The Selective RNA-binding Protein Quaking I (QKI) Is Necessary and Sufficient for Promoting Oligodendroglia Differentiation*

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Quaking I (QKI) is a selective RNA-binding protein essential for myelination of the central nervous system. Three QKI isoforms with distinct C termini and subcellular localization, namely QKI-5, QKI-6, and QKI-7, are expressed in oligodendroglia progenitor cells (OPCs) prior to the initiation of myelin formation and implicated in promoting oligodendrocyte lineage development. However, the functional requirement for each QKI isoform and the mechanisms by which QKI isoforms govern OPC development still remain elusive. We report here that exogenous expression of each QKI isoform is sufficient to enhance differentiation of OPCs with different efficiency, which is abolished by a point mutation that abrogates the RNA binding activity of QKI. Reciprocally, small interfering RNA-mediated QKI knockdown blocks OPC differentiation, which can be partly rescued by QKI-5 and QKI-6 but not by QKI-7, indicating the differential requirement of QKI isoform function in advancing OPC differentiation. Furthermore, we found that abrogation of OPC differentiation, as a result of QKI deficiency, is not due to altered proliferation capacity or cell cycle progression. These results indicate that QKI isoforms are necessary and sufficient for promoting OPC development, which must involve direct influence of QKI on differentiation/maturation of OPCs independent of cell cycle exit, likely via regulating the expression of the target mRNAs of QKI that support OPC differentiation.

Oligodendrocytes (OLs) are responsible for myelination of the central nervous system. The oligodendroglia progenitor cells (OPCs) are derived from pluripotent neural stem cells in the developing brain (1, 2). After fate specification for the OL lineage, OPCs keep proliferating until they are committed for terminal differentiation (3). The maturation of OPC into myelinating OLs is characterized by the extension of numerous branched processes and membrane sheets, along with the expression of myelin-specific structural proteins (4). Adequate OPC proliferation ensures the generation of sufficient numbers of myelin-producing cells, whereas OPC differentiation is a critical prerequisite for myelin formation. Thus, the transition of OPCs from proliferation to differentiation is a key step that governs central nervous system myelin development (5, 6). However, forced cell cycle exit is not sufficient for inducing OPC differentiation (7, 8), suggesting the existence of cell cycle-independent yet undefined mechanisms that directly promote OPC differentiation/maturation.

QKI is a selective RNA-binding protein that controls the homeostasis and subcellular localization of target mRNAs during OL and myelin development (9–13). Deficiency of QKI in OLs results in severe hypomyelination in the quaking viable (qkv) mutant mice (10, 14), which can be rescued by a QKI transgene specifically expressed in the OL lineage (15). Three major QKI protein isoforms with distinct C termini are derived from alternative splicing, named QKI-5, QKI-6, and QKI-7 (16, 17). QKI-5 is predominantly localized in the nucleus, whereas QKI-6 and QKI-7 are mainly detected in the cytoplasm (18), suggesting distinct functional influence by QKI isoforms on their mRNA targets. The common N terminus in QKI isoforms harbors an extended RNA-binding domain homologous to that in the heterogeneous nuclear ribonucleoprotein K (KH) (16), which is essential for the interaction of QKI with its mRNA ligands and the dimerization between QKI isoforms (18). Thus, it is believed that the functional interplay of QKI isoforms governs the stability and subcellular localization of their mRNA targets during central nervous system myelin development (11, 19).

In addition to its essential role in myelination, QKI is expressed in cells at the earliest stage of OL lineage development before they migrate away from the subventricular zone (14, 16, 20), suggesting that QKI may function to advance OL lineage development before actual myelination. Consistent with this idea, QKI is up-regulated in OLs when they extend processes to contact axons (14, 21). In addition, ectopic expression of exogenous QKI-6 and QKI-7 can force cell cycle exit and differentiation of OPCs (19). However, whether endogenous QKI is essential for advancing OL development, and if so, which QKI isoform is functionally required, has not been determined. Whether QKI only accelerates OPC cell cycle exit or whether it also directly promotes OPC differentiation via cell cycle-independent mechanisms is still unknown.
Function of QKI in Oligodendroglia Differentiation

In this study, we report that QKI is necessary and sufficient for OPC differentiation. In addition, QKI isoforms exhibit differential abilities in promoting OPC differentiation, which depends on the RNA binding activity of QKI. Furthermore, in the CG4 OPC cell line, RNA interference-mediated QKI knockdown abrogates differentiation without affecting the proliferation capacity or cell cycle progression. These results provide the first evidence suggesting that QKI can exert direct influence on OPC differentiation via cell cycle-independent mechanisms, most likely by regulating the expression of its mRNA targets that encode key proteins for OPC differentiation.

MATERIALS AND METHODS

Cell Culture—CG4 cells were maintained for proliferation in Dulbecco’s modified Eagle’s medium containing 1% heat-inactivated fetal bovine serum, insulin (5 µg/ml), transferrin (50 µg/ml), PBPS (putrescine (100 mM), biotin (10 ng/ml), progesterone (20 nM), and selenium (30 nM)). Platelet-derived growth factor AA (Sigma) and basic fibroblast growth factor (Promega) were added to the proliferation medium at a final concentration of 10 ng/ml each. Induction of differentiation was achieved by replacing proliferation medium with differentiation medium, which contains Dulbecco’s modified Eagle’s medium, insulin, PBPS, transferrin, tri-iodothyronine (50 nM), and 0.5% fetal bovine serum. Primary cultures of OPCs were isolated as described previously (22). Briefly, mixed glial cultures were prepared from the brains of neonatal rats (P2; Sprague-Dawley, Taconic Farms) and allowed to reach confluence. OPCs were removed by shaking and further purified by immunopanning with A2B5. They were plated onto poly-lysine-coated coverslips and maintained in a defined serum-free medium (22) with platelet-derived growth factor and fibroblast growth factor (10 and 20 ng/ml, respectively) to prevent differentiation. Mature OLs were obtained by culturing the OPCs in a defined medium lacking growth factors.

Introducing siRNA, Plasmids, and Lentivirus into Cells—Synthetic double-strand small interfering RNA (siRNA) specific for the QKI coding region, 5'-GGACUUACAGCUAACACATc-3' (sense strand), 5'-GUUGUUUAGCUGUAUGCtcc-3' (antisense strand), and the Silencer® negative control 1 siRNA, were purchased from Ambion, Inc. The plasmids used for transfection include PC-FLAG-QKI-5, QKI-6, QKI-7 (13), PC-FLAG-QKI-6<sup>ccc</sup> (E48G), GFP-N3, and siRNA-resistant mutant PC-FLAG-QKI-5, -QKI-6, -QKI-7. The siRNA-resistant mutations were generated using the PCR-based GeneTailor® site-directed mutagenesis system (Invitrogen) with the following primers: 5'-ATCCTTGGACCTAGAGGATTGACCGCTAAACAA-CTT-3' (forward), 5'-TCCTCTAGTGCCAGGTATCCTCCCAACTTTAAA-3' (reverse). CG4 cells were transfected using a Nucleofector® kit optimized for OLs (Amaxa, Inc.) following the manufacturer’s protocol, and the GFP-N3 plasmid was co-transfected to mark the transfected cells. Three micrograms of plasmid DNA and/or 200 pmol of siRNA were used to transfect 2 × 10<sup>6</sup> cells/culture. For lentivirus production, pLenti-hU6BX constructs containing either QKI siRNA or the Silencer® negative control 1 siRNA were established by Cellogenetics, Inc. The lentiviruses were produced at the core facility at the Center for Neurodegenerative Diseases at Emory University.

Morphological Analysis—Light-field images and fluorescent images of transfected live CG4 cells (GFP-positive) were captured from randomly selected microscopic fields using the Olympus IX-51 inverted fluorescent microscope. Processes were categorized into three groups based on where they originate and counted: the primary processes are directly from the cell body, the secondary processes are from the primary processes, and the tertiary processes are from the secondary processes. More than 30 randomly selected cells were analyzed from each transfected culture.

BrdUrd Labeling—To examine the proliferation of CG4 cells, BrdUrd (Sigma) was added to the culture medium at a final concentration of 10 µM. After incubation at 37 °C for 1 h, cells were fixed with 70% ethanol at −20 °C for 30 min. HCl (2 N) was used to denature DNA for 1 h at 37 °C. Cells were permeabilized with 0.1% Triton X-100 and blocked with 2% normal goat serum, incubated with anti-BrdUrd (1:1000; Chemicon, Inc.) at room temperature for 1 h followed by secondary antibody incubation and quantitative analysis of cell numbers as described in the legend for Fig. 6.

Flow Cytometry Analysis of Cell Cycle—Cells were harvested, washed with ice-cold PBS, and incubated with prechilled 80% ethanol at 4 °C overnight. After washing, cells were treated with 500 µg/ml RNase A in PBS at 37 °C for 30 min followed by incubation with a propidium iodide (Invitrogen) solution (50 µg/ml propidium iodide, 0.1% sodium citrate, 0.1% Triton X-100 in PBS) at room temperature for 1 h in dark. DNA content of the cells was measured with a FACSCalibur flow cytometer (Beckman Instruments), and results were analyzed with Flowjo software (Tree Star, Inc.).

Immunoprecipitation and Reverse Transcription-PCR—FLAG QKI-6 was transfected into CG4 cells and immunoprecipitated 48 h after transfection using the anti-FLAG M2 antibody (Sigma) as described previously (23). The immunoprecipitated complexes were subjected to RNA extraction followed by reverse transcription-PCR using p27Kip1-specific primers: 5'-AGCTTGGACCGTCTAC-3' (forward) 5'-GGGGAACCGTCTGAAACATT-3' (reverse). PCDNA-transfected cells were processed in parallel as a negative control.
Function of QKI in Oligodendroglia Differentiation

RESULTS

Forced Expression of Exogenous QKI Isoforms Enhances OPC Differentiation, Which Is Abolished by the E48G Mutation That Affects the RNA Binding Activity of QKI—CG4 is an OPC cell line that can be induced for differentiation, recapitulating the morphological changes and maturation marker expression profiles observed in primary cultured OPCs as well as in the developing brain (23–25). One advantage of using CG4 cells over primary cultured OPCs is the consistent high efficiency of transfection, allowing efficient manipulation of QKI expression. We first examined the functional influence of QKI isoforms on the differentiation of CG4 cells by transfecting plasmids that encode FLAG-QKI-5, FLAG-QKI-6, and FLAG-QKI-7 individually into proliferating CG4 cells. Immunofluorescent staining indicated predominant nuclear localization of FLAG-QKI-5, whereas the majority of FLAG-QKI-6 and FLAG-QKI-7 were detected in the cytoplasm, reminiscent of the subcellular localization of QKI isoforms in the developing brain (14). Upon removal of mitogens, the bipolar-shaped proliferating CG4 cells started to differentiate, characterized by the increased number of primary processes and the development of sophisticated secondary and tertiary branches (Fig. 1A). Enhanced morphological differentiation was observed in cells expressing exogenous QKI isoforms, which were marked by the expression of the GFP from the co-transfected plasmid, as compared with that in control cells that were transfected by the GFP plasmid alone (Fig. 1A). Quantitative analysis indicated that the average numbers of secondary and tertiary processes were markedly increased in cells expressing exogenous QKI, whereas the numbers of primary processes were not significantly altered (Fig. 1B). Although all three QKI isoforms were capable of enhancing process development, QKI-5 and QKI-6 apparently enhanced CG4 cell differentiation more efficiently than QKI-7 (Fig. 1B), despite the fact that all three FLAG-QKI isoforms were expressed at similar levels (Fig. 1B, inset). In addition, we did not detect increased apoptosis in cells expressing QKI-7 (data not shown), excluding the possibility that the weaker activity for QKI-7 to advance differentiation of CG4 cells is due to increased apoptosis.

Consistent with a previous report identifying p27Kip1 mRNA as a QKI target (19), we observed association p27Kip1 mRNA with FLAG-QKI-6 in transfected CG4 cells. Immunoprecipitation assay with anti-FLAG M2 beads was performed with cells transfected with either FLAG-QKI-6 or the PCDNA vector (PC). p27Kip1 mRNA is detected by reverse transcription-PCR in the eluate (E) of immunoprecipitate derived from QKI-6-transfected cells but not from control cells. I, input lysate for immunoprecipitation. D, influence of exogenous QKI isoforms on p27Kip1 expression measured by immunoblot. Cells transfected by the PCDNA vector (PC) was processed in parallel as a negative control.

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extended KH domain. This mutation was previously shown to abolish the association of QKI with its RNA ligands (27). In contrast to wild type FLAG-QKI-6, the E48G mutant QKI-6 failed to promote either process branching or O1 expression, suggesting that QKI facilitates OPC differentiation by RNA binding-dependent mechanisms.

RNA Interference-mediated QKI Knockdown Abrogates OPC Differentiation, and QKI Isoforms Display Differential Abilities in Rescuing the Differentiation Defects—To determine whether QKI is functionally required for OPC differentiation, we introduced a small interfering RNA (siRNA) into proliferating CG4 cells specifically targeting the 5′-coding region that is common for all QKI isoforms (QKIsi, Fig. 3A). A negative control siRNA (Ctrlsi) that harbors no sequence homology to any mammalian mRNA (Ambion) was used in parallel transfection. The plasmid encoding GFP was co-transfected to mark cells that received the siRNA. Immunoblot analysis showed that transfection of double-stranded QKIsi eliminated greater than 80% of endogenous QKI isoforms (Fig. 3B). We also generated lentivirus that express QKIsi or Ctrlsi, respectively, and observed similar efficiency for knocking down the endogenous QKI. Process extension and branching in primary cultured rat OPCs was severely abrogated when cells were treated with the QKIsi virus, whereas cells treated with the Ctrlsi virus developed fully extended processes with vigorous branching (Fig. 3C, panels a and b). Similarly, transfection of synthetic QKIsi into CG4 cells significantly attenuated process branching during differentiation as compared with Ctrlsi-treated cells, evident by the markedly reduced secondary processes (Fig. 3C, panels c and d). Quantitative analysis confirmed the significant decrease in the average number of secondary processes in QKIsi-treated CG4 cells (Fig. 3D), suggesting that elimination of QKI arrested OPC differentiation at early stages of morphogenesis. We also examined the effect of QKIsi on expression of the OPC differentiation marker O1 in CG4 cells (Fig. 4). After 72 h of differentiation, 40% of Ctrlsi-treated cells were O1-positive (Fig. 4A, red fluorescence), but very few (5%) of

![FIGURE 2. RNA binding-deficient QKI-6 mutant fails to promote CG4 cell differentiation. A, immunofluorescent staining of the late oligodendrocyte differentiation marker O1 (red) in CG4 cells transfected with GFP plasmid alone or GFP plus the plasmid encoding either the wild type (WT) QKI-6 or the E48G mutant QKI-6. The DAPI staining marks nuclei. Scale bar, 50 μm. B, quantification of the percentage of O1-positive cells in cultures transfected with the aforementioned plasmids. More than 200 cells were counted from each transfected culture, and the percentages of O1-positive cells from three independent experiments were graphically displayed with S.E. indicated.](image)

![FIGURE 3. QKI is functionally required for OPC differentiation. A, schematic presentation for the location of the QKI siRNA in the common coding region for all QKI mRNAs. B, immunoblot analysis of protein extracts of CG4 cells detects endogenous QKI isoforms. QKI-5 co-migrates with QKI-7 above QKI-6. Diminished expression of all endogenous QKI proteins is obvious upon QKI siRNA treatment. Actin was used as a loading control. C, morphology of primary cultured rat OPCs transfected with the lentivirus either expressing control siRNA or expressing QKI siRNA (panels a and b, marked by the co-expressed GFP) and morphology of CG4 cells co-transfected with GFP and the indicated synthetic siRNA (panels c–f). Transfected CG4 cells expressing GFP were shown in panels e and f. Cells were induced to differentiate for 72 h. Scale bar, 25 μm. D, quantification of the number of primary and secondary processes per cell from QKIsi- and Ctrlsi-treated CG4 cells from three independent experiments (n = 3). Black bar, control siRNA; gray bar, QKI siRNA.](image)
the QKIsi-treated CG4 cells expressed O1. Taken together, these data support the hypothesis that QKI is required for the normal differentiation of OPC.

The specific QKI isoform required for OPC differentiation can be determined based on its ability to rescue the differentiation defect in CG4 cells caused by QKIsi. Since siRNA-mediated mRNA cleavage requires perfect sequence match between the siRNA and the mRNA target, we constructed QKIsi-resistant mutant QKI isoforms in which three silent point mutations were introduced into the QKIsi target site (Fig. 5A). When co-transfected with QKIsi, the expression level of each wild type FLAG-QKI isoform was greatly reduced with similar efficiency, whereas the levels of mutant FLAG-QKI isoforms were largely unaffected as compared with that in Ctrlisi-treated cells (Fig. 5B). In our differentiation paradigm, we found

**FIGURE 4.** QKI knockdown results in differentiation arrest of CG4 cells indicated by the differentiation marker O1. A, CG4 cells were transfected with either QKIsi or Ctrlisi and induced to differentiate for 72 h. Immunostaining indicates O1-positive cells (red), with total nuclei labeled by DAPI staining. Scale bar, 50 μm. B, quantification of the percentage of O1-positive cells. More than 200 cells were counted from each transfected culture, and results from three independent experiments (n=3) were graphically displayed with S.E. indicated.

**FIGURE 5.** QKI isoforms display differential abilities in rescuing QKI siRNA-mediated block of OPC differentiation. A, silent mutations introduced into the QKI mRNA at the siRNA target site. Qsi, QKIsi; WT, wild type; Mut, mutant. B, immunoblot analysis indicating that the mutant QKI isoforms are resistant to the QKI siRNA, whereas wild type QKI isoforms are diminished in the presence of QKIsi. Q5, Q6, and Q7, QKI-5, QKI-6, and QKI-7 isoforms, respectively. C, CG4 cells were transfected either with QKIsi alone or with various plasmids encoding siRNA-resistant QKI isoforms and induced to differentiate for 72 h. Immunostaining indicates O1-positive cells (red), with nuclei labeled by DAPI staining (blue). Scale bar, 50 μm. D, quantification of the percentage of O1-positive cells. More than 200 cells were recorded from each transfected culture, and results from three independent experiments were graphically displayed with S.E. indicated.
that co-transfection of CG4 cells with the QKIsi-resistant QKI-5m or QKI-6m together with QKIsi significantly increased the number of O1-positive cells as compared with that in cells treated with QKIsi alone (Fig. 5). Quantitative analysis further revealed that although each QKI isoform is expressed at similar levels, QKI-6 is more efficient than QKI-5 in rescuing CG4 cell differentiation (Fig. 5D). In contrast, QKI-7 failed to rescue differentiation of CG4 cells, regardless of the fact that ectopic overexpression of QKI-7 can promote OPC differentiation (Fig. 1, A and B) (19), albeit with lower efficiency.

The siRNA-mediated QKI Knockdown Abrogates Differentiation of CG4 Cells without Altering Proliferation Capacity or Cell Cycle Progression—Since ectopic expression of QKI can promote cell cycle exit of OPCs (19), we questioned whether QKIsi-mediated suppression of OPC differentiation (Figs. 3–5) is due to the attenuation of cell cycle exit, which would lead to increased number of proliferating OPCs. To address this question, we pulse-labeled QKIsi- and Ctrlsi-treated CG4 cells with BrdUrd and identified cells that had undergone active proliferation based on the incorporation of BrdUrd into the replicating genomic DNA as detected by immunofluorescent staining. As shown in Fig. 6, QKIsi treatment did not alter the numbers of BrdUrd-labeled proliferating cells. Quantitative analysis indicated a similar percentage of BrdUrd-labeled cells in QKIsi- and Ctrlsi-treated cultures, suggesting that endogenous QKI isoforms are not involved in governing the proliferation capacity of OPCs.

To further test whether QKI knockdown may affect cell cycle progression, we performed fluorescence-activated cell sort analysis using CG4 cells that are treated with the QKIsi lentivirus or the control lentivirus. In this experiment, greater than 90% of cells were transduced, marked by the expression of GFP from the same virus. The DNA content in cells was labeled by propidium iodide, which was used to determine the distribution of cells at various steps of cell cycle. As shown in Fig. 7, nearly identical fluorescence-activated cell sort profiles were observed in QKIsi-treated cells and the control cells. Quantitative analysis detected no significant difference between QKIsi-treated cells and control cells on the percentage distribution at the G1/G0 phase (~70%), the S phase (~15%), and the G2/M phase (15%) (Fig. 7B). Although overexpression of exogenous QKI-6 and QKI-7 enhanced p27Kip1 (Fig. 1D), knocking down QKI in CG4 cells does not affect p27Kip1 pro-

FIGURE 6. QKI-siRNA does not increase CG4 cell proliferation. A, BrdUrd (BrdU) incorporation was performed using QKIsi- or Ctrlsi-transfected cells. Cells were stained with the anti-BrdUrd antibody (red), and nuclei were stained with DAPI (blue). B, quantification of BrdUrd-positive cells. More than 200 cells were analyzed from each transduced culture, and results from three independent experiments were graphically displayed with S.E. indicated. No significant difference is observed in the results when comparing QKIsi- and Ctrlsi-treated cells.

FIGURE 7. Cell cycle progression of CG4 is not altered by the QKI-siRNA treatment. A, flow cytometry analysis of DNA content in propidium iodide (PI)-stained CG4 cells. Cells were transduced with either the QKIsi lentivirus or the control (Ctrl) virus and cultured for 48 h before flow cytometry analysis was performed. B, quantitative distribution of cells at various steps in the cell cycle. The percentage of cells at G1/G0, S, and G2/M is calculated, and the mean value from three independent experiments is displayed graphically with S.E. indicated. The black and gray bars represent cells treated with the control virus and the QKIsi virus, respectively. C, QKI knockdown does not affect p27Kip1 protein expression. Western blots were performed with anti-QKI and anti-p27Kip1 antibody using lysates derived from QKIsi- and Ctrlsi-treated CG4 cells. Actin was used as an internal loading control.
tein expression (Fig. 7D), suggesting that endogenous QKI is not necessary for maintaining p27Kip1 expression. These results suggest that QKI isoform-mediated differentiation arrest is not due to delayed cell cycle exit. Instead, QKI deficiency must directly affect differentiation of CG4 cells via cell cycle-independent mechanisms.

**DISCUSSION**

The above studies demonstrated that the selective RNA-binding protein QKI is necessary and sufficient for advancing OPC differentiation. In addition, specific QKI isoforms have differential abilities in promoting OPC differentiation, which requires the RNA binding activity of QKI. Furthermore, we show that endogenous QKI is not necessary for cell cycle control of the CG4 OPC cell line, suggesting that at least part of the function of QKI is achieved by direct influence on OPC differentiation, after proliferating OPCs exit the cell cycle.

The role of QKI in central nervous system myelination is well documented (10). QKI selectively interacts with a subgroup of mRNAs that encode essential myelin structural proteins to control their homeostasis and subcellular localization (11, 12, 23). The best characterized QKI targets for myelination include the myelin basic protein (MBP) mRNA (11–13) and the myelin-associated glycoprotein mRNA (28). QKI deficiency in OLs causes destabilization and mislocalization of the MBP mRNA (12) and dysregulated splicing of the myelin-associated glycoprotein mRNA (28), which are potential mechanisms for the failure of myelination in the quaking (qk') mutant mice. The functional importance of QKI in myelination is further reinforced by the fact that N-ethyl-N-nitrosourea-induced single base pair mutations in the qki gene also cause hypomyelination (29, 30). However, the detection of QKI in the earliest stage of OL lineage development (20, 31) and the association of QKI with mRNAs critical for OL fate specification and differentiation (19, 27) suggest that QKI must also play important roles in early OL development prior to myelin formation. Our results that siRNA-mediated QKI knockdown abrogates OPC differentiation (Figs. 3 and 4) provide the first evidence demonstrating the functional requirement of QKI in OL differentiation.

The fact that the RNA binding-deficient E48G mutant QKI failed to promote OPC differentiation (Fig. 2) suggests that QKI supports OPC development via regulating its mRNA targets. Since QKI exerts its influence on OPC differentiation before the expression of myelin structural genes such as mbp, in the differentiation paradigm, QKI must regulate its mRNA targets that are critical for OPC differentiation but distinct from those that support myelination.

A previous report showed that overexpression of QKI-6 and QKI-7 can enhance OPC differentiation (19). However, the function of QKI-5 in OPC differentiation was not addressed. Our results confirmed the ability of exogenous QKI-6 and QKI-7 in promoting OPC differentiation (Fig. 1) and further indicated that overexpression of the nuclear isoform QKI-5 also promotes OPC differentiation (Fig. 1), regardless of the fact that overexpression of QKI-5 causes nuclear retention of the MBP mRNA and presumably may negatively affect myelination (11). The fact that QKI-5 promotes CG4 cell differentiation but not p27Kip1 expression (Fig. 1) suggests that the nuclear QKI isoform may advance OPC differentiation via distinct mechanisms as compared with that employed by the cytoplasmic QKI isoforms. In fact, QKI-5 is the most abundant isoform in OPCs during embryonic and neonatal development (14) and is the second most efficient QKI isoform in rescuing the differentiation defect caused by the QKI siRNA (Fig. 5). One possible explanation is that QKI-5 may function to achieve nuclear retention of mRNAs that encode suppressors for OPC differentiation, which in turn supports OPC differentiation.

QKI isoforms harbor differential abilities to support OPC differentiation, as indicated by the quantitative effect of each QKI isoform in promoting OPC differentiation (Fig. 1) and in rescuing the defect of differentiation caused by the QKI siRNA (Fig. 5). In both experiments, QKI-6 is the most efficient isoform in supporting OPC differentiation. This is consistent with the recent finding that QKI-6 alone can significantly improve the hypomyelination phenotype of the qk' mutant mice (15). On the other hand, QKI-6 cannot completely rescue the defect of myelin gene expression at the early stage of myelin development (15) or the differentiation arrest by the QKI siRNA (Fig. 5), suggesting the functional importance of other QKI isoforms for the early development of the OL lineage. Among the QKI isoforms, QKI-7 displays weaker activity in supporting OPC differentiation (Figs. 1 and 5). Because QKI-7 is the least abundant isoform in OPCs and is up-regulated at a later stage of myelin development (14), it may perform a major role to support myelin formation and maintenance. This idea is consistent with the observation that preferential reduction of QKI-7 is associated with white matter disruption in schizophrenia patients (32, 33). An alternative possibility is that QKI-7 may depend on the other QKI isoforms to promote OPC differentiation and could not function by itself when endogenous QKI isoforms are eliminated by RNA interference.

Forced expression of QKI-6 and QKI-7 can enhance cell cycle exit of OPCs (19). One proposed mechanism is that QKI binds and stabilizes the mRNA encoding the cyclin-dependent kinase inhibitor p27Kip1 (19), one of the key regulators for cell cycle arrest of OPCs (3, 34). In CG4 cells, exogenous QKI-6 and QKI-7 also enhanced p27Kip1 expression (Fig. 1D). However, although overexpression of p27Kip1 can lead to forced cell cycle arrest of OPCs, it is not sufficient for inducing OPC differentiation (7, 8). Reciprocally, p27Kip1 deficiency increases cell proliferation capacity but does not affect the timing of oligodendrocyte differentiation (31). Thus, the effect of QKI on promoting OPC differentiation must also involve cell cycle-independent mechanisms and is achieved by regulating other mRNA targets in addition to p27Kip1 mRNA. Consistent with this view, our results indicate that exogenous QKI-5 promotes OPC differentiation without enhancing p27Kip1 expression (Fig. 1D). Furthermore, elimination of QKI in CG4 cells does not alter proliferation capacity or cell cycle progression (Figs. 6 and 7), suggesting that the abrogation of OPC differentiation caused by siRNA-mediated QKI knockdown (Figs. 3–5) is not due to blocking cell cycle exit. In fact, knocking down endogenous QKI did not affect p27Kip1 protein expression (Fig. 7D), indicating that QKI is not necessary for governing p27Kip1 expression in CG4 cells. It is important to point out that p27Kip1 expression is under sophisticated regulation by multi-
ple molecular mechanisms at the levels of transcription, translation, and protein stability in addition to the stabilization of p27Kip1 mRNA by QKI (8, 35–38). Therefore, compensatory mechanisms may prevent changes of p27Kip1 expression even when QKI is eliminated, which provides a possible explanation why QKI is unnecessary for p27Kip1 expression and cell cycle exit of CG4 cells (Fig. 7). In this regard, QKI must exert direct influence on OPC differentiation/maturation, most likely via enhanced expression of its mRNA targets that encode proteins critical for OPC differentiation. Thus, siRNA-mediated QKI knockdown causes deficits in expression of these differentiation factors, which in turn attenuates OPC differentiation (Figs. 3–5).

One of the QKI targets that plays key roles in supporting OPC morphological differentiation is the microtubule-associated protein 1B (MAP1B) (39). QKI binds to the 3'-untranslated region of the MAP1B mRNA, and QKI-dependent mRNA stabilization underlies the developmentally programmed up-regulation of MAP1B during OPC differentiation (23). Considering the well characterized function of MAP1B in supporting the dynamic assembly of the microtubule cytoskeleton and the critical role of microtubules in OPC differentiation (39–42), the effect of QKI in facilitating MAP1B up-regulation is a conceivable mechanism for supporting the process extension of OPCs. Although QKI most likely regulates distinct sets of mRNA targets to support myelination and OPC differentiation, the full range of the mRNA targets of QKI at different stages of OL lineage development still remains undetermined. Identification of the downstream mRNA targets that are under control of QKI-dependent posttranscriptional regulation in future studies will provide critical insights regarding molecular mechanisms that govern OPC development in normal myelogenesis, as well as the white matter disruption and repair in various myelin disorders, represented by multiple sclerosis and schizophrenia (33, 43).

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REFERENCES
1. McMahon, A. P. (2000) Genes Dev. 14, 2261–2264
2. Anderson, D. J. (2001) Neuron 30, 19–35
3. Gao, F. B., Durand, B., and Raff, M. (1997) Curr. Biol. 7, 152–155
4. Pfeiffer, S. E., Warrington, A. E., and Bansal, R. (1993) Trends Cell Biol. 3, 191–197
5. Temple, S., and Raff, M. C. (1986) Cell 44, 773–779
6. Barres, B. A., Lazar, M. A., and Raff, M. C. (1994) Development (Camb.) 120, 1097–1108
7. Tang, X. M., Beesley, J. S., Grinspan, J. B., Seth, P., Kamholz, J., and Cambi, F. (1999) J. Cell. Biochem. 76, 270–279
8. Tokumoto, Y. M., Apperly, J. A., Gao, F. B., and Raff, M. C. (2002) Dev. Biol. 245, 224–234
9. Vernet, C., and Artzt, K. (1997) Trends Genet. 13, 479–484
10. Hardy, R. J. (1998) J. Neurosci. Res. 51, 417–422
11. Larocque, D., Pilotte, J., Chen, T., Cloutier, F., Massie, B., Pedraza, L., Couture, R., Lasko, P., Almazan, G., and Richard, S. (2002) Neuron 36, 815–829
12. Li, Z., Zhang, Y., Li, D., and Feng, Y. (2000) J. Neurosci. 20, 4944–4953
13. Zhang, Y., Lu, Z., Ku, L., Chen, Y., Wang, H., and Feng, Y. (2003) EMBO J. 22, 1801–1810
14. Hardy, R. J., Loushín, C. L., Friedrich, V. L., Jr., Chen, Q., Ebersole, T. A., Lazzarini, R. A., and Artzt, K. (1996) J. Neurosci. 16, 7941–7949
15. Zhao, L., Tian, D., Xia, M., Macklin, W. B., and Feng, Y. (2006) J. Neurosci. 26, 11278–11286
16. Ebersole, T. A., Chen, Q., Justice, M. J., and Artzt, K. (1996) Nat. Genet. 12, 260–265
17. Kondo, T., Furuta, T., Mitsunaga, K., Ebersole, T. A., Shichiri, M., Wu, J., Artzt, K., Yamamura, K., and Abe, K. (1999) Mamm. Genome 10, 662–669
18. Chen, T., and Richard, S. (1998) Mol. Cell. Biol. 18, 4863–4871
19. Larocque, D., Galarneau, A., Liu, H. N., Scott, M., Almazan, G., and Richard, S. (2005) Nat. Neurosci. 8, 27–33
20. Hardy, R. J. (1998) J. Neurosci. Res. 34, 46–57
21. Wu, H. Y., Dawson, M. R., Reynolds, R., and Hardy, R. J. (2001) Mol. Cell. Neurosci. 17, 292–302
22. Osterhout, D. J., Wolven, A., Wolf, R. M., Resh, M. D., and Chao, M. V. (1999) J. Cell Biol. 145, 1209–1218
23. Zhao, L., Ku, L., Chen, Y., Xia, M., LoPresti, P., and Feng, Y. (2006) Mol. Cell. Biol. 17, 4179–4186
24. Franklin, R. J., Bayley, S. A., Milner, R., Ffrench-Constant, C., and Blake,more, W. F. (1995) Glia 13, 39–44
25. Louis, J. C., Magal, E., Muir, D., Manthorpe, M., and Varon, S. (1992) J. Neurosci. Res. 31, 193–204
26. Bansal, R., Warrington, A. E., Gard, A. L., Ranscht, B., and Pfeiffer, S. E. (1989) J. Neurosci. Res. 24, 548–557
27. Galarneau, A., and Richard, S. (2005) Nat. Struct. Mol. Biol. 12, 691–698
28. Wu, J. I., Reed, R. B., Grabowski, P. J., and Artzt, K. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 4233–4238
29. Noveroske, J. K., Hardy, R., Dapper, J. D., Vogel, H., and Justice, M. J. (2005) Mamm. Genome 16, 672–682
30. Cox, R. D., Hugill, A., Shedlovsky, A., Noveroske, J. K., Best, S., Justice, M. J., Lehrach, H., and Dove, W. F. (1999) Genomics 57, 333–341
31. Casaccia-Bonnefil, P., Hardy, R. J., Teng, K. K., Macklin, W. B., Kriwacki, R. W., and Slingerland, J. (2007) Cell 128, 281–294
32. Grimmel, M., Wang, Y., Mund, T., Cilensek, Z., Keidel, E. M., Waddell, M. B., Jakel, H., Kullmann, M., Kriwacki, R. W., and Slingerland, J. (2007) Cell 128, 269–280
33. Song, J., O’Connor, L. T., Yu, W., Baas, P. W., and Duncan, I. D. (1999) J. Neurocytol. 28, 671–683
34. Voujouklis, D. A., and Brophy, P. J. (1993) J. Neurosci. Res. 35, 257–267
35. Fischer, I., Konola, J., and Cochary, E. (1990) J. Neurosci. Res. 27, 112–124
36. Meixner, A., Haverkamp, S., Wissel, H., Fuhrer, S., Thalhammer, J., Koerp, N., Bittner, R. E., Lassmann, H., Wiche, G., and Propst, F. (2000) J. Cell. Biol. 151, 1169–1178
37. McInnes, L. A., and Lauriat, T. L. (2006) Neurosci. Biobehav. Rev. 30, 551–561