DYRK1A, located on the Down syndrome (DS) critical region of chromosome 21, was found to be overexpressed in brains of DS and Alzheimer’s disease individuals. DYRK1A was considered to play important roles in the pathogenesis of DS and Alzheimer’s disease; however, the degradation mechanism of DYRK1A was still unclear. In this study, we found that DYRK1A was degraded through the ubiquitin-proteasome pathway in HEK293 cells. The N terminus of DYRK1A that was highly unstable in HEK293 cells contributed to proteolysis of DYRK1A. E3 ligase SCFβTrCP mediated ubiquitination and promoted degradation of DYRK1A through an unconserved binding motif (49SDQQVSALS57) lying in the N terminus. Any Ser-Ala substitution in this motif could decrease the binding between DYRK1A and β-transducin repeat containing protein (βTrCP), resulting in stabilization of DYRK1A. We also found DYRK1A protein was elevated in the G0/G1 phase and decreased in the S and G2/M phase, which was negatively correlated to βTrCP levels in the HEK293 cell cycle. Knockdown of βTrCP caused arrest of the G0/G1 phase, which could be partly rescued by down-regulation of DYRK1A. Our study uncovered a new regulatory mechanism of DYRK1A degradation by SCFβTrCP in HEK293 cell cycle progression.

DYRK1A, a member of the dual specificity tyrosine phosphorylation-regulated kinase family, located on chromosome 21, was reported to be overexpressed and responsible for nervous defects in patients with Down syndrome (DS) (1, 2). Several mouse models showed that DYRK1A knock-in could lead to neurodevelopmental delay, motor abnormalities, mental retardation, learning and memory deficits, and reduced neuronal density (3–5), which could also be observed in DS patients (6–8), indicating the important roles of DYRK1A in the development and functions of the nervous system. Recent studies provided evidence that neural functional defects in DS could be due to proliferative impairment of neural progenitor cells. Decreased proliferative activity in the ventricular zone/subventricular zone and dentate gyrus was observed in DS patients as early as the fetal phase (9–11). As one of the key genes implicated in DS, DYRK1A was considered to be involved in the proliferative regulation of NPCs. Studies showed that overexpression of DYRK1A could inhibit proliferation and promote cell cycle exit of NPCs by phosphorylation on several cell cycle regulators, including cyclin D1 (12–14) and p27Kip (14, 15), which was consistent with the proliferative disruption of NPCs in DS. However, haploinsufficiency of DYRK1A could lead to impaired longevity of neural stem cells, resulting in reduced self-renewal and reservoir of neural stem cells (15, 16). These studies indicate the importance of maintaining the appropriate protein level of DYRK1A in neural development. Our recent study showed that RE1 silencing transcription factor (REST) regulated DYRK1A gene transcription, and both the overexpression and down-regulation of DYRK1A reduced REST levels, further emphasizing the appropriate protein level of DYRK1A for cellular functions (17).

Nearly all DS individuals showed typical features of AD, such as senile plaques and neurofibrillary tangles in their early 40s, making DS an ideal model for AD research (18). Studies showed that DYRK1A could phosphorylate the amyloid protein precursor on Thr-668 (19) and several sites of Tau (20–22). Phosphorylation by DYRK1A could facilitate cleavage of APP by the β-site APP cleaving enzyme 1 (23) and γ-secretase (23, 24), leading to increased production of amyloid-β. DYRK1A-mediated phosphorylation could disrupt the normal biological effect of Tau and make it much more preferable to aggregate (22). The roles of DYRK1A in AD pathogenesis were still not clear. Nonetheless, histological studies showed that there were significantly more DYRK1A-positive cells in AD brains than normal controls (25). The protein level of DYRK1A was also remarkably up-regulated in the sporadic AD cortex, compared with normal individuals (25). However, the detailed mechanism causing abnormal up-regulation of DYRK1A in AD brains was still unclear. The ubiquitin-proteasome pathway, one of the
main routes for protein destruction in eukaryotic cells, has been implicated in maintaining normal functions of nervous systems. Accumulation of ubiquitin conjugates or aggregates has been reported in AD and other neural degenerative diseases (26), implying that ubiquitin-proteasome-mediated protein degradation was impaired during the pathogenesis of neural degeneration. DYRK1A showed strong gene dosage effect, and its transcription and translation were strictly regulated (27). As described above, DYRK1A overexpression in DS patients was due to the extra copy of the DYRK1A gene. However, generally AD patients had normal gene copy number, suggesting that disrupted proteolysis could be responsible for DYRK1A increments in AD patients. Nevertheless, the molecular mechanism of DYRK1A degradation was still unclarified.

In the ubiquitin-proteasome system, ubiquitin was sequentially catalyzed by the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) to be covalently linked to Lys residues of substrates, forming a degradation signal that could be recognized by 26S proteasome (26). SCFβTrCP was the most frequently studied ubiquitin ligase. It could specifically bind to the DpS/GXpxS motif, where serine residues were phosphorylated, and subsequently mediated by ubiquitination of substrates (28, 29). Ubiquitin cluster covalently connected to substrates functioned as a degradation signal and could be recognized by proteasome for degradation (26). SCFβTrCP exerted its roles in neural development by regulating the stability of the RE1-silencing transcription factor, which was a transcriptional repressor of neural genes in non-neuronal cells. SCFβTrCP promoted neuronal differentiation by targeting REST for degradation in the proteasome (30). In our previous study, we found that dysregulation of DYRK1A could impair REST protein stability, making REST vulnerable for proteolysis (17). However, more studies are needed to uncover whether there is an association between SCFβTrCP and DYRK1A in neural development.

In this study, we found DYRK1A was degraded through the ubiquitin-proteasome pathway in HEK293 cells. Its N terminus rather than the PEST-rich region was responsible for the proteasome proteolysis. SCFβTrCP bound to the **SDQQVSALS** motif lying in the N terminus of DYRK1A, mediating the ubiquitination of DYRK1A. Knockdown of βTrCP up-regulated DYRK1A protein levels and prolonged its half-life. We also observed that DYRK1A protein but not mRNA was elevated in the G0/G1 phase and decreased during S and G2/M phase, negatively correlated with the changes of βTrCP in HEK293 cell cycle. Our study proposed a new regulatory role of DYRK1A proteolysis in HEK293 cell cycle progression.

Results

**DYRK1A Degradation Was Mediated by Ubiquitin-Proteasome System**—To determine the half-life of DYRK1A, HEK293 cells were pulse-chased with CHX. Cells were harvested every 12 h after CHX treatment, and DYRK1A protein was analyzed by Western blotting. As shown in Fig. 1A, DYRK1A protein gradually decreased in a time-dependent manner after the addition of CHX. The half-life of DYRK1A in HEK293 cells was 14.07 h by the following calculation, $Q_t = Q_0(1/2)^{t/H}$, where $Q_t$ indicates the protein level at $t$ time; $Q_0$ indicates the protein level at 0 h; and $H$ indicates half-life (Fig. 1A).

 Ubiquitin-proteasome pathway was responsible for degradation of most proteins in eukaryotic cells. To examine whether the ubiquitin-proteasome pathway was involved in the degradation of DYRK1A, HEK293 cells were transfected with pDAYRK1A-MycFLAG and treated with the protea-
some inhibitor lactacystin. Western blotting results clearly showed that treatment with lactacystin significantly increased DYRK1A protein level in a time-dependent (Fig. 1B) and dosage-dependent manner (Fig. 1C). Lactacystin treatment also markedly decreased the degradation of DYRK1A in CHX chase assay (Fig. 1D). These data demonstrated that ubiquitin-proteasome participated in the degradation of DYRK1A.

Because ubiquitination was the essential step for ubiquitin-proteasome pathway-mediated protein degradation, we next investigated whether DYRK1A could be ubiquitinated using co-IP assay. Endogenous DYRK1A was pulled down with DYRK1A monoclonal antibody, and ubiquitinated proteins were detected by ubiquitin antibody. Western blotting exhibited a smeared band, the typical feature of ubiquitination (Fig. 1E), implying that DYRK1A could be ubiquitinated in HEK293 cells. Taken together, our results revealed that DYRK1A was ubiquitinated and degraded by the ubiquitin-proteasome system.

**Down-regulation of DYRK1A Rescued Cell Cycle Arrest at G0/G1 Phase**—In cell cycle progression, E3 ligase SCF^{وبك} showed the highest activity in S phase, and βTrCP was specifically attenuated in the G0/G1 phase and increased in the S phase, implying the important roles of SCF^{وبك} during G0/G1-S phase transition. DYRK1A had been reported to be involved in regulating cell proliferation. We previously showed that overexpression of DYRK1A could cause proliferative inhibition and cell cycle arrest at the G0/G1 phase, indicating that down-regulation of DYRK1A could be necessary for G0/G1-S phase transition (31). To test this possibility, we synchronized HEK293 cells by nocodazole. After 12 h of treatment with nocodazole, more than 90% of cells were arrested at G2/M phase (Fig. 2A). Cell cycle was recovered with fresh medium. We found DYRK1A protein showed the highest expression level at the G0/G1 phase and was comparatively reduced at the S and G2/M phase (Fig. 2B). Interestingly, βTrCP protein level was negatively correlated to DYRK1A, exhibiting an inverse profile in cell cycle (Pearson $r = -0.5655$, $p = 0.0442$, Fig. 2B). The down-regulation of DYRK1A during G0/G1-S phase transition could be due to enhanced degradation, as the mRNA level of DYRK1A did not change significantly in cell cycle (Fig. 2C). Then we established a HEK293 cell line stably expressing βTrCP shRNAs (Fig. 2D), and we evaluated the cell cycle of this cell line. FACs results showed that βTrCP shRNAs reduced the ratio of cells in S phase by 9.12% and increased the ratio of cells in G0/G1 phase by 11.53%. When transfected with DYRK1A interfering constructs, the cell cycle arrest induced by βTrCP knockdown was partly rescued (Fig. 2E). To further confirm our results, we performed an EDU staining assay. Flow cytometry analysis showed that knockdown of βTrCP markedly decreased positive-staining cells from 40.51 to 29.03%, which could be rescued by DYRK1A shRNA transfection (37.90%) (Fig. 2F). Our studies suggested that DYRK1A was inversely regulated by E3 ligase SCF^{وبك} and played important roles in cell cycle progression.

**DYRK1A Degradation Was Mediated by E3 Ligase SCF^{وبك}**—As the key component of the SCF^{وبك} complex, F-box protein βTrCP directly bound to target proteins independent of the SCF complex, promoting ubiquitination of target proteins (26).

To investigate whether SCF^{وبك} was involved in DYRK1A degradation, we first applied co-IP assay to detect the interaction between βTrCP and DYRK1A. Co-IP results showed that both exogenous βTrCP and DYRK1A could be efficiently precipitated by βTrCP antibody (Fig. 3A). To better validate the interaction between βTrCP and DYRK1A, we performed co-IP using HEK293 cells without any treatment. This experiment also revealed that endogenous βTrCP and DYRK1A could be precipitated simultaneously (Fig. 3B). These results indicated that βTrCP interacted with DYRK1A.

To demonstrate whether SCF^{وبك} was involved in DYRK1A protein degradation, we interrupted SCF^{وبك} by knocking down βTrCP expression with siRNAs (Fig. 3C). βTrCP siRNA significantly increased the DYRK1A protein level to 218.51 ± 33.61%, compared with control (Fig. 3D). To further confirm the roles of βTrCP in the degradation of DYRK1A, we assessed the ubiquitination status of DYRK1A when βTrCP was knocked down or overexpressed. We infected HEK293 cells

**FIGURE 2. Expression profile of DYRK1A in cell cycle and down-regulation of DYRK1A rescued cell cycle arrest at G0/G1 phase.** HEK293 cells were treated with nocodazole with a final concentration of 150 ng/ml for 12 h. Then fresh medium was added, and cells were harvested every 2 h. Then FACs (A), Western blotting (B), and real time qRT-PCR (C) were performed to detect cell cycle, DYRK1A and βTrCP protein, and DYRK1A mRNA, respectively. β-Actin was used as internal control for both Western blotting and real time qRT-PCR. Values represented means ± S.E. ($n = 3$). Pearson's correlation test was used for correlation analysis between DYRK1A and βTrCP protein levels ($r = -0.5655$, $p = 0.0442$). D, endogenous βTrCP in HEK293 stably expressing βTrCP shRNAs was determined by Western blotting. β-Actin was used as loading control. E, stably transformed HEK293 cell line was transfected with DYRK1A shRNA expressing or control vectors. Thirty six hours after transfection, cells were stained with propidium iodide and subjected for FACS analysis. F, βTrCP stably transfected HEK293 cell line was transfected with DYRK1A shRNA construct. Thirty six hours after transfection, cells were replaced with fresh medium containing 10 μM EdU. After 10 h of incubation, cells were collected and stained with Apollo 643 reagent. Positive-staining cells were counted on a FACS instruments.
with βTrCP shRNA-expressing lentiviral particles (Fig. 3E). Our results showed βTrCP down-regulation led to a significant decrease of ubiquitinated endogenous DYRK1A levels to 61.72 ± 8.74% (Fig. 3F), whereas overexpression of βTrCP remarkably promoted the ubiquitination of endogenous DYRK1A to 142.73 ± 26.98% (Fig. 3G). To validate whether

FIGURE 3. E3 ligase SCFβTrCP promoted DYRK1A degradation by accelerating its ubiquitination. A, HEK293 cells were co-transfected with DYRK1A and βTrCP-expressing constructs. Co-IP assay was performed to determine the interaction between DYRK1A and βTrCP. βTrCP antibody was applied in the pulldown step. FLAG antibody was used for signal detection. B, co-IP assay was used to detect the interaction between intrinsic endogenous DYRK1A and βTrCP. Co-IP and western blotting (IB) antibody, respectively. IgG (rabbit) was used as the negative control of βTrCP antibody. C, HEK293 cells were transfected with βTrCP siRNAs (siβTrCP) or negative control (siCon). Total RNA was separated by Trizol reagent. The mRNA level of βTrCP was evaluated by qRT-PCR. D, HEK293 cells were co-transfected with βTrCP-expressing plasmid and βTrCP or control siRNAs. DYRK1A protein level was detected by Western blotting using FLAG antibody. β-Actin was used as internal control. Values represented means ± S.E. (n = 3). *, p < 0.05. E, protein level of DYRK1A in βTrCP shRNA lentivirus-infected HEK293 cells was determined by Western blotting. β-Actin was used as internal control. F, co-IP assay was performed to detect the ubiquitinated DYRK1A in cells stably transfected with shβTrCP. IgG was used as negative control of DRK1A (7D10) antibody. The ubiquitination was normalized to pulled down DYRK1A. Values represented means ± S.E. (n = 3). *, p < 0.05. G, ubiquitinated DYRK1A was estimated by co-IP assay in cells transfected with βTrCP expression vector. DYRK1A (7D10) antibody was used as pull-down antibody, and IgG was used as negative control. The ubiquitination was normalized to pull down DYRK1A. Values represented means ± S.E. (n = 3). *, p < 0.05. H, degradation rate of DYRK1A in shCon and shβTrCP lentivirus-transformed HEK293 cells was determined using CHX chase assay. DYRK1A was detected by FLAG antibody. β-Actin was used as internal control. Values represented means ± S.E. (n = 3). *, p < 0.05. I, degradation of endogenous DYRK1A was determined by CHX chase assay. DYRK1A and βTrCP antibody were used to detect endogenous DYRK1A and βTrCP, respectively. β-Actin was used as internal control. Values represented means ± S.E. from three independent experiments. *, p < 0.05. J, DYRK1A was co-transfected with Cullin1 or Cullin2 dominant negative mutants expressing constructs. Protein level of DYRK1A was evaluated by Western blotting using FLAG antibody. β-Actin was used as internal control. Values represented means ± S.E. from three independent experiments. *, p < 0.05.
DYRK1A up-regulation upon βTrCP interference was due to impaired proteolysis, we determined the DYRK1A turnover rate in HEK293 cells with the βTrCP knocked out. CHX chase assay showed that both the exogenous and endogenous DYRK1A proteins (Fig. 3, H and I, respectively) were markedly stabilized in shβTrCP stably transfected HEK293 cells, implying an important role of βTrCP in the proteolytic process of DYRK1A.

The SCFβTrCP E3 ligase complex was mainly composed of four core components as follows: Skp1; F-box protein (βTrCP); cullin; and Rbx1. βTrCP specifically interacted with target proteins, whereas cullin acted as the structural scaffold linking the N terminus of Skp1 to the C terminus of Rbx1 (28). To disrupt the normal function of SCFβTrCP complex, we constructed dominant negative mutants of cullin, in which only the N-terminal 1–452-aa residues of Cullin1 or 20–427-aa residues of Cullin2 were expressed (DN-Cullin1 and DN-Cullin2). These dominant negative forms of cullin, serving as the decoy partner, could assemble a complex with Skp1 and F-box protein (βTrCP), while losing the ability to bind Rbx1 and E2 enzyme, leading to failure of ubiquitination. When overexpressed with DN-Cullin1 or DN-Cullin2, the protein level of DYRK1A was increased to 207.44 ± 30.17 and 213.73 ± 34.50%, compared with control vector, respectively (Fig. 3J). Our studies provided solid evidence that E3 ligase SCFβTrCP was responsible for DYRK1A proteolysis.

**DYRK1A Degradation Was Mediated by Its N Terminus**—DYRK1A protein was composed of the following four domains: N terminus (NT, 1–149 aa); the kinase catalytic domain (150–517 aa); the PEST-rich region (PEST, 478–516 aa) (Fig. 4A) (32). The PEST-rich region was a peptide composed with proline (P), glutamic acid (E), serine (S), and threonine (T) residues, commonly acting as protein degradation signal (33).

Because DYRK1A possessed a PEST-rich region comprising 478–516-aa residues, it is predicted that DYRK1A could be degraded rapidly with a short half-life in cells. We determined whether the half-life of DYRK1A was dramatically changed when the PEST-rich region was truncated (Fig. 4A). CHX chase assay showed that deletion of the PEST-rich region did not significantly alter the degradation rate, compared with wild type DYRK1A (Fig. 4B), indicating other domains of DYRK1A could be responsible for DYRK1A proteolysis. For this purpose, a series of DYRK1A truncation mutants were constructed (Fig. 4A), and the relevant degradation rates were assessed by CHX chase assay. Compared with wild type DYRK1A, the degradation rate of DY-ΔCT was not significantly changed (Fig. 4C), whereas the degradation rate of DY-CT was notably prolonged (Fig. 4D), suggesting the C terminus did not play a significant role in DYRK1A degradation. Noticeably, we found that the N terminus of DYRK1A (DY-NT) was highly unstable and could be rapidly degraded in HEK293 cells (Fig. 4E), compared with other domains of DYRK1A. These results suggested the N terminus could play a vital role in regulating DYRK1A degradation.

**SCFβTrCP Promoted DYRK1A Degradation via Its N-terminal 34–71 Amino Acids**—Considering the importance of E3 ligase SCFβTrCP and N terminus in the degradation of DYRK1A, we proposed that SCFβTrCP could directly bind to the N terminus of DYRK1A. To confirm this, we carried out co-IP assay between βTrCP and a series of DYRK1A truncation mutants (Fig. 4A). Results in Fig. 5, A and B, show that only DYRK1A truncation mutants containing the N terminus could be immunoprecipitated with βTrCP, implying that the SCFβTrCP complex specifically bound to the N terminus of DYRK1A. To further identify the exact residues to which βTrCP bound, we constructed another two truncation mutants, which lacked the N-terminal 33 aa (DY-ΔN33) or 71 aa (DY-ΔN71) residues (Fig. 5C). The co-IP results showed that interaction between DY-ΔN33 and βTrCP was negligibly affected (Fig. 5D, 5th lane versus 4th lane), but the DY-ΔN71 band co-immunoprecipitated with βTrCP was markedly reduced (Fig. 5D, 6th lane versus 4th lane), suggesting that the region of 34–71 aa contained the degradation degron. To further verify this degradation degron, the CHX assay was performed in HEK293 cells trans-
fected with the two deletion constructs. The CHX chase assay demonstrated that the degradation rate of DY-N33 was similar to that of wild type DYRK1A, whereas DY-N71 degradation was noticeably impaired, revealed by slower decrement after CHX addition (Fig. 5E). Collectively, our results suggested the binding site of TrCP on the DYRK1A protein lying in the N-terminal 33–71-aa residues.

SCFβTrCP Bound to an Unconserved Motif in the DYRK1A N Terminus—SCFβTrCP was the substrate-specific E3 ligase that directly interacted with a conserved binding motif DSGX2n(S/T), where serines were phosphorylated by particular kinases (34). Despite the substrate preference, many studies also showed that SCFβTrCP complex could also recognize proteins lacking the perfect DSGX2n(S/T) motif and promote ubiquitination (35–40). However, most of these substrates harbored at least one variant of the DSGX2n(S/T) motif (Fig. 6A). Normally, substitutions between phosphorylated residues and aspartic or glutamic acid existed in these variants (Fig. 6A), forming a functional degron. Comparing DYRK1A N terminus with DSGXXS and its variants, we identified a similar sequence 49SDQQVSALS57 (Fig. 6A).

To investigate the role of SDQQVSALS sequence in the degradation of DYRK1A, we mutated the Ser-49, Ser-54, Ser-57, and Ser-59 residue to alanine in the DY-NT construct. First, we utilized CHX chase assay to determine the degradation rate of wild type DYRK1A N terminus and NT-S49A, NT-S54A, NT-S57A, and NT-S59A mutants. Western blotting results showed that N-terminal proteolysis was significantly slowed down by single amino acid substitution on Ser-49, Ser-54, or Ser-57 but not Ser-59 (Fig. 6B), suggesting 49SDQQVSALS57 motif could be responsible for DYRK1A degradation. To further confirm this, we examined the interactions of DYRK1A N-terminal mutants with βTrCP as described previously. The N terminus of DYRK1A could efficiently interact with βTrCP, and the NT-S59A mutant showed similar binding ability (Fig. 6C). However, S49A, S54A, or S57A mutation significantly
reduced the interaction between the DYRK1A N terminus and βTrCP (Fig. 6C).

In the process of ubiquitination, E3 ligase recruited E2 ligase and substrate and catalyzed the transfer of ubiquitin from E2 ligase to substrate. We speculated that ubiquitination of DYRK1A could be disrupted if its interaction with SCF/βTrCP was impaired. To test this, we pulled down wild type DYRK1A as well as DYRK1A S49A, S54A, S57A, and S59A mutants to assess the comparative ubiquitination level. We found that substitution of amino acid residues on Ser-49, Ser-54, and Ser-57 markedly decreased the relative ubiquitination level (Fig. 6D, 2nd to 4th lanes compared with 1st lane). However, DY-S59A mutant failed to exhibit significant alteration on the ubiquitination status (Fig. 6D). To determine the effect of phosphorylation in the interaction between βTrCP and DYRK1A or its N terminus, λ-PPase was used to remove the phosphate group.

FIGURE 6. SCFβTrCP bound to an unconserved motif of the DYRK1A N terminus. A, lists of unconserved SCFβTrCP substrate sequences and predicted binding motif in DYRK1A. B, DY-NT or S49A, S54A, S57A, and S59A mutants were transfected into HEK293 cells. Thirty six hours later, CHX chase was performed to evaluate the degradation rate. FLAG antibody was applied for Western blotting analysis. β-Actin was used as loading control. The quantification was calculated from three independent experiments. Values represented means ± S.E. **, p < 0.01. C, expressing constructs of βTrCP and DY-NT or S49A, S54A, S57A, and S59A mutants were co-transfected into HEK293 cells. Forty eight hours after transfection, co-IP assay were applied utilizing βTrCP antibody. IgG (rabbit) was used as negative control. Values represented means ± S.E. (n = 3). *, p < 0.01; **, p < 0.01. D, ubiquitin and DYRK1A full-length wild type or point mutants DY-S49A-, DY-S54A-, DY-S57A-, or DY-S59A-expressing plasmids were co-transfected into HEK293 cells. Co-IP was executed using FLAG antibody 48 h later. Precipitated proteins were detected by ubiquitin (Ubi) and FLAG antibody. Values represented means ± S.E. (n = 3). **, p < 0.01. STE, short time exposure. LTE, long time exposure.

DYRK1A Was Degraded by SCFβTrCP

DECEMBER 16, 2016 • VOLUME 291 • NUMBER 51 • JOURNAL OF BIOLOGICAL CHEMISTRY 26405
DYRK1A Was Degraded by SCF<sup>βTrCP</sup>

from Ser or Thr residues before anti-βTrCP immunoprecipitation. The co-IP assay showed that the binding between βTrCP and DYRK1A or its N-terminus was significantly impaired after treatment with λ-PPase (Fig. 6E). These data revealed <sup>49</sup>SDQQVSALS<sup>57</sup> as the degradation degron was responsible for DYRK1A ubiquitination and degradation.

Discussion

In this study, we found that DYRK1A was subjected to degradation in proteasome mediated by E3 ligase SCF<sup>βTrCP</sup> in HEK293 cells. We identified <sup>49</sup>SDQQVSALS<sup>57</sup> as the binding motif of βTrCP, which was located in the N-terminal region but not the PEST-rich region of DYRK1A. The ubiquitin-proteasome pathway was responsible for proteolysis of more than 80% proteins in the eukaryote, playing diverse roles in regulation of proliferation, apoptosis, tumor genesis, and neural development (41). Disruption of the ubiquitin-proteasome pathway played a pivotal role in several neurodegenerative diseases. Complex of ubiquitin, proteasome, and certain proteins was involved in several neurodegenerative diseases. Complex of ubiquitin, proteasome, and certain proteins was found in neurofibrillary tangles of AD, Lewy bodies (LBs) of Parkinson’s disease, LBs in LB dementia, Bunina bodies in amyotrophic lateral sclerosis, and nuclear inclusions of Huntington’s disease, spinocerebellar ataxias, and Kennedy’s syndrome (42). DYRK1A was elevated in DS and AD patients (2, 25); however, the molecular mechanism was unknown. The study here implied that the disruption of ubiquitin-proteasome pathway may contribute to the elevation of DYRK1A in AD patients.

Our study and the recent studies from others proposed that proliferative repression caused by DYRK1A overexpression could be due to cell cycle arrest at G<sub>0</sub>/G<sub>1</sub> phase (14, 31, 43, 44). Consistent with this, we found that DYRK1A protein level was specifically reduced during the transition from G<sub>0</sub>/G<sub>1</sub> to S phase in HEK293 cells. Overexpression of DYRK1A could disrupt HEK293 cell cycle progression. Previous work showed that SCF<sup>βTrCP</sup> actively participated in the regulation of the cell cycle, promoting G<sub>0</sub>/G<sub>1</sub> to S transition by directing degradation of PFKFB (45) and BORA1 (46). More importantly, βTrCP seemed to be up-regulated in the S phase and to promote G<sub>0</sub>/G<sub>1</sub> to S phase transition (45, 47, 48). Our results showed the negative correlation in the cell cycle progression between DYRK1A and βTrCP. DYRK1A down-regulation partially rescued G<sub>0</sub>/G<sub>1</sub> arrest caused by βTrCP knockdown, suggesting that E3 ligase SCF<sup>βTrCP</sup> was affected on HEK293 cell cycle progression partly through its regulation of DYRK1A degradation. The study used HEK293 cells to study the cell cycle progression, which is a cancer cell line and may not reflect the cell cycle profile of normal cells.

Recent studies in human fetuses and mouse models with DS (9–11) (9, 49, 50) revealed reduced cell proliferation of ventricular and subventricular zones and dentate gyrus, the two neurogenic regions where NPCs mainly existed, suggesting the impaired NPC reservoir could effectively contribute to the pathology of DS. Studies by Hammele et al. (15), Yabut et al. (12), and Park et al. (44) showed that overexpression of DYRK1A could induce cell cycle exit and proliferative inhibition of NPCs (12, 15, 44), and the proliferative suppression induced by DYRK1A overexpression could be markedly rescued by specific repression of DYRK1A activity by siRNAs, dominant negative inhibitor, or small molecules (15, 19, 51, 52), directly demonstrating the regulatory effects of DYRK1A in NPC self-renewal. The abnormal expression of DYRK1A in DS and AD brains may lead to the incompetence in NPC self-renewal, which may subsequently be attributed to neurodegeneration.

Materials and Methods

Cell Culture—HEK293 and HEK293T cells were cultured in high glucose Dulbecco’s modified Eagle’s medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) at 37 °C and 5.0% CO<sub>2</sub> atmosphere. Before the day of transfection, cells were seeded at 60–70% confluence. Transfection was carried out using Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer’s protocols. Lactacystin (Lac) (Sigma) or CHX (Sigma) was added to medium 24 or 36 h after transfection, respectively. For synchronization, cells were treated with 150 ng/ml nocodazole (Sigma) for 12 h and then cultured in fresh medium.

To establish a stable transfection cell line with βTrCP knocked down, HEK293 cells were plated in a 6-well plate with a density of 50,000/well. On the 2nd day, cells were supplemented with shβTrCP lentiviral or control particles. After a 24-h incubation, the medium containing lentiviral particles was removed and replaced with fresh medium. Three days after infection, puromycin (Sigma) was added with a final concentration of 1 µg/ml for 2 weeks to the cell culture for screening, which was sustained for 2 weeks. After screening, the stable transfection cell lines were maintained with puromycin at a low concentration of 0.1 µg/ml.

For transfection, cells are plated into culture dishes at a density of 50,000/cm<sup>2</sup>. The next day, when the cells reach the confluency of 70%, cells were transfected with plasmids or siRNAs utilizing Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer’s instructions.

Western Blotting and Antibodies—To perform Western blotting analysis, cells were harvested and washed in ice-cold PBS twice, and then lysed by ultrasonication in 0.1% SDS-RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) in the presence of protease inhibitor mixture (Roche Applied Science). Whole-cell extracts were quantified using the DC protein assay kit (Bio-Rad) and separated by 10% glycine SDS-PAGE. Proteins were transferred onto nitrocellulose membrane under 100 V for 2 h. Nitrocellulose membranes were blocked in 5% bovine serum albumin in TBS-T for 2 h and then incubated in primary antibody dilutions (TBS-T with 1% BSA) overnight. The next day, after washing three times in TBS-T for 5 min, membranes were incubated in fluorescence-conjugated secondary antibody for 30 min at room temperature. Signals were detected on LI-COR Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). PageRuler Prestained Protein Ladder was purchased from Fermentas (Vilnius, Lithuania). Primary antibodies used in this study were as follows: FLAG antibody (rabbit) (Sigma); FLAG antibody (Sigma); DYRK1A antibody (7D10) (Sigma); βTrCP antibody (D13F10) (CST, Danvers, MA); cyclin B1 antibody (V152) (CST, Danvers, MA). Secondary antibodies IRDye 680 goat anti-rabbit IgG and...
IRDye 800CW goat anti-mouse IgG were both purchased from LI-COR Biosciences.

**Cycloheximide Chase Assay**—One day before transfection, HEK293 cells were seeded in 6-well plates. The next day, cells were transfected using Lipofectamine 2000 reagent (Invitrogen). Thirty hours after transfection, cells were treated with 150 μg/ml CHX and harvested every 4 or 12 h for Western blotting analysis. Half-life of protein was calculated using $Q_t = Q_0(1/2)^{t/H}$ (where $Q_t$ is protein level at time $t$; $Q_0$ is protein level at 0 h; and $H$ is half-life).

**Co-IP Assay**—For co-IP assay, cells were harvested and lysed in 1% Nonidet P-40 lysis buffer (1% Nonidet P-40, 50 mM Tris-base, 150 mM NaCl, pH 7.4) containing protease inhibitor mixture (Roche Applied Science). Then Sepharose CL-4B (Sigma) with a 10% total lysate volume was added and then shaken gently for 1 h on ice. We centrifuged the lysate bead mixture at 15,000 rpm at 4 °C for 10 min and carefully remove the supernatant into a new 1.5-ml tube. Twenty microliters of supernatant were isolated and used as input. The target protein was immunoprecipitated by shaking overnight at 4 °C with primary antibodies and protein A/G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA). Mouse or rabbit IgG (Beyotime Institute of Biotechnology, Haimen, China) was used as a negative control. The next day, agarose beads were aggregated by centrifugation at 3000 rpm. The pellet was washed with 1% Nonidet P-40 lysis buffer once and ice-cold PBS twice. Then the samples were resuspended in 1× loading buffer (Beyotime Institute of Biotechnology, Haimen, China) diluted with 0.1% SDS-RIPA lysis buffer, denatured on a metal bath at 95 °C for 5 min, and analyzed on 10% glycerin SDS-PAGE.

**Cell Cycle Analysis**—Cell cycle was analyzed by flow cytometry. Cells were harvested and washed twice in PBS and then fixed in 75% alcohol overnight at 4 °C. The next day cells were washed twice with cold PBS and resuspended in 1 ml of PBS with 40 mg of propidium iodide and 100 mg of RNase A (Sigma). Then the cells were incubated at 37 °C for 30 min. Samples were then analyzed on a FACS machine (Beckman, CA) with 20,000 counted.

**Production of Lentivirus**—HEK293T cell was plated onto a 10-cm dish 1 day before transfection, reaching 80% confluency. The next day, cells were transfected with expressing plasmid, packaging plasmid, and envelope plasmid (20, 15, and 6 μg, respectively) using Lipofectamine 2000 reagent (Invitrogen), and medium was replaced 7 h after transfection. Two days later, supernatant containing lentiviral particles was collected into fresh 50-ml tubes and maintained on ice. Supernatant was continuously collected twice every 24 h and mixed with supernatant previously harvested (supernatant could be passed through a 0.45-μm filter if necessary to produce purer lentiviral particles). Then we added 40% PEG8000 into supernatant to make a final concentration of 10%. The tubes were incubated on ice overnight. The supernatant/PEG8000 mixture was centrifuged at 4500 rpm at 4 °C for 15 min. Supernatant was aspirated, and tubes were centrifuged again at 4500 rpm at 4 °C for 5 min. We carefully removed the entire supernatant. For a 10-cm dish, precipitant was resuspended by 500 μl of Opti-MEM medium, aliquoted in five 1.5-ml tubes, and stored at −80 °C.

| Sequence harbored | Vector | Primers | Templates | Restriction enzymes |
|-------------------|--------|---------|-----------|--------------------|
| Cells1 D5S       | pDNA4-MycHis_A1 | F-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′, R-5′CGGAAGGGACACCACATGACTGATGAG3′ | cDNA1 | BamHI / XhoI |
| Cells2 D5S       | pDNA4-MycHis_A1 | F-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′, R-5′CGGAAGGGACACCACATGACTGATGAG3′ | cDNA2 | BamHI / XhoI |
| NT               | pCMV2-vector | T7, NT-R-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| CT               | pCMV2-vector | XL-30, CT-F-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| N-CE-DIPEST      | pCMV2-vector | XL-30, N-CE-DIPEST-F-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| ΔΔ               | pCMV2-vector | XL-30 | pDAYK1A-mycFLAG | Act I / XhoI |
| ΔΔΔ              | pCMV2-vector | XL-30 | pDAYK1A-mycFLAG | Act I / XhoI |
| ΔΔΔΔ             | pCMV2-vector | XL-30 | pDAYK1A-mycFLAG | Act I / XhoI |
| NT-SHA           | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| NT-SHA           | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| NT-SHA           | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| NT-SHA           | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| DYRK1A-SHA       | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| DYRK1A-SHA       | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| DYRK1A-SHA       | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |
| DYRK1A-SHA       | pCMV2-vector | T7, NR-5′CGAGGACGCACCATCTGTCATCAACCACGAGG3′ | pDAYK1A-mycFLAG | Bgl II / XhoI |

**Plasmids and siRNAs**—DYRK1A expressing vector pCMV-DYRK1A (pDAYRK1A-MycFLAG) and control vector were both purchased from ViGene Bio (Jinan, China). pcDNA4-MycHis_A(+), pDNA4-MycHis_A(-) were both purchased from Invitrogen. βTrCP expressing plasmid p4489 FLAG-βTrCP was obtained from Addgene (ID 10865, Cambridge, MA). The kits to produce shβTrCP and control lentiviral particles were purchased from Genechem (Shanghai, China). Ubiquitin-expressing construct was kindly provided by Dr. Weihong Song (University of British Columbia, Vancouver, Canada). Generally, to construct new expressing vectors, the coding sequences were synthesized using polymerase chain reaction or interception from existing plasmids. The coding sequences and the corresponding vectors were both digested with 2–3 restriction endonucleases (New England Biolabs, Beverly, MA). Then the digestion products were separated by agarose gel, ligated, and transformed into Escherichia coli DH5α competent bacteria. Ampicillin or kanamycin (Sigma) was used as screening antibiotic.

**DYRK1A shRNA-expressing vectors** were constructed by a former colleague of our laboratory (17). For another DYRK1A-expressing vector pDYRK1A-HA, pDAYRK1A-MycFLAG was digested with BglIII and Xhol, and the short fragment was subcloned into pCMV-C-HA (Beyotime Institute of Biotechnol-
**DYRK1A Was Degraded by SCF-TrCP**

ogy, Haimen, China). For ΔPEST mutant, pDAYRK1A-Myctag-FLAG was digested by BglII, Xhol, and PvuII, and the two short fragments were inserted into pCMV6-ENTRY. The construction strategies for other plasmids were described in Table 1. For siRNAs, sense GUG GAA UUU GUG GAA CAU CTT and antisense GGU GUU CCA AUA CUA CCA CTG were synthesized by GenePharma (Shanghai, China) as described previously (33). To knockdown the expression, siRNAs were transfected into cells using Lipofectamine 2000 reagent (Invitrogen) at 100 pmol/well in a 6-well plate. Forty eight hours later, cells were harvested for analysis.

**RNA Extraction and Quantitative RT-PCR (qRT-PCR)**—Total RNA was purified from cells by TRIzol reagent (Invitrogen), according to the manufacturer’s instructions, and cDNA was synthesized from 1 μg of total RNA-utilizing reverse transcription kit (Takara, Dalian, China). For agarose gel electrophoresis analysis, PCR was applied using PCR master mix from BioTeke (Beijing, China). Real time qPCR was performed in an ABI 7900HT Fast Real Time PCR system (Foster City, CA) utilizing SYBR Green PCR Master Mix (Toyobo, Japan) in a 20-μl volume. β-Actin mRNA was used as the internal control. A comparative Ct method (2 − ΔΔCt) was used for relative gene expression analysis. Primers were as follows: ACTB F, 5′-CAGTCGTAAGGGGCTACAGG-3′, and ACTB R, 5′-TCATACACATTGGCAATGAG-3′; βTrCP F, 5′-CTGCG-GCTGGGACCAAAGG-3′, and βTrCP R, 5′-AGACCCTGCTGGGGCCAGCTG-3′; and DYRK1A F, 5′-GCAAATCTC-GCTTCTTGG-3′, and DYRK1A R, 5′-TTATCACCAG-GCTTGTTGTTG-3′.

**EdU Staining Analysis**—One day before staining, cells were plated in a 6-well plate and cultured overnight. The next day, EdU was added to the medium with a final concentration of 10 μM. After 10 h of culture, the cells were trypsinized and stained with Apollo 643 reagent according to the manufacturer’s instruction (Ribobio, C00041, China). Stained cells were analyzed on a FACS machine (Beckman, CA) with 20,000 counted.

**Data Analysis**—All the experiments were repeated three times or more. For Western blotting and qRT-PCR, one representative image was shown, and the quantifications were calculated from three or more independent experiments. The values represented the means ± S.E. Student’s t test and Pearson’s correlation test were used for statistical analysis.

**Author Contributions**—X. S. designed the experiments. Q. L. performed the experiments. X. S. and Q. L. analyzed the data and wrote the paper. N. L., F. L., P. W., and H. L. helped perform the experiments and prepared reagents. Y. T. and L. C. helped to perform the experiments during the revision.

**References**

1. Antonarakis, S. E., Lyle, R., Dermitzakis, E. T., Reymond, A., and Deutsch, S. (2004) Chromosome 21 and Down syndrome: from genomics to pathophysiology. Nat. Rev. Genet. 5, 725–738.

2. Dowjat, W. K., Adayev, T., Kuchna, I., Nowicki, K., Palmiinelli, S., Hwang, Y. W., and Wiegel, J. (2007) Trisomy-driven overexpression of DYRK1A kinase in the brain of subjects with Down syndrome. Neurosci. Lett. 413, 77–81.

3. Smith, D. J., Stevens, M. E., Sudanagunta, S. P., Bronson, R. T., Makinson, M., Watabe, A. M., O’Dell, T. J., Fung, J., Weier, H. U., Cheng, J. F., and Rubin, E. M. (1997) Functional screening of 2 Mb of human chromosome 21q22.2 in transgenic mice implicates minibrain in learning defects associated with Down syndrome. Nat. Genet. 16, 28–36.

4. Altajaf, X., Dierssen, M., Baamonde, C., Martí, E., Visa, J., Guimerà, J., Oset, M., González, J. R., Flórez, J., Fillat, C., and Estiviil, X. (2001) Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing DYRK1A (minibrain), a murine model of Down’s syndrome. Hum. Mol. Genet. 10, 1915–1923.

5. Ahn, K. J., Jeong, H. K., Choi, H. S., Ryooy, S. R., Kim, Y. J., Goo, J. S., Choi, S. Y., Han, J. S., Ha, I., and Song, W. J. (2006) DYRK1A BAC transgenic mice show altered synaptic plasticity with learning and memory defects. Neurobiol. Dis. 22, 463–472.

6. Winniewski, K. E. (1990) Down syndrome children often have brain with maturation delay, retardation of growth, and cortical dysgenesis. Am. J. Med. Genet. Suppl. 7, 274–281.

7. Vicari, S. (2006) Motor development and neuropsychological patterns in persons with Down syndrome. Behav. Genet. 36, 355–364.

8. Lott, I. T., and Dierssen, M. (2010) Cognitive deficits and associated neurological complications in individuals with Down’s syndrome. Lancet Neurol. 9, 623–633.

9. Contestabile, A., Fila, T., Ceccarelli, C., Bonasoni, P., Bonapace, L., Santini, D., Bartesaghi, R., and Ciani, E. (2007) Cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinial matrix of fetuses with Down syndrome and in Ts65Dn mice. Hippocampus 17, 665–678.

10. Larsen, K. B., Kraush, H., Graem, N., Samuelsen, G. B., Bogdanovic, N., and Pakkenberg, B. (2008) Reduced cell number in the neocortical part of the human fetal brain in Down syndrome. Ann. Anat. 190, 421–427.

11. Guidi, S., Bonasoni, P., Ceccarelli, C., Santini, D., Gualtieri, F., Ciani, E., and Bartesaghi, R. (2008) Neurogenesis impairment and increased cell death reduce total neuron number in the hippocampal region of fetuses with Down syndrome. Brain Pathol. 18, 180–197.

12. Yabot, O., Domogauer, J., and D’Arcangelo, G. (2010) DYRK1A overexpression inhibits proliferation and induces premature neuronal differentiation of neural progenitor cells. J. Neurosci. 30, 4004–4014.

13. Chen, J. Y., Lin, J. R., Tsai, F. C., and Meyer, T. (2013) Dosage of DYRK1A shifts cells within a p21-cyclin D1 signaling map to control the decision to enter the cell cycle. Mol. Cell 52, 87–100.

14. Soppa, U., Schumacher, J., Fili, I., Cascan, E., Sánchez, M., and Pakkenberg, B. (2008) Reduced cell number in the neocortical part of Down’s syndrome. Brain Pathol. 18, 180–197.

15. Fillat, C., de la Luna, S., Arbones, M., and Farinas, I. (2010) Targeted regulation of kinase DYRK1A during asymmetric neural stem cell division is critical for EGFR-mediated biased signaling. Cell Stem Cell 7, 367–379.

16. Lu, M., Zheng, L., Han, B., Wang, L., Wang, P., Liu, H., and Sun, X. (2011) REST regulates DYRK1A transcription in a negative feedback loop. J. Biol. Chem. 286, 10755–10763.

17. Tanzi, R. E. (1996) Neuropathology in the Down’s syndrome brain. Nat. Med. 2, 31–32.

18. Ryooy, S. R., Cho, H. I., Lee, H. W., Jeong, H. K., Radaabazar, C., Kim, Y. S., Kim, M. I., Son, M. Y., Seo, H., Chung, S. H., and Song, W. J. (2008) Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A-mediated phosphorylation of amyloid precursor protein: evidence for a functional link between Down syndrome and Alzheimer’s disease. J. Neurochem. 104, 1333–1344.

19. Woods, Y. L., Cohen, P., Becker, W., Jakes, R., Goedert, M., Wang, X., and Proudt, C. G. (2001) The kinase DYRK phosphorylates protein-synthesis initiation factor eIF2B epsilon at Ser539 and the microtubule-associated protein tau at Thr212: potential role for DYRK as a glycogen synthase kinase 3-priming kinase. Biochem. J. 355, 609–615.
21. Ryoo, S. R., Jeong, H. K., Radnaabazar, C., Yoo, J. J., Cho, H. J., Lee, H. W., Kim, I. S., Cheon, Y. H., Ahn, Y. S., Chung, S. H., and Song, W. J. (2007) DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease. J. Biol. Chem. 282, 34850–34857

22. Liu, F., Liang, Z., Wegiel, J., Hwang, Y. W., Iqbal, K., Grundke-Iqbal, I., Ramakrishna, N., and Gong, C. X. (2008) Overexpression of DYRK1A contributes to neurofibillary degeneration in Down syndrome. FASEB J. 22, 3224–3233

23. Lee, M. S., Kao, S. C., Lemere, C. A., Xia, W., Tseng, H. C., Zhou, Y., Neve, R., Ahlijianian, M. K., and Tsai, L. H. (2003) APP processing is regulated by cytoplasmic phosphorylation. J. Cell Biol. 163, 83–95

24. Vingtdeux, V., Hamdane, M., Gompel, M., Bégard, S., Drobecq, H., Ghes-tem, A., Grosjean, M. E., Kostanjeveci, V., Grognet, P., Vanmechelen, E., Bué, L., Delacourte, A., and Sergeant, N. (2005) Phosphorylation of amyloid precursor carboxyl-terminal fragments enhances their processing by a γ-secretase-dependent mechanism. Neurobiol. Dis. 20, 625–637

25. Ferrer, I., Barrachina, M., Puig, B., Martinez de Lagrán, M., Martí, E., Avila, J., and Dierssen, M. (2005) Constitutive Dyrk1A is abnormally expressed in Alzheimer disease, Down syndrome, Pick disease, and related transgenic models. Neurobiol. Dis. 20, 392–400

26. Ciechanover, A. (1994) The ubiquitin-proteasome proteolytic pathway. Cell 79, 13–21

27. Dierssen, M., and de Lagrán, M. M. (2006) DYRK1A (dual-specificity tyrosine-phosphorylated and -regulated kinase 1A): a gene with dosage effect during development and neurogenesis. ScientificWorldJournal 6, 1911–1922

28. Frescas, D., and Pagano, M. (2008) Deregulated proteolysis by the F-box protein SKP2 and β-TrCP: tipping the scales of cancer. Nat. Rev. Cancer 8, 438–449

29. Low, T. Y., Peng, M., Magliozzi, R., Mohammed, S., Guardavaccaro, D., and Heck, A. J. (2014) A systems-wide screen identifies substrates of the SCFβTrCP ubiquitin ligase. Sci. Signal. 7, rs8

30. Westbrook, T. F., Hu, G., Ang, X. L., Mulligan, P., Pavlova, N. N., Liang, A., Leng, Y., Maehr, R., Shi, Y., Harper, J. W., and Elledge, S. J. (2008) SCFβ-TrCP controls oncogenic transformation and neural differentiation through REST degradation. Nature 452, 370–374

31. Liu, Q., Liu, N., Zang, S., Liu, H., Wang, P., Ji, C., and Sun, X. (2014) Tumor suppressor DYRK1A effects on proliferation and chemoresistance of glioblastoma rapidly degraded proteins: the PEST hypothesis. Science 3488–3495

32. Kentrup, H., Becker, W., Heukelbach, J., Wilmes, A., Schürmann, A., Huppertz, C., Kainulainen, H., and Joost, H. G. (1996) Dyrk, a dual specificity tyrosine-phosphorylated and -regulated kinase 1A: a gene with dosage effect during development and neurogenesis. J. Neurosci. 16, 27659–26770

33. Liu, F., Liang, Z., Wegiel, J., Hwang, Y. W., Iqbal, K., Grundke-Iqbal, I., Ramakrishna, N., and Gong, C. X. (2008) Overexpression of DYRK1A contributes to neurofibillary degeneration in Down syndrome. FASEB J. 22, 3224–3233

34. Lee, M. S., Kao, S. C., Lemere, C. A., Xia, W., Tseng, H. C., Zhou, Y., Neve, R., Ahlijianian, M. K., and Tsai, L. H. (2003) APP processing is regulated by cytoplasmic phosphorylation. J. Cell Biol. 163, 83–95

35. Vingtdeux, V., Hamdane, M., Gompel, M., Bégard, S., Drobecq, H., Ghes-tem, A., Grosjean, M. E., Kostanjeveci, V., Grognet, P., Vanmechelen, E., Bué, L., Delacourte, A., and Sergeant, N. (2005) Phosphorylation of amyloid precursor carboxyl-terminal fragments enhances their processing by a γ-secretase-dependent mechanism. Neurobiol. Dis. 20, 625–637

36. Ferrer, I., Barrachina, M., Puig, B., Martinez de Lagrán, M., Martí, E., Avila, J., and Dierssen, M. (2005) Constitutive Dyrk1A is abnormally expressed in Alzheimer disease, Down syndrome, Pick disease, and related transgenic models. Neurobiol. Dis. 20, 392–400

37. Ciechanover, A. (1994) The ubiquitin-proteasome proteolytic pathway. Cell 79, 13–21

38. Dierssen, M., and de Lagrán, M. M. (2006) DYRK1A (dual-specificity tyrosine-phosphorylated and -regulated kinase 1A): a gene with dosage effect during development and neurogenesis. ScientificWorldJournal 6, 1911–1922

39. Frescas, D., and Pagano, M. (2008) Deregulated proteolysis by the F-box proteins SKP2 and β-TrCP: tipping the scales of cancer. Nat. Rev. Cancer 8, 438–449

40. Low, T. Y., Peng, M., Magliozzi, R., Mohammed, S., Guardavaccaro, D., and Heck, A. J. (2014) A systems-wide screen identifies substrates of the SCFβTrCP ubiquitin ligase. Sci. Signal. 7, rs8

41. Westbrook, T. F., Hu, G., Ang, X. L., Mulligan, P., Pavlova, N. N., Liang, A., Leng, Y., Maehr, R., Shi, Y., Harper, J. W., and Elledge, S. J. (2008) SCFβ-TrCP controls oncogenic transformation and neural differentiation through REST degradation. Nature 452, 370–374

42. Liu, Q., Liu, N., Zang, S., Liu, H., Wang, P., Ji, C., and Sun, X. (2014) Tumor suppressor DYRK1A effects on proliferation and chemoresistance of AML cells by downregulating c-Myc. PLoS One 9, e98853

43. Kentrup, H., Becker, W., Heukelbach, J., Wilmes, A., Schürmann, A., Huppertz, C., Kainulainen, H., and Joost, H. G. (1996) Dyrk, a dual specificity protein kinase with unique structural features whose activity is dependent on tyrosine residues between subdomains VII and VIII. J. Biol. Chem. 271, 3488–3495

44. Rogers, S., Wells, R., and Rechsteiner, M. (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234, 364–368

45. Hattori, K., Hatakeyama, S., Shirane, M., Matsumoto, M., and Nakayama, K. (1999) Molecular dissection of the interactions among 14-3-3, FWD1, and Skp1 required for ubiquitin-mediated proteolysis of 14-3-3. J. Biol. Chem. 274, 29641–29647

46. Watanebe, N., Arai, H., Nishihara, Y., Taniguchi, M., Watanebe, N., Hunter, T., and Osada, H. (2004) M-phase kinases induce phospho-dependent ubiquitination of somatic Weel by SCFβ-TrCP. Proc. Natl. Acad. Sci. U.S.A. 101, 4419–4424

47. Limón-Mortés, M. C., Mora-Santos, M., Espina, A., Pintor-Toro, J. A., López-Román, A., Tortorelo, M., and Romero, F. (2008) UV-induced degradation of securin is mediated by SKP1-CUL1-β TrCP E3 ubiquitin ligase. J. Cell Sci. 121, 1825–1831