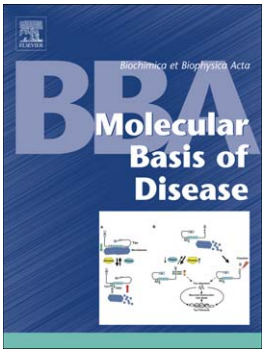
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Invited review for BBA-Molecular Basis of Diseases

Re-expression of cell cycle markers in aged neurons and muscles: Whether cells should divide or die?

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Abstract

Emerging evidence revealed that abrogated cell cycle entry into highly differentiated mature neurons and muscles is having detrimental consequences in response to cell cycle checkpoints disruption, altered signaling cascades, pathophysiological and external stimuli, for instance, Aβ, Parkin, p-tau, α-synuclein, impairment in TRK, Akt/GSK3β, MAPK/Hsp90, and oxidative stress. These factors, reinitiate undesired cell division by triggering new DNA synthesis, replication, and thus exquisitely forced mature cell to enter intoa disturbed and vulnerable state that often leads to death as reported in many neuro-and myodegenerative disorders. A pertinent question arises how to reverse this unwanted pathophysiological phenomenon is attributed to the usage of cell cycle inhibitors to prevent the degradation of crucial cell cycle arresting proteins, cyclin inhibitors, chaperones and E3 ligases. Herein, we identified the major culprits behind the forceful cell cycle re-entry, elucidated the cyclin re-expression based on disturbed signaling mechanisms in neuromuscular degeneration together with plausible therapeutic strategies.

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Key words: Post-mitotic cell division; signaling cascade; neuromuscular degeneration; cyclins; therapeutics

1. Introduction

The etiology of neuromuscular degeneration is characterized by the canonical deposition of non-functional/toxic proteins such as amyloid β, tau, parkin, α-synuclein, mutant huntingtin, and chronic inflammations in neuromuscular disorders (NMDs) including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Inclusion body myositis (IBM) and Poly myositis (PM). The deposition of pathological proteins or inclusions create a physiological burden on cell that triggers multiple signaling cascades including mitogen-activated protein kinase (MAPK), c-Jun N-Terminal Kinases (JNK), Phosphatidylinositol 3-kinase/serine threonine kinase Akt/Glycogen synthase kinase 3 (PI3K/Akt/GSK3β), Notch and apoptotic signaling pathways. The consequent over expression of cell cycle markers have been observed with the symptoms of cell cycle re-entry (CCR) [1]. Moreover, the co-localisation of cell cycle markers with pathological proteins has reinforced the role of cell cycle machinery as a trigger to degeneration [2]. Further evidence suggests that re-expression of cell cycle markers occurs at prodromal stages before the appearance of pathological hallmarks in these neuromuscular disorders. A plethora of studies have demonstrated the involvement of oxidative stress, aging, mutation, mitochondrial dysfunction; ubiquitin proteasome system (UPS) shut down and loss of function of several protective proteins in the pathogenesis of these NMDs. For instance, prolonged activation or under the influence of acute insults such as DNA damage, oxidative stress, neurotrophic factor deprivation and altered micro RNA (miR) function; cell cycle machinery turns detrimental and drives the cascade of DNA synthesis and consequent neuronal

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death culminating into neurodegeneration [3]. Interestingly, numerous studies have established cell cycle re-entry as a true phenomenon in neurodegeneration with actual DNA synthesis and cannot be viewed as an epiphenomenon of other processes. Moreover, cell cycle markers' re-expression in terminally differentiated neurons is also reported to fulfill the physiological demands pertaining to synaptic plasticity, neuronal migration and maturation [4]. Importantly, current studies advocate that aberrant cell cycle re-entry is not a consequence, but rather a cause of neuromuscular degeneration, depicting the importance of targeting cell cycle re-entry as a therapeutic window for treating neuromuscular disorders. These days several compounds/biomolecules have been implicated for targeting cell cycle re-entry in neurons and muscles. Moreover, potential role of ubiquitin E3 ligases and heat shock proteins have also been demonstrated to revert the damaging effect caused by cell cycle re-entry in neuromuscular disorders.

2. Relevance of Cell cycle markers (Cyclins, CDKs, CDKIs) in maintaining Cell cycle homeostasis

The eukaryotic cell cycle is orchestrated machinery that accomplishes fundamental roles in cell replenishment such as DNA replication, cell growth, repair and birth of new daughter cells from the native mother cell. The coordination of the cell cycle requires complex interplay between the levels of different cyclins and cyclin dependent kinases (CDKs) at different checkpoints. For instance, Cyclin C/Cdk3 complex mark the transition of cells into G1 i.e. preparatory phase. Further, G1/S transition is controlled by the crucial action of cyclin D/Cdk4/6 and cyclin E/Cdk2 complex [5]. The DNA replication in S phase is triggered by the cyclin A/Cdk2 and Cyclin A/Cdk1 complex. Cell division in M phase is tightly regulated by the activity of cyclin

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B/Cdk1complex [6]. The integrity of the cell cycle is maintained by the timely degradation of cyclins and consequent inactivation of CDKs, which is brought about by a class of cyclin dependent kinase inhibitors (CDKIs). These CDKIs are categorized into Ink family and Cip/Kip family [7]. The Ink family is comprised of $p15^{Ink4b}$, $p16^{Ink4a}$, $p18^{Ink4c}$ and $p19^{Ink4d}$ that bind with Cdk4/6 and are known to inhibit its association with cyclin D, thereby promoting quiescent stage [8]. On contrary, members of Cip/Kip family comprised of $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ that bind and regulate specific Cyclin/Cdk complexes during different stages of cell cycle progression [9]. Additionally, activation of different signaling cascades tightly regulates the checkpoints, which are strategically distributed throughout the cell cycle. The checkpoints ensure the correct completion of previous phase before the beginning of new cell cycle phase, and ascertain the correction of any defect and provide protection against the transmittance into daughter cells by halting the cell cycle until the repair has been done or by alternatively triggering cell death pathways. For instance, the G1 phase is regulated by two checkpoints in its early and late phase to assess cell size and growth factors required for promotion to S phase [10]. While, the S phase is regulated by tumour suppressor gene p53 in response to DNA damage wherein the cell cycle is halted for repair or alternatively triggering apoptosis if the damage cannot be repaired [11]. Furthermore, the G2/M transition is tightly controlled by phosphorylation dynamics of Cdk1 where the activatory phosphorylation of Cdk1 is carried out by Cdk-activating kinase complex at Thr160/Thr161 while inhibitory phosphorylation is mediated by Myt1 and Wee1 kinase at Thr14 and Tyr15 respectively. These phosphorylation events can be reversed by the action of cell division cycle 25 (Cdc25) thereby tightly regulating Cdk1 activity [12]. In this way, cell cycle markers play a discrete role in the normal physiology of neuronal cells. However, post mitotic

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expression of these markers is responsible for the onset of cell cycle re-entry mediated neuromuscular dysfunction.

3. Causative agents that force cell cycle for re-entry into neurons and muscles

There are various factors that are responsible for triggering cell cycle re-entry in neurons and muscles, including environmental toxins, genetic factors, and other cellular stresses. These factors have different mechanistic approaches for eliciting cell cycle abnormalities in neurons and muscles that have been discussed below and elucidated in **Figure 1.**

PLEASE INSERT FIGURE 1 HERE

Figure 1: Triggering factors associated with the forceful cell cycle re-entries. Hypoxia and oxidative stress are two critical factors responsible for triggering CCR. Hypoxic exposure increases HIF-1α that causes up-regulation of growth factors and subsequent reduction of p27 expression. Reduced p27 expression and increased HIF-1α transactivation inducecyclin/Cdk complex thereby activating cell cycle progression. Further, ROS mediated oxidative stress could lead to aberrant CCR viz. several different pathways. First, ROS accumulation can result in cumulative DNA damage and consequent alteration in new DNA synthesis/replication, chromatin remodeling and transcriptional activation, signaling cascade activation and subsequent activation of cell cycle markers and thus CCR. Further, redox signaling activates pro-inflammatory cytokines (IL-1, IL-6, TNF-α) that triggers NF-κβ mediated arachidonic acid pathway metabolism and consequent CCR. In addition, ROS triggers Aβ mediated Tau phosphorylation and consequent dysfunction of ubiquitin-proteasome system and activation of mitogenic signaling pathways that also induce cell cycle progression. Hif1α-Hypoxia-inducible factor 1-alpha; Hif1β-Hypoxia-inducible factor 1-beta; CDK-Cyclin dependent Kinase; ROS-Reactive oxygen species; JNK-c-Jun N-terminal kinase; JAK/STAT-Janus kinase/signal transducers and activators of transcription; PI3K-Phosphoinositide 3-kinase; FOXO-Forkhead box protein; PL-Phospholipid; pRb-Phosphorylated retinoblastoma; IL-Interleukin; TNFα-Tumor necrosis factor-α; EETs-Epoxyeicosatrienoic acids; Cox-Cyclooxygenase.

3.1 Potential Cell cycle re-entry triggering factors

3.1.1 Environmental Factors

Recently, certain environmental factors have been identified, which contributed in the pathogenesis of several neuromuscular disorders. These environmental toxins comprised of heavy metals, pesticides and metal based nanoparticles, which are currently being studied for their role in CCR mediated neuromuscular degeneration. For instance, administration of methyl

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mercury (MeHg) is found to alter the expression of cell cycle regulators p16 and p21 in rat embryonic cortical neuronal stem cells. Moreover, compromised cell proliferation and a significant decrease in global DNA methylation were observed subsequent to MeHg treatment in NSCs, supporting the idea that its long-term exposure would lead to neurodevelopmental or neurodegenerative disorders [13]. Further, exposure to various other toxins likekainic acid, quinolinic acid and heavy metals, including cobalt, lead and iron was shown to be responsible for reactive gliosis. Accordingly, induced reactive gliotic changes led to cell cycle re-entry attempts in neurons [14]. Recently, fluoride and aluminium were found to disrupt neuronal morphology and cell cycle induced progressive cell death by triggering the activation of cell cycle markers and lysosomal proteins [15]. Additionally, a renal carcinogen OchratoxinA (OTA) induced cell cycle aberrations in renal tubular cells [16]. Moreover, environmental factors are found to exacerbate the neuromuscular degeneration in action with other triggering factors. There are numerous reports demonstrating the toxic effect of heavy metals, pesticides and nanoparticles in neurodegeneration, but very few showing the lethal effect of these toxins in the cell cycle re-entry mediated neuromuscular degeneration. Therefore, significant researches need to be carried out to determine the involvement of heavy metals and pesticides in cell cycle induction related neuromuscular toxicity.

3.1.2 Oxidative Stress

Oxidative stress is the most vulnerable and obvious cause for inducing cell cycle irregularities in post mitotic neurons and muscles reported till the date. Reactive oxygen species (ROS) generation is the prime factor responsible for causing oxidative stress, which in turn alters the

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components of mitogenic signaling cascade by activating various signaling molecules. For instance, MAPK, nuclear factor κB (NF-κB) and growth factor receptors are activated via ROS and initiate cell cycle re-entry in postmitotic neurons [17]. Interestingly, ROS also induced DNA damage; chromosomal breaks and base mis-incorporation in cell cycle events [18]. Moreover, ROS was also found to abort the activity of DNA repair proteins associated with the DNA replication process in AD. Numerous studies have reported ROS mediated unrepaired, oxidized nitrogenous bases and DNA strand breaks in post-mortem tissues of patients with different neuromuscular disorders [19]. Hypoxia has emerged as a critical player of cell cycle induction in neurons and muscles. Additionally, various studies have reported DNA replication in post-mitotic neurons upon exposure to hypoxia. Apart from DNA damage, oxidative stress is proposed to trigger cell cycle re-entry in response to UPS dysfunction [20]. However, UPS is evident to regulate the cell cycle in two disparate manner via degrading either cyclin D1 or Cdk inhibitors p21/p27 [18]. Likewise, altering the levels of HSPs including HSP 27, HSP 70 and HSP90, also trigger cell cycle re-entry via controlling different stages of the cell cycle. For instance, blocking of HSP90 action with geldanamycin, halted cell cycle at G0/G1 transition by obstructive action of HSP90-specific client proteins Cdc37 and FKBP52 [21]. Besides Kwon et al demonstrated the interaction between HSP70 and cyclin D or E1 in IBM and PM biopsy samples depicting their role in cell cycle progression [22]. Another study reported the association between Hsc70 and cyclin D1 to regulate its assembly with Cdk4 and Cip/Kip proteins and formation of a catalytically active complex [23]. Therefore, oxidative stress in concordance with cell cycle re-entry markers contributed significantly in the etiology of neuromuscular degeneration.

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3.1.3 Double stranded DNA break (DSB)

DSB accumulation and its attainment of non-repairable conformation is another crucial factor for initiating cell cycle re-entry in post-mitotic neurons [24]. DNA damage activates ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR) and E2F transcription factor 1 (E2F1), and other proteins, which regulate cell cycle; DNA damages repair, and apoptosis in post-mitotic neurons [25]. Chk1 and chk2 are the checkpoint kinases that are induced in response to DNA damage in the cell cycle. ATM is a well-known checkpoint in the cell cycle which regulates dsDNA break response in a p53 dependent manner and controls cell cycle arrest, apoptosis and mediates protection against oxidative stress [26]. Moreover, phosphorylated ATM has been shown to be elevated in cerebellar dentate neurons of AD patients when compared with age-matched controls [27]. Further, increased ATM expression accompanying down regulation of TP53-inducible glycolysis and apoptosis regulator (TIGAR) protein constituted a key feature of dementia associated with Alzheimer's disease. Furthermore, DSB mediated accumulation of functionally inactive monomers and dimers of TP53 have been reported in AD brain [28]. Likewise, E2F complexes are associated with histone-modifying enzymes, histone modifications, including histone acetylation and methylation, and therefore, essential for cell cycle re-entry that may play a regulatory role in DNA repair or apoptosis [25]. Moreover, evidence for DSB had also been reported in s-IBM muscles that resulted in muscle fiber maturation arrest [29]. Importantly, recent studies are going on to explore the relationship between DNA damage and cell cycle re-expression in different systems.

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3.1.4 Micro RNAs (miR)

miR has recently gained attention due to their strategic control of cell cycle regulation in post mitotic neurons. For instance, over-expression of miR26 has been reported to trigger aberrant cell cycle re-entry, DNA replication, and tau hyper phosphorylation in rat primary post mitotic neurons. Moreover, elevated level of miR-26b triggered the re-expression of cell cycle proteins cyclin E1, pRb, PCNA, Ki67 and BrdU in post-mitotic neurons [30]. Additionally, miR26b over-activation elicited Cdk5 mediated enhanced tau phosphorylation *in vitro* and *in*-vivo studies. It also attenuated the action of cell cycle inhibitors p15 and p27 [31]. In another study, increased miR-26b level induced cyclin E1 up-regulation and downregulated $p27^{kip1}$ expression mediated by Rb1/E2F protein complex, signifying its role in cell cycle regulation [30]. Further, genetic and epigenetic evidence revealed that NF-κB-regulated micro RNAs including miRNA-9, miRNA-125b, miRNA-146a and miRNA-155 are involved in the down regulation of small brain and retinal cell related family of target mRNAs causing complement factor H (CFH) deficiency leading to inflammatory neurodegeneration in AD and age-related macular degeneration (AMD) [32]. Likewise, disruption of miR34a and miR-132 has been linked with AD and other NDDs. For instance, Aβ mediated suppression of miR-34a resulted in unscheduled cell cycle re-entry and apoptosis via MEK-ERK pathway mediated degradation of tumor suppressor TAp73 in AD mouse model. Interestingly, numerous studies are evident for the over-expression of miRNAs in temporal cortex and hippocampus region of AD patients signifying that erroneous miRNA regulation is involved in the etiology of various NDDs [33]. Recently, altered miRNA expression has been observed in IBM, PM and other muscular dystrophies that are involved with myoblast

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differentiation and muscular regeneration [34]. However, few studies reported the role of microRNA in the cell cycle re-entry, but micro RNA research is still at preliminary stage.

3.1.5 Brain derived Neurotrophic Factor (BDNF)

BDNF is a member of neurotrophin family of growth factors that plays a crucial role in neuronal cell survival, growth and differentiation. It protects neurons against glutamate and oxidative stress, i.e. NMDA or H_2O_2 mediated toxicity, might be through inhibiting Ras-MAPK pathway. However, altered BDNF has been implicated in the cell cycle re-entry mediated pathogenesis of neuromuscular disorders during oxidative or excite toxic stress responses [35]. For instance, BDNF downregulatedcyclin B and Cdk1 levels (generally localized in NFTs in AD) in differentiating tetraploid neurons in the retina through TrkB neurotrophic receptor-mediated activity and thereby halted the cell cycle at G_2/M checkpoint. The inability of this complex to translocate into the nucleus likely played a role in blocking G_2/M transition [36] and abnormal tau phosphorylation, thereby contributing in the pathogenesis of AD [35]. Additionally, BDNF found to trigger inhibition of Cdk1 activity through phosphorylation of the site Tyr15, thereby halting G2/M transition in tetraploid neurons [36]. Another study identified that nerve growth factor (NGF) induces cell cycle re-entry through a novel, Cdk4/6-independent pathway, i.e. p^{38}/MAPK-Dependent E2F4 Phosphorylation mediated neurodegeneration [37]. The neurotrophin system also exhibits a role in myogenesis and regeneration in skeletal muscle biology. Moreover, differential study of BDNF receptors identified p75 neurotrophin receptor (p75NTR) to be predominantly expressed in human myocytes [38]. Presently, BDNF deprivation

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was well implicated in the cell cycle re-entry mediated pathogenesis in neurons, but its investigation in muscles is still desired.

3.2 Altered signaling cascades in aberrant cell cycle re-entry induced neuromuscular degeneration

Although there are several risks, factors identified to be involved in aberrant CCR but the demonstration of consequent alteration of signaling cascade is still at preliminary stage. However, a growing body of evidences have highlighted the involvement of multiple signaling pathways such as Notch, Wnt/β-catenin, Akt/GSK3β, P^{38}/MAPK and dyotic signaling in the biology of cell cycle re-entry **(Figure 2).** For instance, treatment with kainic acid is reported to activate notch signaling that augmented the aberrant CCR via activation of CyclinD1-Rb-E2F1 axis. Moreover, this abundance of cyclin D1 expression was accomplished by Akt/GSK3β pathway in AD [39]. Similarly, elevated levels of β-catenin through wnt/β-catenin signaling in PS1 FAD brain has been found to accelerate CCR simply by upregulating cyclin D1 transcription [40]. Further, elevated cyclin D1 contributes towards the apoptotic response mediated neuronal cell death. Additionally, disturbed Cdk5 associated neurotrophic signals have also been reported to cause neuronal dysfunction. For instance, Cdk5 mediates phosphorylation of Ape1, MEF2D and GSk-3β and contribute in the pathophysiology of neuromuscular disorders [41]. Ape1 plays a crucial role in DNA repair mechanism while MEF2D, a pro-survival transcription factor assists in memory development [42]. Moreover, Cdk5 also inhibits protein phosphatase-1 (PP1) and promotes P^{38}/MAPK activity, which in turn leads to tau hyperphosphorylation and consequent microtubule dissociation [43]. Further, Cdk5 also involved with Parkin phosphorylation and glutamate or dopamine mediated neurotoxicity in PD and HD respectively [44]. Likewise, Cdk5

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is also responsible for altering Drp1 activity thereby increasing mitochondrial fission in HD. Besides, it is also reported to induce tau hyperphosphorylation and consequent increased Perikaryal inclusions of neuro-filaments (NF) in the SOD1 amyotrophic lateral sclerosis (ALS) mice model [45]. Furthermore, the increased expression of procaspase-3 and cleaved caspase-3 and 9 i.e. apoptotic signaling is observed in IBM/PM sample to be associated with Aβ accumulation. Since, accumulated Aβ in IBM/PM has evidenced to downregulate the Akt expression and subsequent alteration in G1/S phase transition thereby triggering aberrant CCR [22]. Recently, dyotic signaling has also been identified to be associated with CCR in post mitotic neuron. Since, this signaling trigger altered AβPP processing and neuronal hypertrophy in M phase of cell cycle, thus governing aberrant CCR in AD [40]. Moreover, recent studies are going on to identify the cell cycle markers associated signaling disturbances in post mitotic neurons and muscles to demonstrate the pathophysiology behind the cell cycle re-entry mediated neuromuscular degeneration. Such altered signaling associated with cell cycle markers in different neuromuscular disorders have been summarized in **Table 1**. Nevertheless, numerous signaling pathways are currently being explored in different models to unravel the complexity of cell cycle re-entry mechanisms in various NMDs.

PLEASE INSERT FIGURE 2 HERE

Figure 2: Disturbed signaling cascades associated with an aberrant cell cycle re-entry in neuromuscular degeneration. Multiple signaling pathways including Notch, Wnt/β-catenin, Akt/GSK3β, P^{38}/MAPK and dyotic signaling get affected in response to neurotophic signals, ROS production and neurotoxins attack and contribute towards the pathogenesis of neuromuscular disorders. ROS induced dyotic signaling alters AβPP processing and neuronal hypertrophy in M phase of cell cycle in post mitotic neurons. Neurotrophic signals mediated Cdk5 activation triggers neuronal dysfunction via phosphorylating Ape1, MEF2D and GSK-3β that govern the onset of NMDs. Further, Cdk5 also inhibits protein phosphatase-1 (PP1) and promotes P^{38}/MAPK activity, which in turn leads to tau hyperphosphorylation and consequent microtubule dissociation. In addition, it also directs Parkin's

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phosphorylation and glutamate or dopamine mediated neurotoxicity in PD and HD respectively. Similarly, altered Drp1 activity is also mediated via Cdk5 activity that augments mitochondrial fission in HD. Importantly, in case of ALS, mutant SOD1 impairs Cdk5 activity and subsequently causes tau hyper phosphorylation mediated Perikaryalneurofilaments (NF) accumulation. Further, neurotoxins induced Notch signaling causes aberrant CCR via activation of CyclinD1-Rb-E2F1 axis in AD. Similarly, increased levels of β-catenin viz. wnt/β-catenin pathway accelerate CCR via upregulating cyclin D1 transcription in PS1 FAD brain. Additionally, Aβ mediated increased expression of procaspase-3 and cleaved caspase-3 downregulatesAkt expression and subsequent alteration in G1/S phase transition i.e. CCR. Furthermore, EGFR mediated MK2 activation inhibits Cdc25B, which in turn promote Cdc2 and Cyclin B complex and cause neuronal dysfunction. ROS-Reactive oxygen species; Cdk5-Cyclin dependent kinase 5; GSK3β-Glycogen synthase kinase 3β; CREB-cAMP responsive element binding protein; PP1-Protein phosphatases 1; MEF2D-Pro-survival transcription factor; Ape1-Human AP-endonuclease 1; MK2-MAPK-activated protein kinase-2; NF-Neurofilaments; EGFR-Epidermal growth factor receptor; PS1-Presenilin 1.

Table 1: Consequences of altered cell signaling in the post mitotic cell division

4. Aberrant cell cycle re-entry in Neuromuscular Disorders

Scientists are investigating whether the re-expression of various cell cycle markers symbolizes an authentic cell cycle, or it is just a consequence of other pathological events. The growing evidence supports the notion that cyclin's re-expression is a true phenomenon representing actual re-activation of the cell cycle in post-mitotic neurons and muscles. However, cell cycle re-entry in terminally differentiated neurons and muscles is lethal and has been widely implicated in the pathogenesis of degenerative disorders like AD, PD, IBM/PM, HD and ALS **(Figure 3)**.

4.1 Alzheimer's Disease

Alzheimer's disease is the most common progressive neurodegenerative disorder, well characterized by the intracellular deposition of hyper-phosphorylated form of microtubule associated protein; Tau and extracellular aggregation of amyloid beta (Aβ) protein manifesting in neurofibrillary tangles (NFT) and senile plaques respectively. However, increasing evidencehas defined the role of cell cycle re-entry as one of the keys causal phenomenon in neuronal death

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associated pathogenesis of AD. Moreover, cell cycle aberrations are directly linked with the increase in pathological accumulation of Aβ and tau hyper-phosphorylation. Furthermore, cell cycle re-entry preceded NFT and senile plaque formation in AD and cell cycle markers were found to co-localize with NFTs, further reinforcing that cell cycle re-entry posits an early laid phenomenon in the pathogenesis of AD [24,63]. The cdc7 dependent phosphorylation of mini-chromosome maintenance protein 2 (Mcm2) at Ser40/41 has been reported in AD neuronal cytoplasm and NFTs, supporting the evidence of cell cycle re-entry in a DNA replication manner. However, it is interesting to note that the Mcm2 expression has not been reported in non-proliferating tissues, rather, its accumulation is observed in the cells re-entering G1 phase. Moreover, phosphorylated Mcm2 gets accumulated in the cytoplasm and is supposed to alter the minichromosome maintenance protein complex (MCM) to prevent cytokinesis in AD neurons [64]. Interestingly, mitotically active neuronal cells also trigger tau phosphorylation with the help of CDKs, which in turn stimulate the NFT formation. In a study of human tau expressing Drosophila model, Tau phosphorylation was carried out by Checkpoint kinase 1 & 2 (chk1/2) at Ser 262, which is an AD-related site [65]. In addition, Over-expressed chk2 mediated Ser 262 phosphorylation resulted in increased tau-led neurodegeneration [66]. Likewise, APP is also notably upregulated in response to mitogenic stimuli by cell cycle markers. The APP-BP1 adaptor protein, which plays an important role in APP cleavage, is a cell cycle protein involved in S-M phase transition. Augmented APP-BP1 level drives S phase mediated DNA replication in neurons accompanied by expression of cyclin B1 and cdc2 [67]. Similarly, *in-vitro* studies revealed that Presenilin 1 & 2 (PS1/PS2) over expression arrested the cycle in G1 phase while its knockdown promoted G1/S transition [40]. Further, transgenic mice studies with human APP

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mutant model (K670M/N671L), also corroborated the findings of upregulated G1/S cell cycle proteins like cyclin D1 and cyclinA in frontal cortical layers [68]. The cyclins re-expression intervened mitogenic signaling is thought to be induced by advanced glycation end products (AGEs) released from activated microglia and astroglia upon Aβ trigger. Further, in an apolipoprotein E-deficient mice model, AGEs deposition triggered cyclin D1 expression and consequent DNA replication through RAGE-ERK1/2 MAP Kinase Signaling Pathway [69]. Further, reports suggest that Aβ induced cell cycle re-entry in neurons can only be driven in the presence of tau phosphorylation carried out by non-receptor tyrosine kinase- fyn, cAMP-regulated protein kinase A and calcium-calmodulin kinase II at Y18, S409 and S416 sites respectively [70]. Moreover, it is also notable that Aβ is itself mitogenic *in-vitro*, thereby controlling the cell cycle events in AD. The evidence for Aβ induced cell cycle re-entry in post-mitotic neurons was marked by the re-expression of cyclin D1, PCNA and BrdU incorporation, evident of new DNA synthesis. Moreover, Aβ exerted a dose dependent effect on the expression of various cyclins. For instance, low Aβ triggered cyclin D1 expression, while high Aβ induced cyclin B1 [63]. Further, cell cycle reactivation is evident of non-homologous end joining (NHEJ) events in differentiated neurons. Recent studies demonstrated the elevated expression of cyclinD1, phosphorylated retinoblastoma protein (pRb), global cell cycle regulators such as Ki-67 and Mcm2 in relation with NHEJ response [24]. Cell cycle in AD is driven through G1/S phase, which is evident, by the increased level of cyclin E in different regions of the hippocampus in AD brains in comparison with age-matched controls. Moreover, upregulated cyclin E activity matched well with neuritic plaque burden in the neocortex region [46]. The affected neurons in AD also exhibit a crucial role of Cdk5 in cell cycle regulation. It has been

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demonstrated that nuclear Cdk5 plays a pivotal role in keeping neurons in the post-mitotic state as they mature while loss of nuclear Cdk5 leads to cell cycle entry [71]. Thus, increasing evidences confirm that Aβ and tau have a temporal relationship with the cell cycle re-entry and consequent neurodegeneration.

4.2 Parkinson's Disease

The signature, toxic accumulation of alpha-synuclein (α-syn) protein, characterizes the pathology of Parkinson's disease. The phenomenon of cell cycle re-entry has also been observed in PD. For instance, expression of E2F-1, pRb and proliferating cell nuclear antigen (PCNA), a marker of dividing cells has been evident in around 3-6% of substantia nigra pars compacta (SNpc) neurons in PD brains with respect to controls [55]. Moreover, neurons also showed aneuploidy of selected chromosomes signifying the involvement of DNA replication machinery. Further, the markers of G1/S phase; cyclin D and cyclin E were upregulated upon MPP^+ exposure in neuronal culture, suggesting the activation of Cdk4/6 and Cdk2 respectively. Moreover, experimental results suggest that Cdk2/cyclin E complex is more vulnerable than Cdk4/cyclin D or Cdk6/cyclin D complex [72]. Another study reported increased level of cyclin D in SNpc subsequent to rotenone administration in rats. Additionally, elevated levels of DNA polymerase β induced endoreplication were also found to be involved in the pathogenesis of PD [73]. Furthermore, microarray expression analysis revealed the association between cyclin-G-associated kinase (GAK) polymorphism rs1564282 and α-synuclein in post-mortem frontal cortex of PD brain [74]. The non-classical cell cycle protein Cdk5 possibly caused phosphorylation of Parkin, thereby reducing its E3 ligase activity and preventing the ubiquitination of toxic substrates. Moreover, several studies reported that Cdk5 also mediated

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MPTP induced neuronal cell death. Accordingly, MPTP induced activation of calpains, which triggered cleavage of p35 into p25 thereby inducing over-activation of Cdk5, which, in turn resulted in neuronal apoptosis. Furthermore, Cdk5 mediated higher levels of phosphorylated Peroxiredoxin-2 (Prx2), a neuronal antioxidant is observed in SNpc neurons in PD indicating its involvement in neuronal death [55]. This evidences suggested a potential connection between Cdk5 and cell cycle re-entry in PD. In addition, MPP induced ROS generation also triggered pRb dependent cell cycle reentry and subsequent ATM mediated DNA damage in animal models as well as in PD patients [75]. Thus, experimental studies strongly advocate the causal role of CCR in Parkinson's disease pathogenesis.

4.3 Inclusion Body Myositis and Polymyositis

IBM is the most common, progressive and debilitating, neuro inflammatory muscle fibre disease among the elderly that is primarily characterized by intra-muscular fibre aggregation of congophilic, misfolded/toxic multi-proteins and vacuolization [76]. Most importantly, IBM shows similar pathogenic manifestations as AD such as Aβ deposits, paired helical filaments containing hyperphosphorylated tau, aggregates of Aβ precursor protein (AβPP) and presenilin1 [77]. Similarly, the common morbid feature between IBM and PD has also been noticed including, enrichment of α-synuclein, Parkin and oxidized DJ-1 in muscle fibres. Such sharing of pathological features between AD, PD and IBM clearly points towards a common underlying switch to neuro-muscular degeneration marked by alteration of these proteins. However, PM has been much debated as to co-exist with IBM than as an independent phenomenon. PM is regarded as a T-cell mediated autoimmune disease typically manifesting as a painless, proximal myopathy [34]. The actual cause of occurrence and series of events leading to IBM/PM has not been fully

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understood as is the case with NDDs, though multiple factors, including toxic protein burden, UPS failure, aging and oxidative stress remained the common triggering mechanisms. Recently, Aβ induced aberrant CCR has been shown to be involved in the pathogenesis of IBM and PM. The markers of G1/S transition and proliferation, including cyclin D1, E, and Ki67 were also found to be up-regulated in both IBM and PM samples along with a parallel decrease in the level of cell cycle inhibitor p21. Further, cyclin D1 and E were shown to interact with HSP70 whose level was significantly increased in IBM and PM where heat shock proteins might be playing a role in stabilizing the cyclins [22]. Moreover, the level of various tau-phosphorylating cell cycle kinases including ERK, Cdk5 and GSK3β have been found to co-localize with inclusions containing hyper-phosphorylated tau in degenerating muscle fibres in IBM [78]. Further, co-localized ERK in tau containing paired helical filaments (PHFs) strongly suggested its role in directing tau hyper-phosphorylation led pathogenesis in IBM muscle fibres. Additionally, ERK activation was also proposed to contribute in the Activator protein 1 (AP-1) transcription factor mediated phosphorylation of AβPP gene thereby altering Aβ synthesis and processing. Furthermore, postsynaptic activation of ERK at neuromuscular junctions in normal muscles and at non-junctional regions in IBM muscle fibres provided evidence for its role in mediating junctional protein dynamics in health and disease respectively [79]. More evidence of cell cycle activation in IBM/PM has been provided by studies involving the action of NAD^+-dependent histone deacetylase (HDAC), Sirtuin 1 (Sirt1). Sirt1 was shown to promote cell cycle progression in adult skeletal muscle stem cells; muscle precursor cells (MPC) and are involved in the growth, repair and regeneration capability of adult skeletal muscle fibres. The over-expression of Sirt1, induced MPC proliferation through cell cycle activation in G1/S phase as

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evidenced by Rb phosphorylation and decreased expression of cell cycle inhibitor p21 & p27. Further, Sirt 1 induced cell cycle activation progressively into S phase and directed new DNA synthesis as indicated by the elevated level of PCNA and BrdU[80]. On contrary, the deacetylase activity of Sirt 1 is neuroprotective and its increased level has been shown to rescue neurons against Aβ induced toxicity through NF-κB inhibition or by inducing α-secretase in various models of tauopathies including AD and ALS [81]. However, the deacetylase activity and protein level of Sirt1 in the nucleus was found to be reduced in human sporadic IBM muscle fibres. Further, Sirt1 proteins are evident for forming cytoplasmic aggregates in response to ER stress. Moreover, increased AβPP and Aβ accumulation in IBM muscle fibres along with NF-κB activation is attributed to decreased Sirt 1 activity. Besides, upregulation of NF-κB was also reported in PM [82]. Taken together, muscular degeneration in IBM/PM seems to be a manifestation of aberrant CCR mediated cell death through DNA synthesis, as is the case with AD, PD and Poly Q diseases.

4.4. Other Neurodegenerative Disorders

Numerous studies on post-mortem brain revealed the pathological evidence of cell cycle re-entry in various other neurodegenerative disorders such as epilepsy, ALS, traumatic brain injury (TBI) and cerebral hypoxia-ischemia. The cell cycle re-entry was marked by the formation of cyclin D/Cdk4 complexes (G0/G1 transition) in these NDDs. Recent studies pointed towards oxidative stress as the main mechanism responsible for CCR. Experimental evidence suggests that oxidative stress mediated DNA damage activate ATM, which in turn activate the expression of E2F1 and consequent neuronal apoptosis. Furthermore, E2F-1 induced apoptosis may be p53-dependent or independent where multiple mitochondrial pathways might be activated including

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caspase dependent and independent signaling cascades. Similar evidence is observed in the western blot analysis of post-mortem brain samples in Huntington's patients [58]. Further, mHTT-perinuclear inclusions mediated cell cycle re-entry has been observed in primary cortical neurons of transgenic R6/2-J2 HD mice [83]. Another study reported an alteration in the ATF6α/Rheb pathway in HD patients triggering CCR [84]. Another finding identified the role of p25/Cdk5 in dopamine and glutamate neurotoxicity linked with HD [44]. Further, erroneous cell cycle entry and neuronal death has been demonstrated by kainic-acid induction through Notch dependent mechanism *in-vivo*. Additionally, they identified increased bioavailability of Cyclin D1 through Akt/GSK3β pathway and subsequent CCR mediated neurodegeneration via CyclinD1-Rb-E2F1 axis [39]. Recently, mutant cyclin F, a component of an E3 ubiquitin-protein ligase complex in neuronal cells has been detected through whole exome sequencing of ALS (Amyotrophic lateral sclerosis) and FTD (Frontotemporal dementia) patients. These mutants caused neuronal degeneration by altering ubiquitination process and precipitating ubiquitinatedproteins, including TDP-43 [85]. Previous reports on ALS also suggested the involvement of mutant SOD1 in Cdk5 mediated deregulation at G1-S checkpoint in neuronal death [86]. Since there is little evidence of cell cycle re-entry in other neurodegenerative disorders, further researches are required to demonstrate the involvement of cell cycle re-entry in traumatic brain injury (TBI), epilepsy and other neurodegenerative disorders of the CNS.

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Figure 3: Cell cycle re-entry mechanisms in neuromuscular disorders. The pathogenesis of HD is mediated by altered activity of Cdk5, micro RNA-124 and mutant HTT, which triggers the activation of cyclins, disruption of nuclear membrane and thus subsequent CCR. While Neurotoxin (3NP) induced cyclin re-expression is also responsible for CCR in HD. Similarly, Aβ and Sirt-1induced re-activation of Cyclin D1, Cyclin E, Ki67, PCNA, Rb and reduced p27 level is responsible for CCR in case of IBM/PM. While in case of ALS, mutant SOD1 contributes towards CCR via induction of Cdk5 and reduction of p27. Similarly, microRNA-19 modulates the expression of CCR critical genes and thereby contributes towards CCR in ALS. Likewise, in case of AD, Aβ mediated Tau hyperphosphorylation triggers CCR and causes Cdk5 activation via NMDA induced Calmodulin activation. Further, MPTP activity that induced various factors such as Calpain and ATM, which triggers the cell cycle markers and contributes towards CCR mediated pathogenesis of PD. Additionally, Parkin and NOS also altered the activity of Cyclin E, p53 and α syn that triggers the apoptosis as well as impaired neuronal differentiation in PD progression via CCR. PHF-Paired helical filaments; Cdk-Cyclin dependent Kinase; miR-Micro RNA; 3NP-3-Nitropropionic acid; mtHTT-Mutant huntingtin; Sirt1-Sirtuin 1; PCNA-Proliferating cell nuclear antigen; mtSOD1-Mutant Superoxide dismutase1; Bcl2-B-cell lymphoma 2; PKA-Protein kinase A; CaMKII-Calmodulin-dependent protein kinase II; NMDA-N-methyl-D-aspartate receptor; CaM-Calmodulin; MPTP-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NOS-Nitric oxide synthase; MPP^+-1-methyl-4-phenylpyridinium; ATM-ataxia-telangiectasia mutated; Bax-Bcl-2-associated X protein; Rb-Retinoblastoma.

5. Post-mitotic cell division: Imperative or Impulsive?

Earlier neurons were believed to be 'truly' post-mitotic and thus being life-long arrested in the quiescent stage G_0. Paradoxically, mounting evidence suggested the exit of resting neurons, which actively participated in the untimely cell cycle re-entry upon receiving mitogenic stimuli, synthesized DNA and were subsequently crucified [64]. One of the possible explanations to the contradictory premise of 'permanent' post-mitotic neuronal state is to consider that neurons had to constantly suppress the urge of cell cycle reactivation in lieu of their healthy state or face the reverberation of death. For instance, Rb protein is one such cell cycle marker, which serves crucial function of life-long cell cycle suppression in adult neurons [87]. Another candidate ataxia-telangiectasia mutated (ATM) protein triggered checkpoints during the DNA damage response under physiological conditions and maintained neurons in quiescent stage in adult CNS [88]. Moreover, deregulation of ATM signaling is observed in at-risk neurons in AD brains.

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Likewise, Cdk5, a non-classical Cdk is found to ensure post-mitotic state of neurons by sequestering transcription factor E2F1 and thereby preventing its interaction with the activation subunit. While in response to the encountered neuronal stress, Cdk5 is translocated to the cytoplasm, thus relieving its cell cycle inhibition and inducing cell cycle re-entry. Further ATM, breakdown of Cdk5 also contributed to the pathogenesis of AD [71]. Similarly, the ubiquitin E3 ligase APC/Cdh1 also acted as the suppressor of a cell cycle in the adult brain [89].

However, increasing evidence showed the presence of different cell cycle markers in adult neurons, which was attributed to their requirement in neuronal maturation, migration and synaptic plasticity. Evidences also suggest that cell cycle activation is a crucial event for non-homologous end joining (NHEJ) repair for the double stranded breaks incorporated by excessive ROS under chronic inflammation. It has also been noticed that post mitotic neurons undergoing DNA repair did not enter S phase, but remain in G1 phase. While unconquerable DNA damage proceed towards G1/S transition evidenced by increased BrdU, CDK2 and cyclin E expression, subsequently leading to apoptosis. For instance, Rb protein served a key post-mitotic role in neuronal migration in conjunction with transcription factor E2F3 [90]. Further, ATM regulated synaptic functions, including Long-Term Potentiation and was involved in trafficking in adult neurons [89]. Another marker, cell-division cycle protein-20 (cdc20) subunit of ubiquitin E3 ligase APC/C induced NeuroD2 degradation found to promote presynaptic axonal differentiation. Further, cdc20 mediated CaMKIIβ driven retraction and pruning of dendrites. Likewise, Cdh1 modulated growth and patterning in axons, and its inhibition favoured axonal growth. Furthermore, p27 has been proposed to be a microtubule binding protein owing to its

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binding and disruption of Cofilin-actin complex upon Cdk5 phosphorylation, thereby promoting neuronal migration [91]. Similarly, p57 mediated cell cycle exit and promoted differentiation and maturation in post-mitotic neurons. Thus, the cell cycle machinery is 'repurposed' to serve important demands of the post-mitotic cells. Yet, such re-expression of cell cycle proteins under various stressors such as oxidative stress, DNA damage and neurotrophic factor deprivation forced mature neurons to re-enter the cell cycle and die at G_1/S transition before actual mitosis could begin. The DNA content and consequent cell death in at-risk neurons in AD has been proposed to corroborate with advancing disease stage. These neurons had diploid DNA content in early AD which shifted to hyperploid DNA in advanced stages of the disease. Additionally, the tetraploid neurons were positive for cyclin B1 which was indicative of re-entry into cell cycle. However, since neurons are mitotically incompetent, only about 2% of these completed DNA synthesis and had double DNA content. Moreover, majority of the tetraploid neurons remained arrested in S phase and at G2/M transition without completing DNA synthesis [92]. Further, these hyperploid neurons represented attenuated viability, breakdown of differentiation control and thus were selectively sacrificed during the progression of the disease [93,94]. Thus, aneuploidy marked an early signature of neuronal death in AD [95]. On the other hand, though a fraction of healthy neurons had more than double DNA content, these lacked the expression of cyclin B1 thus delineating their 'non-cycling' status. The tetraploid neurons in normal brains can be attributed to chromosomal missegregation during mitosis. Further, the constitutive expression of cyclin B1 in normal adult brains can be similarly attributed to extra-cell cycle roles pertaining to microtubule dynamics, neuronal plasticity, differentiation and tau phosphorylation [92]. Since, during normal development, tetraploid neurons are arrested at G_2/M and any attempt to go

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beyond this checkpoint induces apoptosis [96]. Therefore, it is not surprising that the affected neurons in AD completed S phase, arrested the cell cycle at G_2/M transition phase and remained in the tetraploid state for some years. These neurons never entered mitotic phase [40]. The evidence of failed mitosis was further agreed by shunted chromatin condensation and spindle formation. The role of various cell cycle markers including the evidence of DNA synthesis in CCR mediated NMD is outlined in **Table 2**. To sum up, erroneous cell cycle re-entry offers two choices to neurons; either they can divide and die in S phase or remain alive with double DNA content at G_2/M transition. Since neurons inherently lack mitotic competence, and no evidence of M phase entry has been reported in NDD, cell cycle re-entry in terminally, differentiated neurons drive them to death after successfully synthesizing new DNA. Thus, cell cycle re-entry into adult neurons constituted an early signature of neuromuscular degeneration and CNS injury.

Table 2. A consolidated list of various Cell cycle markers that actively participate in post mitotic neurons and related disorders as listed (shaded in grey); (–) indicates no reported action of that particular cell cycle markers

6 Therapeutic strategies for targeting cell cycle re-entry in Neuromuscular degeneration

The ectopic cell cycle re-entry causes abnormal cyclins and CDKs expression and alters the activity of various CDK inhibitors thereby contributing towards cellular imbalances. To target or reverse the chronic effects associated with cell cycle aberrancy in neuromuscular degeneration is a tedious task for neurobiologists. Till date, multiple targets and signaling pathways of the altered cell cycle have been reported that need to be addressed. In order to overcome such dyshomeostatis, numerous therapeutic strategies are currently being devised that slow down or attenuate the toxicity associated with the abnormal cell cycle re-entries in various neuromuscular

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disorders. One such strategy involves the implication of various CDK-inhibiting compounds for targeting NMDs. However, these days several compounds/biomolecules that possess anti-apoptotic, anti-proliferative and antioxidant properties have been shown to target various abnormally expressed CDKs and cyclins in NMDs. For instance, the protective role of Flavopiridol has been reported in neurodegenerative disorder like AD, PD and ALS. Flavopiridol is a semi-synthetic flavonoid that found to inhibit all CDKs and reduces cyclin D1 mRNA transcription by arresting G1 and G2/M phase transition of the cell cycle [106]. Similarly, Simvastatin (SIM) has found to inhibit the serum-mediated enhancement of cell proliferation in AD by blocking the events critical for G1/S transition. Further, it also induces a partial blockade of retinoblastoma (Rb) protein phosphorylation and is also responsible for inhibition of cyclin E/Cdk2 activity associated with increased levels of the CDK inhibitors p21(Cip1) and p27(kip1) [107]. Likewise, the protective role of Interleukin-1β (IL-1β) has also been identified in neurodegeneration through cell cycle arrest mechanism. It induced cell cycle arrest and apoptosis in neural precursor cells (NPCs) through an oxidative stress-dependent mechanism that resulted in p53-mediated induction of the CDK inhibitor p21 and the proapoptotic Bcl-2 family members Puma (p53-upregulated modulator of apoptosis) and Noxa[108]. Moreover, there are numerous other compounds identified so far that have been implicated for targeting aberrant CCR. The mechanism associated with therapeutic action of such compounds at different cell cycle check points are outlined in **Table 3**. Most of these biological compounds prevent the neurons and muscles from S phase entry and thereby provide protection against subsequent NMDs. Furthermore, these compounds have defined a beginning point for driving forward screening of potential therapeutic agents with desired pharmacological activity.

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Table 3: Different drugs and biomolecules to attenuate the cell cycle progression

6.1 Emerging therapies for preventing Cell cycle re-entry

Although, the important role of various bio-chemical compounds have been explored extensively to target altered cell cycle re-entry in neuromuscular disorders, yet, several new therapeutic strategies have been recently identified to target such alterations. Various studies have pointed towards the employment of UPS in the correction of CCR induced NMDs. The UPS plays a pivotal role in modulating cell cycle checkpoints, CDKs, transcription factors and other molecules critical for CCR. For instance, when a neuronal cell enters S phase, Cdk5 is transported to the cytoplasm, where it is ubiquitinated and rapidly degraded by the action of ubiquitin E3 ligase APC-Cdh1 complex. Further, APC-Cdh1 is responsible for degrading cyclin B1 where its accumulation was observed in the degenerating brain areas of AD patients [114]. Currently, p10, the N-terminal domain of p35, has also been shown the protective role against Cdk5/p25-induced toxicity in neurons [115]. Since, altered role of Cdk5 has been identified in disease pathology of NDDs. Further, it has been observed that raised expression of Skp1 protein corrects the malfunction of the E3 ligase SCF complex. Since, malfunction of the E3 ligase SCF complex subsequently results in dysregulation of cell cycle regulators of the G_1/S phase transition like cyclins, CDKs, and cell cycle inhibitors thereby leading to neurodegeneration [116]. Likewise, SCF complex controls the G1/S and G2/M transitions by maintaining the level of β-catenin, p21, p27 and cyclin E in HD. Another Ubiquitin E3 ligase, Parkin protected neurons against cyclin E accumulation by associating with a protein complex, including hSel-10 and Cullin-1 [117]. Importantly, molecular chaperones in general preserve intracellular protein homeostasis in cooperation with the UPS. Therefore, HSPs are also being induced upon the cell

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cycle trigger in neurons and muscles that regulate the G1 phase. For instance, increased expression of chaperone HSP70 along with cyclins E/D1 during cell cycle progression has been reported in both IBM and PM muscle and in AD brain. Moreover, the interaction between HSP70 and cyclins E/D1 has crucial importance during cell cycle progression in the inflammatory myopathies, probably controlling cyclin maturation or degradation. Similarly, elevated levels of HSP90 in human IBM and PM biopsy specimens have been reported as, presumably in a stress-protective role [22]. Further, targeting cell cycle proteins could be another potential therapeutic approach for treating NMDs. For instance, inhibition of Cdk4 prevents both inductions of Bim expression and thrombin-induced neuronal apoptosis. Since, exposure of neuronal cultures to thrombin causes induction of cell cycle proteins cyclin E and cyclin D1, at both mRNA and protein levels thereby triggering cell cycle re-entry [118].

Interestingly, abnormal activation of the MEK-ERK MAP kinase pathway could be a potential modulator for cell cycle proteins. For instance, aberrant activation of this signaling axis regulates Cyclin D1 expression and forces neurons into the S phase, leading to cell cycle-related neuronal apoptosis (CRNA) [119]. On contrary, Cyclin D1, in turn, prevents the activation of Cdk5 by its neuronal cyclin-like activator p35 and provides neuroprotection [120]. Similarly, ablating Notch signaling in neurons provides neuroprotection against kainic acid-induced neuronal death in AD. Since, kainic-acid treatment *in vivo* elicits erroneous CCR and neuronal death through a Notch-dependent mechanism, which further increases the bioavailability of CyclinD1 through Akt/GSK3β pathway, leading to aberrant CCR via activation of CyclinD1-Rb-E2F1 axis [39]. Furthermore, treatment with quercetin has currently reported to act as a disruptor of the β-catenin/TCF transcription complex and consequently, reduced cyclin D1 levels and reversed the cell cycle/cell death phenotype in the PS1 Familial Alzheimer's disease (FAD) brain [40].

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However, elevated expressions of β-catenin in the PS1 FAD brain accelerate CCR simply by increasing cyclin D1 transcription. Further, the induction of numerous cyclin-dependent kinase inhibitors, including, p16, p19, p21 and p27, have been reported as a protective molecule in AD. These inhibitors attenuate the phosphorylation activity of Cdk4 and Cdk6 thereby protecting neurons from apoptotic insults [121]. Currently, the regulatory role of Smad protein has been observed in AD brain via regulating the transcriptional activation of Cdk4. In the normal adult brain, Smad proteins are constitutively phosphorylated and predominantly localized in neuronal nuclei. Under neurodegenerative conditions such as AD, the subcellular localization of phosphorylated forms of Smad protein is heavily disturbed, lead to a loss of neuronal differentiation control and successive cell cycle re-entry [122]. Further, the involvement of histone deacetylases inhibitors such as suberoylanilidehydroxamic acid (SAHA) and sodium butyrate (NaB) and rapamycin, an inhibitor of mechanistic target of rapamycin (mTOR) has recently been reported to normalize the proliferation of PD lymphoblasts via preventing the over-expression of the cyclin D3/Cdk6/pRb signaling cascade. These drugs are having neuroprotective roles in both human neuroblastoma SH-SY5Y cells and primary rat mid-brain dopaminergic neuronal cultures toxicity induced by 6-hidroxydopamine [56]. Furthermore, humungous amount of researchers are still required in near future to explore the potential and probable therapies against cell cycle aberrancy to alleviate the disease atmosphere of neuromuscular disorders.

7. Conclusion

Re-expression of cell cycle markers in aged neurons and muscles is identified as an intriguing process for aggravating neuromuscular disorders. Earlier its re-expression was confounded by their multiplicity of functions in a *bonafide* mitotic process, but later it is reported to be showing partial mitotic cell division and subsequent cell death. The mechanism behind the cell cycle re-

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entry includes the homeostatic imbalances among the levels of different cyclins and Cycin dependent kinases. The cell cycle markers play a pertinent role in marking the cell for mitotic division or for controlling other functions like synapse regulation and damage repair. The recent research has identified the disturbances in multiple signaling cascades controlling the process of cell proliferation and apoptosis. Such CCR mediated disturbed signaling includes Notch, Wnt/β-catenin, Akt/GSK3β, P^{38}/MAPK and dyotic signaling pathways in post mitotic neurons and muscles. Moreover, numerous triggering factors forcing cell cycle re-entry has been identified, which includes, environmental factors like toxins, pesticides and metal based nanoparticles; oxidative stress, double-stranded DNA break (DSB), UPS dysfunctions, Micro RNAs, and deprivation of the brain derived neurotrophic factor (BDNF). Further, cell cycle aberrations have been widely studied in AD, while its investigation in other neurodegenerative disorders and muscular degenerative disorders like IBM and PM is still at preliminary stage. The upcoming research prospects are to device the potential methods for targeting the cyclin/CDKs dysregulation in neuromuscular degeneration. Currently, in order to target aberrant CCR or reverse the chronic effects of such abnormalities, numerous biomolecules and chaperones mediated therapeutic strategies have been adopted to obviate the disease symptoms. For instance, Flavopiridol, Roscovitine, and Olomoucineas CDKs inhibitor are used as a drug for the treatment of AD, PD, and ALS. Likewise, the potential protective role of numerous flavonoids such as Resveratrol, Apigenin and Epigallocatechin-gallate has also been observed for inducing cell cycle arrest at different checkpoints in AD, PD and HD. Moreover, numerous ubiquitin E3 ligases such as APC-Cdh1 complex has shown the protection against Cdk5/p25-induced toxicity in neurons via its proper ubiquitination and degradation. Similarly, HSP70 along with cyclins

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E/D1 during cell cycle progression in both IBM and PM muscle, AD brain has shown promising role in controlling cyclin maturation or degradation. Further, it is reasonable to proclaim that the current knowledge's on the involvement of aberrant CCR in the pathogenesis of NMDs are still at an elementary stage. However, in light of its substantial progression from both clinical research and therapeutic application perspectives, we look forward to major research efforts being drawn in this field and more approaches being formulated in the near future.

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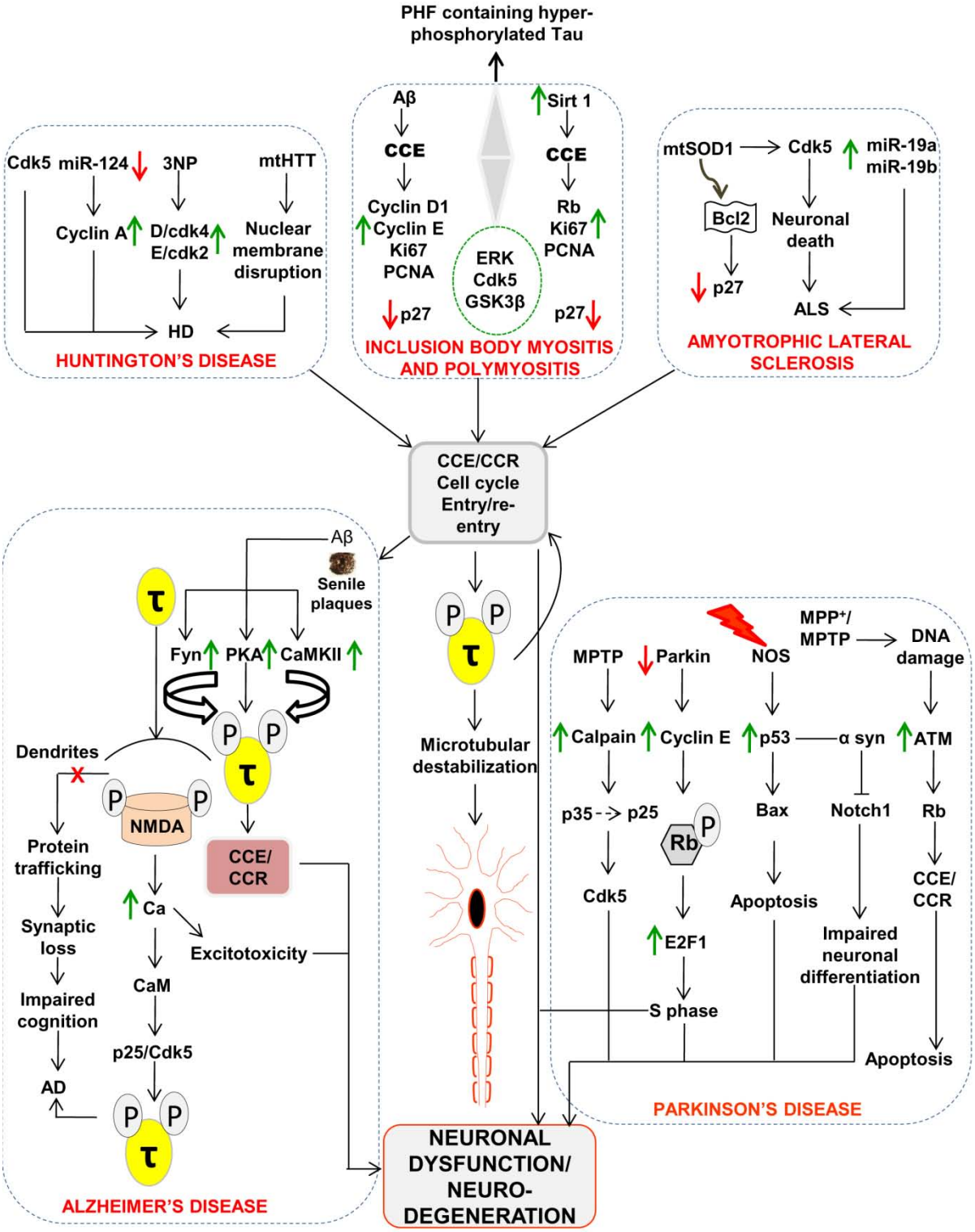
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Invited review for BBA-Molecular Basis of Diseases

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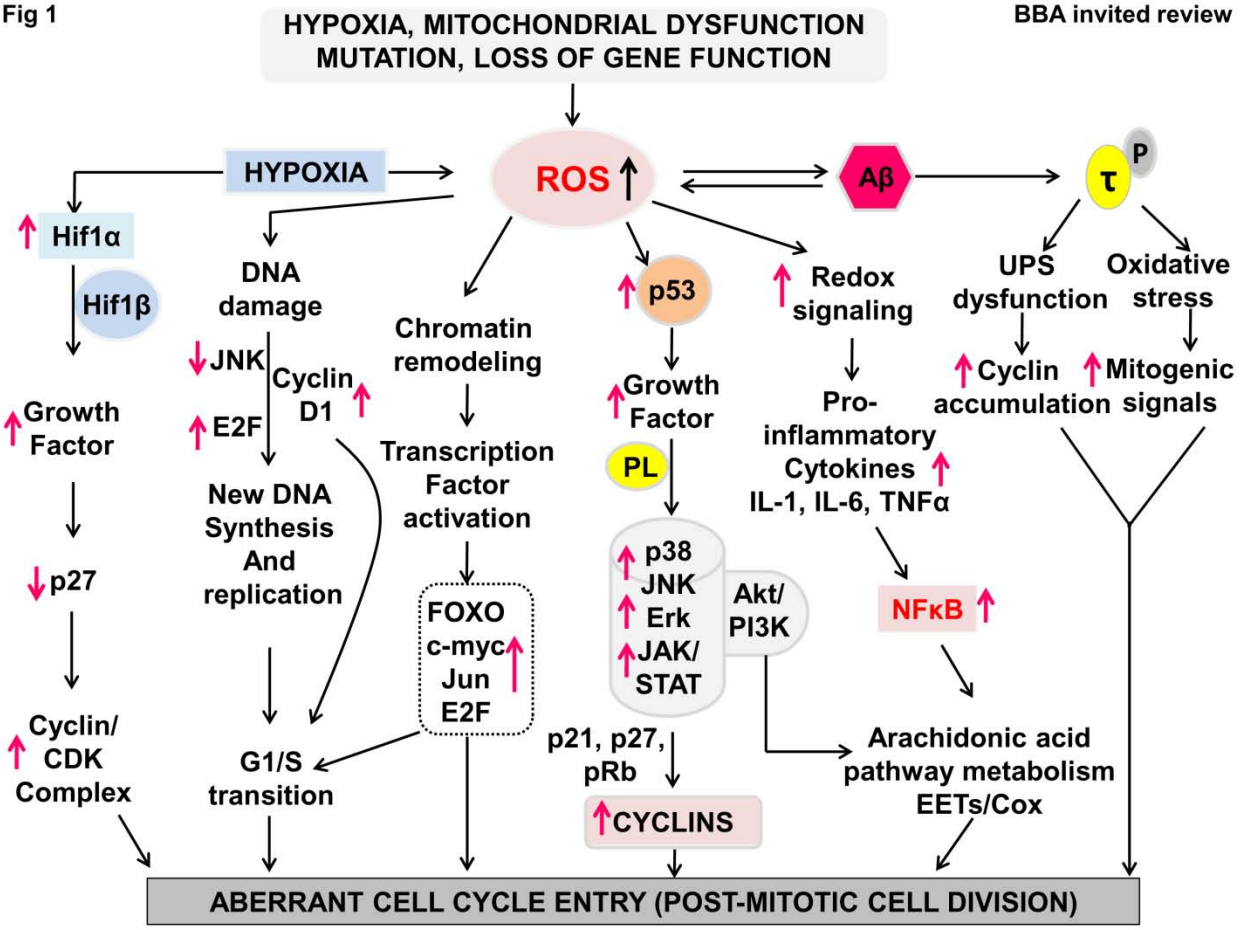
Graphical Abstract



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Fig 1

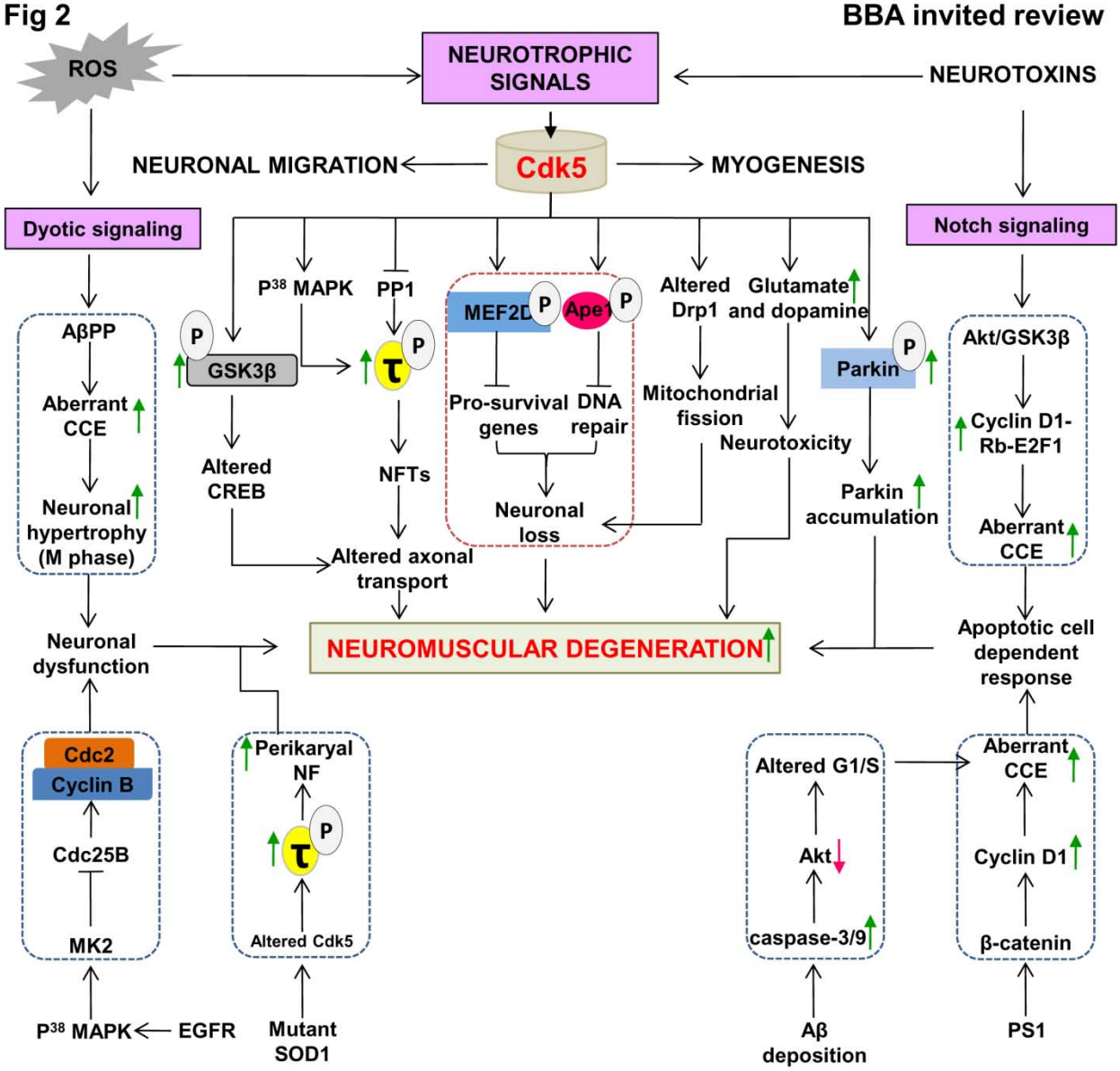
BBA invited review



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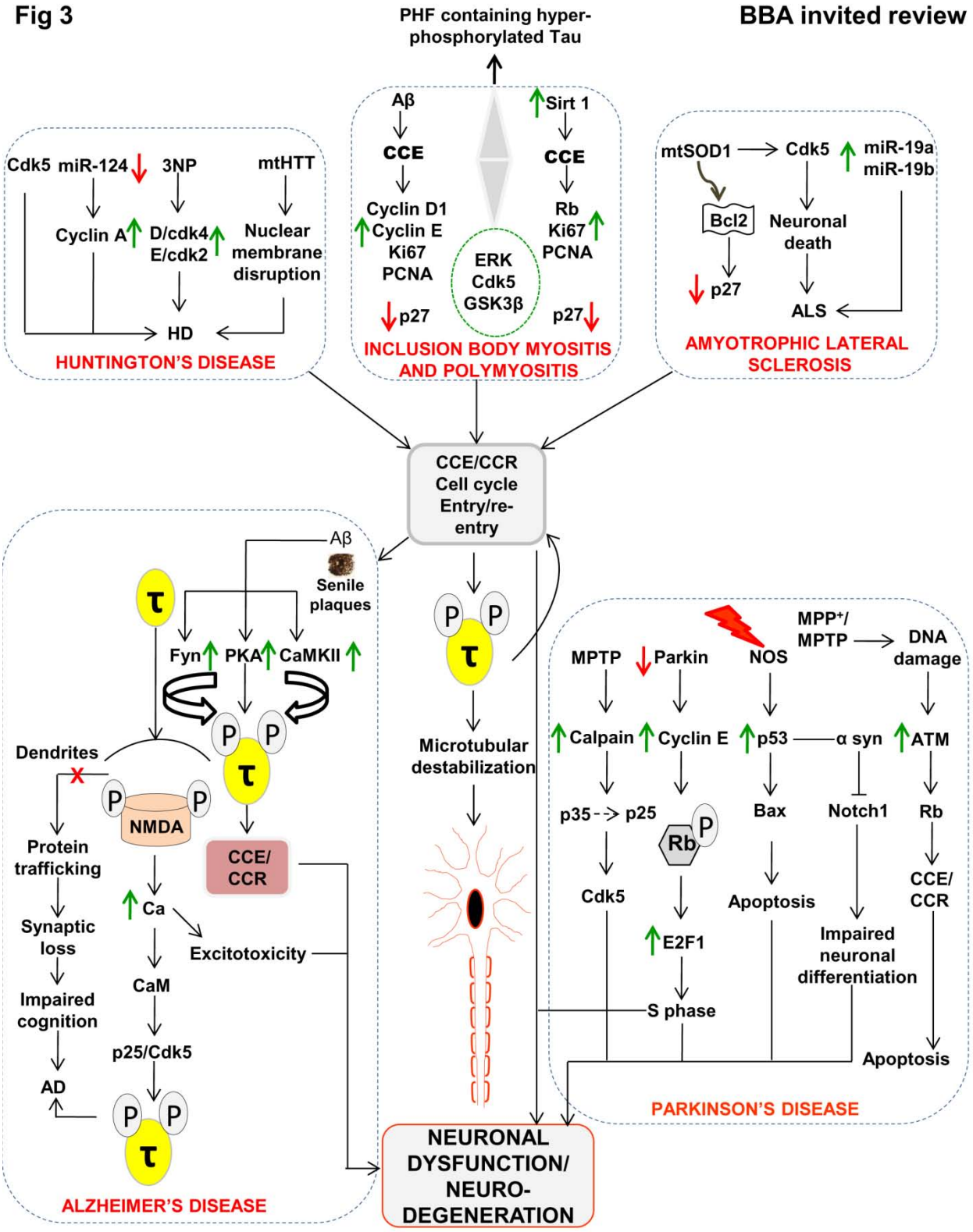
Fig 2 **BBA invited review**



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Fig 3

BBA invited review



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Invited review for BBA-Molecular Basis of Diseases

TABLE 1: CONSEQUENCES OF ALTERED CELL SIGNALING IN THE POST MITOTIC CELL DIVISION

PRINCIPLE PHENOTYPE	SIGNALING CASCADES	CHECK POINTS	CYCLINS EXPRESSION	CDK/CDC/CDKI/TFS INVOLVED	REFERENCES
AD	MEK-ERK MAP pathway/p38 MAPK	G0 to G1 and G1/S phase	Cyclin D1 and Cyclin E1	Cdk4 and Ki67 (MKI67)	[46]
	Notch signaling and Akt/GSK3β pathway	G0 to G1 and G1/S phase	CyclinD1	Cdk4/Cdk6 and Rb-E2F1	[39]
	β-catenin/TCF transcription complex (Wnt signaling)	G1/S phase	Cyclin D1	Cdk4/Cdk6 and Rb-E2F1	[40]
	Not defined	G1/S transition and G2/M phase	Cyclin B, Cyclin C, cyclin D1 and Cyclin E	Cdk1, Cdk2, Cdk4/Cdk6, Cdk5, Cdk7, Cdk11, p15, p16, p18, p19, p21, p27, pRb/p107, Ki67, mpm-2, E2F-1, PCNA, MORF4-related transcription factor and p53	[47-52]
PD	Not defined	G1 to S phase	Cyclin D and Cyclin E	Cdk2, Cdk4, Rb-E2F1	[53]
	MEK-ERK MAP pathway/p38 MAPK	G0/G1 phase	Cyclin D3	Cdk5, Cdk6/pRb, p21, p25 and p35/p39, Prosurvival transcription factor MEF2D, E2F-1 and p53	[54-57]
HD	Not defined	G0/G1 phase and G1/S phase	Cyclin A2, Cyclin D1 and Cyclin E	Cdk2, Cdk4, Cdk5 and p25, E2F-1	[58-61]
ALS	Apoptotic signaling	G1 to S phase	Cyclin D1 and Cyclin F	Cdk4, Cdk5, Cdk6, p25, p35/p39 and pRb/p107, E2F-1	[62]
IBM/PM	Apoptotic signaling and Akt signaling	G1/S phase	Cyclins E and Cyclin D1	Cdk2, Cdk4, Cdk6, Ki-67, PCNA and p21	[22]

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TABLE 2: A CONSOLIDATED LIST OF VARIOUS CELL CYCLE MARKERS THAT ACTIVELY PARTICIPATE IN POST MITOTIC NEURONS AND RELATED DISORDERS AS LISTED (SHADED IN GREY); (–) INDICATES NO REPORTED ACTION OF THAT PARTICULAR CELL CYCLE MARKERS

Cell cycle markers	AD	PD	HD	ALS	IBM/PM
Ki67		-	-	-	
BrdU		-	-		
PCNA			-	-	
Mcm2		-	-	-	-
Cyclin A	-				
Cyclin B	-		-	-	-
Cyclin C		-	-		-
Cyclin D					
Cyclin E					
Cyclin G	-		-		-
Cyclin F	-	-	-		-
References	[22,97-100]	[56,73,74,98,101-103]	[58-61]	[85,104,105]	[22]

AD- Alzheimer's disease; PD- Parkinson's disease; HD- Huntington's disease; ALS- Amyotrophic lateral sclerosis; IBM-inclusion body myositis; PM- Polymyositis.

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TABLE 3: DIFFERENT DRUGS AND BIOMOLECULES TO ATTENUATE THE CELL CYCLE PROGRESSION

S.NO.	COMPOUNDS	INVOLVED CHECK POINTS	INVOLVED MECHANISMS	DISEASES	REFERENCES
1	Roscovitine	G1 and G2/M phases of cell cycle	Well-known purine analogue, responsible for suppressing the activation of both Cdk2 and Cdk5. Further, it also inhibits the activity of ERK1 and ERK2 signaling cascade at its higher concentration	AD, PD and ALS	[106]
2	Olomoucine	G1 and G2/M phases of cell cycle	Well-known purine analogue which modulates the activity of Cdk2 and Cdk5 by competitively binding the ATP-binding site. Moreover, neuronal cell death induced by p27 reduction is also inhibited by olomoucine administration	AD, PD and ALS	[106]
3	Resveratrol (RSV)	G_0/G_1 phase of cell cycle	It potentiates SIRT1 activity and induces an indirect inhibition of p53 that further inhibit the GSK3β and p53 interaction. Since, GSK3β overactivity leads to increased levels of plaques and tangles and p53 activity induces tau phosphorylation. Further, RSV also protects neuronal cells by toxic effects of mHtt, potentiating SIRT1 activity and inducing an indirect inhibition of p53	AD and HD	[109]
4	Ibuprofen	S phase of cell cycle	Non-steroidal anti-inflammatory drug Ibuprofen has been shown to abrogate the cell cycle arrest at S phase	AD and PD	[110,111]
5	Naproxen	G0/G1 phase of cell cycle	It blocks alterations in brain microglia as well as neuronal cell cycle events (CCEs) in the absence of detectable alterations in AβPP processing and Aβ metabolism	AD	[110]
6	Apigenin	G2/M phase of cell cycle	It induces cell cycle arrest preferentially in the G2/M phase	AD and PD	[111]
7	Epigallocatechin-gallate (EGCG)	G0-G1 phase of cell cycle	It is a natural product that has been shown to abrogate the cell cycle at the G0 to G1 phase in various tissues and is known as a cell cycle inhibitor	AD	[46]
8	Tamoxifen	G0-G1 phase of cell cycle	It is a well-tolerated anti-proliferative cancer drug that has been reported to abrogate the cell cycle progression from G0 to G1 in various tissues	AD	[46]
9	Butyrolactone I	G1-S phase of cell cycle	A Cdk5 inhibitor, provides protection against Aβ toxicity in SEK1-AL-expressing cells, advocating that Cdk5 and JNK activation independently contributed to this toxicity	AD	[112]
10	Arimoclomol	Not defined	It upregulates the HSP70 expression therefore it might be used as a potential molecule to treat IBM. Since, the interaction between HSP70 and cyclins E/D1 has crucial importance during cell cycle progression in the inflammatory myopathies, probably controlling cyclin maturation or degradation	IBM	[113]

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Invited review for BBA-Molecular Basis of Diseases

HIGHLIGHTS

Cyclin/CDKs mediated cell cycle homeostasis and re-expression of cyclins in response to stress.

Potential triggering factors that force mature neuron and muscles to re-enter into cell division post mitotically

Altered signal transduction cascade, which causes disruption in cell cycle homeostasis.

Postmitotic cell division in the progression of AD, PD, HD, ALS and IBM diseases.

Therapeutic strategies using cell cycle inhibitors, chaperones, UbE3 ligase for the reversal of aberrant cell cycle entry.

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