell cycle inhibition. A detailed description of the cell cycle and its components follows before we discuss the specific interactions that neurons have with cell cycle proteins.

Classical proteins and regulators of the cell cycle

The cell cycle is the series of events that lead to cell replication (Norbury and Nurse, 1992; Dirks and Rutka, 1997; Malumbres and Barbacid, 2001; Schwartz and Shah, 2005). In brief, the release of cells from a quiescent state (G0) results in their entry into the first gap phase (G1), during which the cells prepare for DNA replication in the synthetic phase (S). This is followed by the second gap phase (G2) and mitosis phase (M). When cells cease proliferating, either due to the presence of specific anti-mitogenic signals, or the absence of pro-mitogenic signals, they exit the cycle and enter the G0 quiescent phase. A majority of types of newly divided G0 cells can re-enter the cell cycle after passing specified checkpoints, whereas some types of cells, such as neurons, cannot. Because such a large number of molecules involved in the cell cycle have been discovered and characterized, we will provide a brief overview of these below.

Cyclin-dependent kinases and cyclins

Cyclin-dependent kinases (Cdks) are a group of serine/threonine kinases that form active heterodimeric complexes following binding to their regulatory subunits, cyclins (Malumbres and Barbacid, 2001). There are two main families of cyclins: (1) mitotic cyclins (e.g., cyclin A, cyclin B) and (2) G₁ cyclins (e.g., cyclin C, cyclin D, cyclin E) (Dirks and Rutka, 1997). Several Cdks — mainly Cdk4, Cdk6, Cdk2, Cdk1, and possibly Cdk3 — cooperate to drive

cells through the cell cycle (Malumbres and Barbacid, 2001). For example, Cdk4 and Cdk6 form active complexes with the D-type cyclins (cyclins D1, D2 and D3), which are thought to be involved in early G1 (Baldin et al., 1993; Ohtsubo and Roberts, 1993; Quelle et al., 1993; Resnitzky et al., 1994). The complexes of Cdk2 with cyclins E1 and E2 are required to complete G1 and initiate S phase (Resnitzky et al., 1994; Ohtsubo et al., 1995), whereas Cdk2 with cyclin A control S/G transition (Dirks and Rutka, 1997). Translocation of cyclin B with Cdk1 from cytoplasm into the nucleus heralds the onset of mitosis (together, cyclin B and Cdk1 are also called 'mitosis promoting factor'), and the destruction of cyclin B is required for exit from mitosis (King et al., 1994; Pines, 1995). The role of Cdk3 is still obscure, mainly due to its low expression levels (Malumbres and Barbacid, 2001).

Cyclin-dependent kinase inhibitors

There are two subclasses of cyclin-dependent kinase inhibitors (CdkIs) - the Ink4 family (including p16^{Ink4a}, p15^{Ink4b}, p18^{Ink4c} and p19^{Ink4d}) that prevents the formation of cyclin/Cdk complexes; and the Cip/Kip family (including p21^{Cip1}, p27^{Kip1} and p57^{Kip2}) that inhibits the kinase activity of the already formed cyclin/cdk complexes (Shankland and Wolf, 2000; Nagata et al., 2003; Copani and Nicoletti, 2005). Thus, these inhibitors regulate the cell cycle via assessing damage and arresting progress at any of several defined checkpoints (Sherr and Roberts, 1995; Dirks and Rutka, 1997; Carnero et al., 2000; Yang and Herrup, 2007).

Cdk substrates

The primary substrates of Cdk4/6 and Cdk2 in G1 progression are members of the retinoblastoma protein (Rb) family, including p107 and p130 (Lundberg and Weinberg, 1998; Harbour et al., 1999; Ezhevsky et al., 2001). Rb family members are phosphorylated by activated Cdk4/6/cyclin D and Cdk2/cyclin E complexes (Morgan, 1997; Adams, 2001). The pRb is released from the transcription factor complex E2F/DP, which then activates genes required for transition to the S phase (Malumbres and Barbacid, 2001).

Cell cycle re-entry in post-mitotic neurons results in death

Under physiological conditions, neurons are subjected to a variety of stimuli and signals. These include mitogenic signals that promote re-entry into the cell cycle, and also a series of anti-mitogenic factors that strive to maintain the neuron at rest (Copani and Nicoletti, 2005). However once brain injuries occur, this balance is lost. For example, some cell cycle proteins (e.g., cyclin D, PCNA and GADD34) are produced in mature neurons very soon after experimental rat brain ischemia (Guegan et al., 1997; Li et al., 1997; Katchanov et al., 2001; Imai et al., 2002). In addition, expression of cell cycle proteins was also observed in the brains of AD patients who had mild cognitive impairment (Yang et al., 2001), and 6–8 months before the onset of amyloid beta (A β) deposition in the A β precursor protein (APP) transgenic mouse models of AD (Yang et al., 2006; Varvel et al., 2008). These findings suggest that the initiation of cell cycle protein expression is an early event in these disease processes that may eventually lead to the death of mature neurons.

However, the expression of cell cycle proteins is not always associated with cell cycle re-entry by neurons. Recent studies have demonstrated that some core cell cycle proteins serve diverse post-mitotic functions that span various developmental stages of a neuron, including neuronal migration, axonal elongation, axonal pruning, dendrite morphogenesis, and synaptic maturation and plasticity (Frank and Tsai, 2009; Kim et al., 2009). Additionally, we, and others, have observed sporadic expression of cyclin D in unperturbed normal primary neurons, but there was no active Cdk4 detected in those neurons (Rao et al., 2007; Liu et al., 2008). Since G0/G1 transition is dependent on cyclin D/Cdk4 complex formation, cyclin D expression without active Cdk4 means that the control neurons could not re-enter the cell cycle (Rao et

al., 2007; Liu et al., 2008). When subjected to a mitogenic stimulus like thrombin, the neurons did re-enter the cell cycle, ultimately dying via apoptosis (Rao et al., 2007; Liu et al., 2008).

This supports the idea of a “two hit hypothesis”, similar to that first proposed by Zhu et al. and Yang et al. (Zhu et al., 2004; Yang and Herrup, 2007; Zhu et al., 2007). In this case the two conditions that must be met in order for aberrant cell cycle re-entry to occur in neurons are: (1) an elevation in cell cycle proteins and (2) an increase in promitogenic signals. Thus, even though mature neurons may express some cell cycle proteins, the amount produced is not sufficient on its own to drive the mature neuron to re-enter the cell cycle. The final death of the neurons likely requires the stimulus of additional pro-mitogenic molecules, such as thrombin, A β , reactive oxygen species (ROS), nitric oxide (NO), and others, which when elevated will trigger the mitogenic signal cascades in the injured neurons. Once mitogenic signaling is stimulated beyond a certain threshold, neurons appear to exit their quiescent state and re-enter the cell cycle.

The extent to which neurons proceed into the cell cycle and the phases whereby cell cycle re-entry leads to apoptosis vary in response to the mitogenic stimuli. Thrombin-treated neurons do not proceed to S phase, but rather die via apoptosis after the G0/G1 transition (Rao et al., 2007; Liu et al., 2008). This may be facilitated by the thrombin-induced decreases in G1/S regulators cyclin C and Cdk3, Cdk2 and Cdk1 (Rao et al., 2008). However, A β challenged neurons can proceed through S phase before dying (Copani et al., 1999). Yang *et al.* further confirmed the G1/S transition of cell cycle reactivated neurons by using fluorescent *in situ* hybridization (FISH) on samples from AD patients (Yang et al., 2003). This was a direct indication that DNA replication does occur in the mature neurons, as the FISH methodology differentiates between DNA replication and other synthetic events such as DNA repair.

However, these neurons that re-enter the cell cycle become trapped in S phase. They neither finish dividing nor revert to their G0 quiescent state (Katchanov et al., 2001; Yang et al., 2003; Kuan et al., 2004; Pallas and Camins, 2006). The most plausible explanation is that several factors involved in cell cycle progression are lost or inhibited in mature neurons. On the other hand, a neuron that re-enters the cell cycle cannot revert to an earlier G0 either, because the transitions through the mitotic cell cycle are irreversible processes (Novak et al., 2007). Presumably, the failure to complete the cell cycle in these stressed neurons triggers specific apoptotic or other cell death mechanisms to rid the tissue of these cells. Since cell cycle inhibition can prevent a neuron from reentering the cell cycle, and neuron death (or loss) is a general consequence in neurological diseases (Brasnjevic et al., 2008), cell cycle inhibition appears to be a candidate strategy for the treatment of these diseases.

Aberrant cell cycle re-entry in disease

Aberrant cell cycle re-entry is the hallmark of many tumor cells (Bartkova et al., 1996; Smith et al., 2007; Ishikawa et al., 2009). The cell cycle inhibitors (e.g., Cdk inhibitors) have been widely studied as cancer therapeutics. They have been used to inhibit growth of several types of tumor cells in numerous preclinical studies, both *in vitro* and *in vivo* (Drees et al., 1997; Schwartz et al., 1997; Arguello et al., 1998; Erickson et al., 1998; Chien et al., 1999; Shapiro et al., 1999; Tirado et al., 2005). Several Cdk inhibitors (Flavopiridol, UCN-01, Roscovitine, AT7519) have advanced to human clinical trials for evaluation as treatment for a broad range of solid tumors and hematological malignancies such as chronic lymphocytic leukemia (CLL) (Senderowicz, 1999; Senderowicz and Sausville, 2000; Whittaker et al., 2007; Wyatt et al., 2008).

Although tumor cells undergo uncontrolled proliferation, many tumors originate from adult tissues, in which the majority of cells are in the G0 quiescent phase (Malumbres and Barbacid, 2001). Thus, the cells that go on to form tumors and mature neurons share a common G0 state

of quiescence. However, if tumor cells re-enter the cell cycle, they survive and often proliferate, whereas mature neurons will die. Therefore, cell cycle inhibition helps protect neurons, but kills tumor cells. This is strongly supported by experiments that show Cdk inhibition prevents the death of nerve growth factor (NGF)-differentiated PC12 neuronal cells, but promotes the death of naive PC12 (pheochromocytoma) tumor cells (Park et al., 1996; Park et al., 1997). Thus, cell cycle inhibition may be useful for treatment of CNS diseases wherein cell cycle re-entry occurs. This is very plausible at present since there are many innovative approaches being tested as cancer therapies, such as miRNA-based gene therapy techniques (Tong and Nemunaitis, 2008).

In neurons, Cdk activation occurs prior to the release of cytochrome c, mitochondrial dysfunction, and caspase activation on the path to neuronal death (Endres et al., 1998; Fink et al., 1998; Stefanis et al., 1999; Greene et al., 2007). Cdk inhibition provides long-term rescue from death and prevents release of cytochrome c, loss of mitochondrial transmembrane potential, and prevents caspase activation and processing (Stefanis et al., 1999). In contrast, general caspase inhibitors do not affect early cytochrome c release and do not prevent the loss of mitochondrial transmembrane potential (Stefanis et al., 1999). Moreover, caspase inhibitors protect neurons from this rapid apoptotic death, but do not prevent them from undergoing delayed death in which nuclear features of apoptosis are absent (Stefanis et al., 1999). Thus, cell cycle re-entry (Cdk4 activation, G0/G1 transition) lies upstream of these other pathways producing neuronal death, and mature neurons that re-enter the cell cycle are likely programmed to die not only via caspase-mediated pathways, but also via non-caspase-mediated pathways.

Though no clinical trials of Cdk inhibitors are reported in the treatment of CNS diseases, preclinical experiments demonstrate that Cdk inhibitors (Flavopiridol, Olomoucine, Roscovitine, or Quinazolines) improve behavioral outcomes and increase neuronal survival in a series of CNS disease models such as AD (Copani et al., 2001; Jorda et al., 2003; Verdaguer et al., 2004b; Kruman, 2006; Mark Robert Barvian et al., 2006), PD (Kruman, 2006), stroke (Osuga et al., 2000; Wang et al., 2002), TBI (Di Giovanni et al., 2005; Hilton et al., 2008), spinal cord injury (SCI) (Di Giovanni et al., 2003; Tian et al., 2006), excitotoxic stress (Park et al., 2000; Verdaguer et al., 2003b; Verdaguer et al., 2003a; Verdaguer et al., 2004a) and optic nerve transection (Lefevre et al., 2002) (Table 1). Various side effects may arise because of the non-specificity of those Cdk inhibitors, which also makes it difficult to rule out actions on other molecules (Bain et al., 2003; Sausville, 2003; Blagden and de Bono, 2005; Sridhar et al., 2006; Bain et al., 2007). Fortunately, efforts are underway to develop compounds with increased selectivity for specific Cdks (Hirai et al., 2005; Sridhar et al., 2006).

“Expanded cell cycle” molecules and pathways

Clearer understanding of the mechanisms for neuronal cell cycle re-entry could lead to identification of new pharmacological targets for prevention of cell death and disease progression that lay outside the traditional cell cycle members (Table 1). One approach would be to develop targets based on a broadened or expanded sense of the cell cycle – one which includes not only the cell cycle proteins mentioned above, but also mitogenic molecules and the signaling pathways that interact with them.

Mitogenic molecules can function either as physiological signals or initiators of pathological events depending on their concentrations and activation states (Donovan and Cunningham, 1998; Strigrow et al., 2000; D'Autreaux and Toledano, 2007; Chiang et al., 2008; Liu et al., 2008). Increases in the level and activation of these molecules are an indication of increased mitogenic potential, particularly within the injured brain (Akiyama et al., 1992; Hua et al., 2003; Xi et al., 2003; Grammas et al., 2006; van Groen et al., 2006; Xi et al., 2006; Sokolova

and Reiser, 2008). This growing list of mitogenic molecules, besides thrombin, A β , ROS and NO, noted above, includes excitatory amino acids such as glutamate, various inflammatory cytokines such as interleukin-1 (IL-1), IL-2, IL-6, IL-18, prostaglandin E2 (PG E2), lipopolysaccharide (LPS), tumor necrosis factor- α (TNF- α) and others (Copani et al., 1999; Monfort et al., 2002; Paris et al., 2002; Li et al., 2003; Ishikawa et al., 2006; Caltagarone et al., 2007; Liu et al., 2008; Ojala et al., 2008; Xing et al., 2008). A wide variety of mitogenic molecules are recruited even by a single CNS disease. Each molecule often has a specific ligand-receptor interaction, but may affect multiple downstream signaling pathways (Demoulin and Renauld, 1998; Nathan, 2003; Caltagarone et al., 2007; Sturm et al., 2008; Sun et al., 2008).

The mitogenic signaling of one molecule is often modified or augmented by another. For example, mitochondrial failure results in the release of ROS, which enhance A β production. Intracellular A β accumulation in turn promotes ROS generation, producing a vicious cycle (Standridge, 2006). The signaling can also be accelerated by one molecule on its own, such as the autocrine cycling of NO, mediated by the inducible enzymes NO/ Ras/ Raf/ MEK-1/ ERK 1, 2/ NF- κ B/ eNOS/ NO (Copani and Nicoletti, 2005). These kinds of positive feedback make it possible to elevate molecules abruptly, either as a normal physiological response to disease, or as the cause of disease-induced damage itself.

The actions of mitogenic molecules are both diverse and overlapping, which provides for functional redundancy within mitogenic signaling transduction pathways. As biological cofactors that are enhanced by specific pathological conditions, mitogenic molecules activate specific pathways to mediate cell cycle re-entry and neuronal death. Examples of some mitogenic pathways that overlap and commonly lead to cell cycle re-entry include: (1) FAK/ Src/ Ras/ Raf/ MEK1, 2/ ERK1, 2 $\rightarrow$ cell cycle re-entry (Copani et al., 1999; Williamson et al., 2002; Wang and Reiser, 2003b; Ohnishi et al., 2007; Varvel et al., 2008); (2) Ras/ Rac1/ MEK3, 6/ P38 $\rightarrow$ cell cycle re-entry (Guan et al., 2005; Segarra et al., 2006); (3) PLC/ IP3/ PKC/ JNK $\rightarrow$ cell cycle re-entry (Dwivedi and Pandey, 1999; Lopez-Bergami and Ronai, 2008); (4) PI3K/ Akt/ mTOR/ Tau $\rightarrow$ cell cycle re-entry (Zhu et al., 2000; Khurana et al., 2006; Xing et al., 2008); and (5) JAK/ STAT $\rightarrow$ cell cycle re-entry (Goody et al., 2007; Tan, 2008). In addition, many molecules, including Ca²⁺, ROS, NO and PGE2, etc., can directly or indirectly increase the intensity of mitogenic signaling (Fiebich et al., 2001; de Bernardo et al., 2004; Copani and Nicoletti, 2005; Qian et al., 2006; Li et al., 2009; Mao et al., 2009).

MicroRNAs, which are endogenous, non-coding, single-stranded RNA molecules of 19–25 nucleotides in length, have recently attracted attention due in part to the fact that each miRNA can potentially regulate hundreds of genes. It is predicted that over one third of all human genes may be regulated by miRNAs (Bartel, 2004; Esquela-Kerscher and Slack, 2006; Chen and Rajewsky, 2007; Filip, 2007; Guarnieri and DiLeone, 2008). Several miRNAs modulate the major proliferation pathways through direct interaction with transcripts of critical regulators such as Ras, PI3K or ABL, members of the retinoblastoma family, cyclin-Cdk complexes and cell cycle inhibitors of the p27, Ink4 or Cip/Kip families (Gillies and Lorimer, 2007; Bueno et al., 2008). A complex interaction between miRNAs and E2F family members also exists to modulate cell cycle-dependent transcription during cellular proliferation (Brosh et al., 2008).

Agents that interfere with molecules and pathways of the “expanded cell cycle”

In theory any part of the “expanded cell cycle” could be a potential target for drug discovery. For example, an intracerebral hemorrhage would activate thrombin through the coagulation cascade and thrombin would go on to activate src family kinase members (Wang and Reiser, 2003a; Liu et al., 2008). Src family kinases will activate MAPK which will activate cdk4/

cyclinD complexes and promote cell cycle re-entry (Wang and Reiser, 2003b; Fujimoto et al., 2007; Liu et al., 2008). Thus, these molecules (thrombin, src kinase, and MAPK), while not considered traditional components of the cell cycle, would all be part of the “expanded cell cycle”. Similarly, other protein kinases (including JAK, Akt, PKC, JNK, ERK, P38, GSK-3 β , Cdk, etc.) are also important molecules in the mitogenic pathways leading to neuronal cell cycle re-entry. However, unlike the Cdk-specific inhibitors noted above, many of these kinase inhibitors are currently approved for human use, primarily for the treatment of cancer (Fabian et al., 2005). Since the theory of neuronal cell cycle re-entry was proposed, some of the kinase inhibitors have recently been examined experimentally in the treatment of CNS diseases (Table 1). However, these experiments have been challenging because many kinases play important roles in essential biological processes and many of the kinase inhibitors lack specificity for their targets.

Treatments using antioxidants, NMDA-receptor modulators, cytokine inhibitors, i/eNOS inhibitors, COX-2 inhibitors, and others have often worked fairly well in animal models of brain disease, but have generally failed individually in clinical trials with a few exceptions (Richard Green et al., 2003; Robinson and Keating, 2006; Abe, 2008). Many of these evaluations occurred before cell cycle re-entry was implicated as a mechanism for neuronal death. Even now, their direct effects on the cell-cycle have not been comprehensively studied, and combinations of some of these compounds may be useful for the purpose of cell cycle inhibition experimentally and/or clinically as treatment for CNS diseases.

It is now clear that neurogenesis occurs in the brain of adult mammals (Liu et al., 1998; Brandt and Storch, 2008; Neundorfer, 2008; Shen et al., 2008). This neurogenesis may be associated with maintenance or restoration of neurological function in animal models of CNS diseases, suggesting that neurogenesis is functionally important to recovery (Palmer et al., 2000; Ohab et al., 2006; Abdipranoto et al., 2008; Chesnokova and Pechnick, 2008; Liu et al., 2009a). Neurogenesis arises from brain progenitor cells, rather than from differentiated adult neurons.

Therapies directed at any component inhibiting the cell cycle must be as specific as possible considering cell cycle re-entry contributes to both the death of mature neurons and the genesis of neuroprogenitor cells in adult brain. Therefore, any therapeutics that prevent neuronal death by blocking mitogenic signaling may have limited benefit because they may also prevent neurogenesis. This may provide at least a partial explanation for the questionable efficacy of some currently approved drugs, such as the NMDA receptor modulator Memantine, in the clinical treatment of AD, since NMDA receptor activation has been shown to enhance progenitor cell proliferation and lead to increased neurogenesis (Suzuki et al., 2006). This is consistent with the clinical reports that cognitive dysfunction arises when cell cycle inhibition strategies are used in cancer therapeutics (Dietrich et al., 2008; Konat et al., 2008).

This cognitive dysfunction may also be explained by the fact that current cell cycle inhibition strategies are not cell-specific and also block the proliferation of important brain progenitor cells, thus impairing adult brain neurogenesis. Thus, it appears that cell cycle inhibition strategies could help protect neurons and improve disease and injury outcomes, as long as they do not interfere with the growth of other important cells in the brain. If drugs that block the cell cycle are used to prevent neuronal death in CNS diseases, it is likely that compounds would need to directly (or indirectly) block neuronal cell cycle re-entry and yet not affect the ongoing process of neurogenesis. This will only be possible if the signaling mechanisms are different in adult progenitor cells that divide in the adult brain, versus adult neurons that re-enter the cell cycle.

Conclusions

Cell cycle inhibition protects mature neurons from death. However, it is likely that to truly protect neurons, the best strategy may be to ensure they do not leave the G0 phase at all, since the mere entrance into the initial cell cycle may lead to unavoidable cell death. Moreover, future studies aimed at understanding the respective cell cycle pathways of mature neurons and neuronal progenitors are probably necessary before choosing the best drug targets for treating CNS diseases.

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Table 1

Agents that interfere with mitogenic signaling pathways and molecules of the “expanded cell cycle”.

Target	Agent	Stage/CNS Diseases
Tau solvent	TRx-0014	Clinical use in AD (TauRx-Therapeutics-Ltd., 2007; Williams, 2009).
Antioxidants	Edaravone NXY-059 Coenzyme Q10 Vitamin E Melatonin Trolox SOD NAC PBN	Clinical use in stroke in Asia (Wang and Shuaib, 2007; Abe, 2008) Clinical trials in stroke (Wang and Shuaib, 2007), AD (Berman and Brodaty, 2004; Vina et al., 2004; Park and Jin, 2008), ALS (Ferrante et al., 2005; Levy et al., 2006; Yoshino and Kimura, 2006). Experimental trials in ALS (Ito et al., 2008), PD (Yuan et al., 2008), SCI (Tanabe et al., 2009).
Glutamatergic modulators	Riluzole Ceftriaxone Talampanel MK-801 NBQX	Clinical use in ALS (Andrews, 2009). Clinical trials in ALS (Andrews, 2009). Experimental trials in ICH (Ardizzone et al., 2004), stroke (Meden et al., 1993).
NMDA-receptor modulators	Memantine	Clinical use in AD (Danysz and Parsons, 2003; Molinuevo et al., 2005; Robinson and Keating, 2006; Iraqi and Hughes, 2009). Clinical trials in ALS (Andrews, 2009). Off-label use in psychiatric disorders (Zdanys and Tampi, 2008).
IL-1 inhibitors	Kineret Rytvela	Experimental trials in hypoxic-ischemic newborn brain injury (Quiniou et al., 2008).
TNF- α inhibitors	Thalidomide CNI-1493	Experimental trials in AD (Li et al., 2004; Tweedie et al., 2007); PD (Li et al., 2004; Tweedie et al., 2007); stroke (Meistrell et al., 1997; Nawashiro et al., 1997; Barone and Parsons, 2000; Li et al., 2004; Tweedie et al., 2007).
Thrombin inhibitors	Hirudin	Experimental trials in ICH (Xue et al., 2006; Sun et al., 2008; Liu et al., 2009a).
Thrombin receptor-1 antagonist	BMS-200261	Experimental trials in stroke (Junge et al., 2003); PD (Hamill et al., 2007).
Secretase inhibitors	OM 99-1 OM 99-2 BMS 289948 BMS 299897	Experimental trials in AD (Roberds et al., 2001; Ghosh et al., 2002; Selkoe, 2003; Anderson et al., 2005).
i/eNOS inhibitors	l-NAME AMT NPA	Experimental trials in ICH (Lee da et al., 2006); SCI (Tanabe et al., 2009).
Cox-2 inhibitors	Rofecoxib Celecoxib NS-398	Clinical trials in AD (Etminan et al., 2003; Warner and Mitchell, 2004). Experimental trials in ICH (Lee da et al., 2006).
Ras inhibitors	Exoenzyme C3 Lovastatin	Experimental trials in motor neuron disorders (Donovan et al., 1997; Smirnova et al., 2001).
Ca ²⁺ channel blockers	Flunarizine	Clinical trials in stroke (Franke et al., 1996).
Calpain inhibitors	MDL-28170	Experimental trials in AD (Jordan et al., 1997; Di Rosa et al., 2002; Battaglia et al., 2003).
Src Inhibitors	PP1 PP2	Experimental trials in ICH (Ardizzone et al., 2007; Liu et al., 2008; Liu et al., 2009a); AD (Williamson et al., 2002).
PI3K inhibitors	LY 294002	Experimental trials in AD (Zhao et al., 2004).
JAK/Stat Inhibitors	EGCG	Experimental trials in AD, PD, HIV associated Dementia, multiple sclerosis (MS), ALS, or Pick's Disease (Tan, 2008).

Target	Agent	Stage/CNS Diseases
GSK-3 β inhibitors	Lithium Kenpaullone Indirubin SB 216763 SB 415286	Experimental trials in AD (Cross et al., 2001; Bhat et al., 2004; Jope and Johnson, 2004; Su et al., 2004; Dunn et al., 2005; Pallas and Camins, 2006). Clinical trials in ALS (Fornai et al., 2008; Andrews, 2009).
ERK1/2 kinase pathway	PD98059	Experimental trials in ICH (Fujimoto et al., 2007; Ohnishi et al., 2007).
P38 kinase pathway	SB203580 SB239063	Experimental trials in ICH (Fujimoto et al., 2007); PD (Karunakaran et al., 2008); stroke (Barone et al., 2001).
JUN kinase pathway	CEP-1347 Colostrinin SP600125	Experimental trials in ICH (Fujimoto et al., 2007; Ohnishi et al., 2007); AD (Leszek et al., 2002; Wang et al., 2004); stroke (Kuan and Burke, 2005); PD (Wang et al., 2004; Kuan and Burke, 2005).
CDK inhibitors	Flavopiridol Olomoucine Roscovitine Quinazolines	Experimental trials in AD (Copani et al., 2001; Jorda et al., 2003; Verdaguer et al., 2004b; Kruman, 2006; Mark Robert Barvian et al., 2006); PD (Kruman, 2006); stroke (Osuga et al., 2000; Wang et al., 2002); TBI (Di Giovanni et al., 2005; Hilton et al., 2008); SCI (Di Giovanni et al., 2003; Tian et al., 2006); excitotoxic stress (Park et al., 2000; Verdaguer et al., 2003b; Verdaguer et al., 2003a; Verdaguer et al., 2004a); optic nerve transection (Lefevre et al., 2002).
NF- κ B pathway	KINK-1	Experimental trials in stroke (Herrmann et al., 2005).

4-Amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo(3,4-d)pyrimidine (PP1) 4-Amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazol(3,4-d)pyrimidine (PP2)

2-Amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT)

2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX)

Epigallocatechin-3-gallate (EGCG)

Methythionium chloride (TRx-0014)

5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK 801)

N-acetyl-L-cysteine (NAC)

N(G)-nitro-L-arginine methyl ester (L-NAME)

N(omega)-propyl-L-arginine (NPA)

Phenyl-N-tert-butyl nitrone (PBN)

Superoxide dismutase (SOD)