KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome

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Rett syndrome is a severe form of autism spectrum disorder, mainly caused by mutations of a single gene methyl CpG binding protein 2 (*MeCP2*) on the X chromosome. Patients with Rett syndrome exhibit a period of normal development followed by regression of brain function and the emergence of autistic behaviors. However, the mechanism behind the delayed onset of symptoms is largely unknown. Here we demonstrate that neuron-specific K^+-Cl^- cotransporter2 (*KCC2*) is a critical downstream gene target of *MeCP2*. We found that human neurons differentiated from induced pluripotent stem cells from patients with Rett syndrome showed a significant deficit in KCC2 expression and consequently a delayed GABA functional switch from excitation to inhibition. Interestingly, overexpression of KCC2 in MeCP2-deficient neurons rescued GABA functional deficits, suggesting an important role of KCC2 in Rett syndrome. We further identified that RE1-silencing transcriptional factor, *REST*, a neuronal gene repressor, mediates the *MeCP2* regulation of *KCC2*. Because KCC2 is a slow onset molecule with expression level reaching maximum later in development, the functional deficit of KCC2 may offer an explanation for the delayed onset of Rett symptoms. Our studies suggest that restoring KCC2 function in Rett neurons may lead to a potential treatment for Rett syndrome.

Rett syndrome | MeCP2 | human iPSC | disease modeling | KCC2

Rett syndrome is a severe form of autism spectrum disorders (ASDs). De novo mutations in the methyl CpG binding protein 2 (*MECP2*) gene in humans are responsible for more than 80% of Rett syndrome cases (1, 2). Rett patients suffer from seizures, progressive spasticity, and mental retardation but with a developmental delay in disease onset after birth (3, 4). Previous studies from animal models of Rett syndrome have revealed that MeCP2 binds extensively to the genome (5) and regulates the expression of a variety of downstream genes (6–8). Neuronal deficits including reduced excitatory synapse number (9, 10) and disturbed GABAergic neurotransmission (11, 12) have been described. Cultured human neurons derived from iPSCs of patients with Rett syndrome displayed similar synaptic transmission deficits (13–15). Whereas a number of signaling molecules, including brain-derived neurotrophic factor (BDNF) (16, 17), mammalian target of rapamycin (mTOR) (15, 18), and insulin-like growth factor-1 (IGF1) (13, 19) have been shown to play a regulatory role in animal or cell culture models of Rett syndrome, the mechanism for a consistent delay in the disease onset has yet to be understood.

KCC2, a membrane K^+-Cl^- cotransporter, is the major Cl^- transporter in neurons that is largely responsible for setting the transmembrane chloride gradient (20). Because $GABA_A$ receptors ($GABA_A$-Rs) are coupled with membrane Cl^- channels, proper maintenance of the transmembrane Cl^- gradient is critical for the polarity and efficacy of GABAergic function (21). KCC2 expression level is typically very low during early brain development in both humans and rodents (22, 23). Disruption of KCC2 function has been demonstrated in a number of neurological disorders, including epilepsy (24), stroke (25), spinal cord injury spasticity (26), and schizophrenia (27, 28). Interestingly, animal models with KCC2 deficiency develop pathological features similar to those observed in *MeCP2* knockout mice, including breathing irregularity, lower body weight, and impaired learning and memory (29, 30). However, it is unknown whether KCC2 is involved in the pathogenesis of Rett syndrome.

In this study, we report a direct link between KCC2 and MeCP2. Using human neurons derived from induced pluripotent stem cells (iPSCs) from patients with Rett syndrome (Rett neurons), we discovered a significant deficit of KCC2 in Rett neurons, leading to an impaired GABA functional switch from excitation to inhibition. KCC2 overexpression or IGF1 treatment of Rett neurons rescued the functional deficits in the GABA functional switch. The causal relationship between MeCP2 and KCC2 was confirmed by knocking down MeCP2 in cultured mouse cortical neurons, which leads to a decreased KCC2 expression level and delayed the GABA functional switch. Mechanistically, we demonstrate that RE1-silencing transcriptional factor (REST) is an important mediator linking MeCP2 to KCC2 expression. Taken together, our data suggest that KCC2 is a critical downstream target of MeCP2, and that restoring KCC2 function may offer a potential new therapy for the treatment of Rett syndrome.

Results

We have previously demonstrated that neurons derived from iPSCs from patients with Rett syndrome showed significant glutamatergic deficits (13). Here we investigated GABA function

Significance

Rett syndrome is a devastating neurodevelopmental disorder that currently has no cure. In this work, we demonstrate that human neurons derived from patients with Rett syndrome show a significant deficit in neuron-specific K^+-Cl^- cotransporter2 (KCC2) expression, resulting in a delayed GABA functional switch. Restoring KCC2 level rescues GABA functional deficits in Rett neurons. We further demonstrate that methyl CpG binding protein 2 regulates KCC2 expression through inhibiting RE1-silencing transcriptional factor. Our data suggest a potential therapeutic approach for the treatment of Rett syndrome through modulation of KCC2.

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in human iPSC-derived neurons from patients with Rett syndrome. Human neurons were derived from iPSCs obtained from a male patient with Rett syndrome (Q83X, clone 1), which carried a *MeCP2* mutation at the amino acid residue 83 from glutamine to a premature stop codon, resulting in truncation and degradation of the MeCP2 protein. Immunostaining for MeCP2 confirmed the absence of MeCP2 signal in neurons derived from Rett patient Q83X, whereas control neurons derived from his father (WT83, clone 7, healthy control) had strong MeCP2 staining in the nuclei (Fig. 1 *A* and *B*). When we stained for KCC2 in WT83 neurons, we found a gradual increase in KCC2 expression over 3 mo (Fig. 1 *D* and *E*), whereas Q83X neurons showed little KCC2 signal even after 3 mo in culture (Fig. 1 *F* and *G*). These results suggest that GABA function may be altered in Rett neurons. We have previously demonstrated that IGF1 can rescue glutamatergic deficits in Rett neurons (13). Therefore, we treated the Q83X Rett neurons with IGF1 and found that, whereas MeCP2 levels were not increased in the nucleus (Fig. 1*C*), the KCC2 staining was significantly increased (Fig. 1 *H* and *I*), suggesting that IGF1 may upregulate KCC2 independently of MeCP2. Fig. 1*J* shows the developmental change of the KCC2 expression levels in WT83, Q83X, and Q83X + IGF1 groups during 1–3 mo of culture on astrocytes. We also used Western blot to compare KCC2 expression levels among 2-mo-old neurons from different groups (WT83, Q83X, and Q83X + IGF1). Compared with WT83 control, Q83X Rett neurons showed a significant reduction in the expression of KCC2, which was rescued by IGF1 (Fig. 1 *K* and *L*).

If the lack of KCC2 in Q83X Rett neurons is due to the absence of MeCP2, we reasoned that overexpressing MeCP2 in Q83X Rett neurons would rescue the KCC2 deficit. Indeed, whereas expression of GFP as a control had no effect on KCC2 expression (Fig. 2 *A–D*), overexpression of MeCP2 in Q83X neurons significantly restored the KCC2 expression level (Fig. 2 *E–H*). As another control, we overexpressed KCC2 itself in Q83X neurons and verified that the KCC2 level was dramatically increased (Fig. 2 *I–L*; quantified data in Fig. 2*M*). Therefore, the absence of MeCP2 in Rett neurons induces a significant deficit of KCC2, which can be rescued by MeCP2 reexpression or IGF1 treatment.

KCC2 has been shown to play an important role during neural development (23, 31). KCC2 functions in transporting Cl^- from intracellular to extracellular space to maintain low intracellular Cl^- concentration ($[Cl^-]_i$) in mature neurons (32). Because $GABA_A$-Rs are also Cl^- channels, the Cl^- reversal potential for $GABA_A$-Rs (E_{GABA}) is typically governed by KCC2 (23, 33). Because KCC2 expression has a delayed onset during early brain development, immature neurons often have high $[Cl^-]_i$ and then switch to low $[Cl^-]_i$ after KCC2 level increases in mature neurons (33, 34). Such $[Cl^-]_i$ changes lead to a well-studied phenomenon

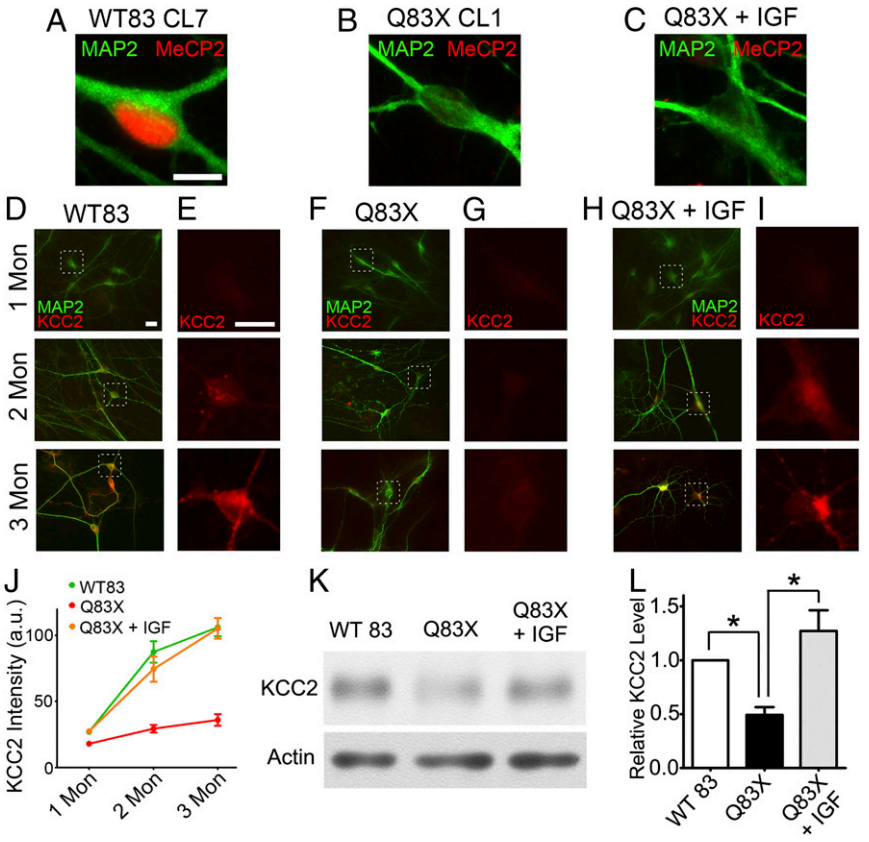

Fig. 1. Human neurons differentiated from iPSCs from patients with Rett syndrome show deficits in KCC2 expression and GABA functional switch. (*A–C*) MeCP2 immunostaining in MAP2-positive human neurons differentiated from WT83 (*A*, clone 7, healthy control, father of Q83X) or Q83X (*B*, clone 1, patient with Rett) iPS cell lines. Note the absence of MeCP2 in the nucleus of Q83X neurons, IGF1 treatment of Q83X Rett neurons (*C*) could not rescue MeCP2 expression. (*D–I*) Representative micrographs showing KCC2 immunoreactivity (red) in MAP2-positive WT83 neurons (*D* and *E*), Q83X neurons (*F* and *G*), or Q83X neurons treated with IGF1 (*H* and *I*) at different culture stages. Columns of *E*, *G*, and *I* are enlarged views of boxed areas in *D*, *F*, and *H*, respectively. (*J*) Quantification of the time course of KCC2 expression in WT83, Q83X, or Q83X neurons treated with IGF1 during 3-mo of culture on astrocytes. (*K*) Representative Western blot probing KCC2 levels among WT83 neurons, Q83X neurons, and Q83X neurons treated with IGF1. (*L*) Quantified data showing that comparing to WT83 neurons, Q83X Rett neurons had a significant reduction in KCC2 expression level (49 ± 7% of WT83 level. $n = 3$ independent repeats; $P < 0.02$, Student's t test). The KCC2 deficit in Q83X neurons was rescued by IGF1 treatment ($P < 0.03$ comparing to Q83X neurons; $P > 0.2$ comparing to WT83 neurons). (Scale bars: *A*, *D*, and *E*, 10 μm.)

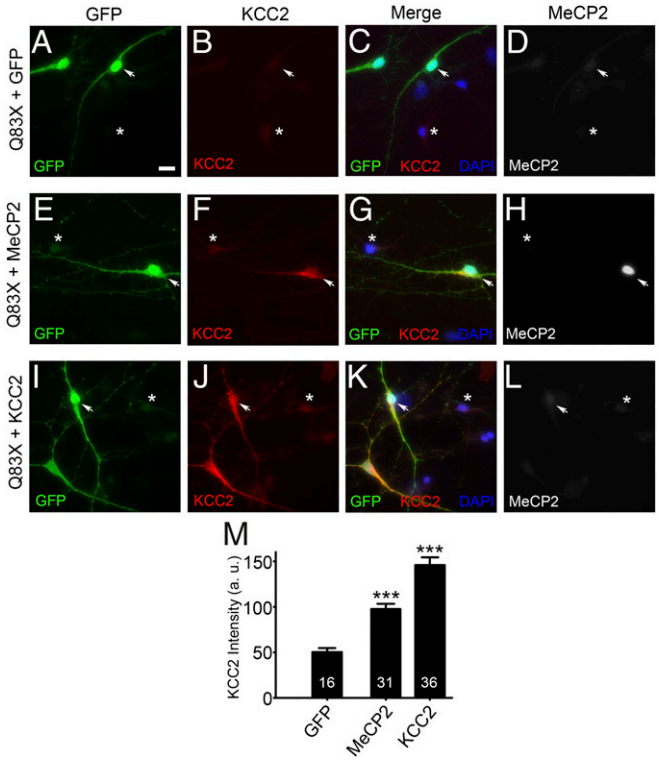

Fig. 2. Rescue of KCC2 deficit in Q83X Rett neurons. (*A–D*) Control experiment showing that transfection of GFP into Q83X neurons had no effect on the KCC2 expression level. (Scale bar, 10 μm.) (*E–H*) Transfection of MeCP2 in Q83X neurons significantly increased the KCC2 expression level. Arrowhead indicates transfected neuron; * indicates nontransfected neuron. (*I–L*) Transfecting KCC2 in Q83X neurons also increased the KCC2 expression level. (*M*) Quantified data showing a significant increase in KCC2 immunoreactivity in Q83X neurons after overexpressing MeCP2 or KCC2 (GFP, 49 ± 4 a.u., $n = 16$; MeCP2, 98 ± 6 a.u., $n = 31$; KCC2, 146 ± 9 a.u., $n = 36$; ***$P < 0.001$, one-way ANOVA with Bonferroni correction).

of GABA functional switch from excitation to inhibition, which is crucial for normal brain development and function (23).

The lack of KCC2 expression in Q83X Rett neurons led us to examine whether GABA function was altered by measuring E_{GABA}, an index for $[Cl^-]_i$ that is controlled by KCC2. For control WT83 neurons, E_{GABA} showed a clear developmental shift from −50 mV to −70 mV when neurons gradually matured during 3-mo of culture on astrocytes (Fig. 3 *A* and *D*), indicating a normal GABA functional switch from excitation to inhibition. In contrast, Q83X Rett neurons did not show a significant change in E_{GABA} even after 3-mo of culturing on astrocytes (Fig. 3 *B* and *D*). Interestingly, IGF1 treatment significantly rescued the alterations of E_{GABA} in Q83X Rett neurons (Fig. 3 *C* and *D*), consistent with its rescue of KCC2 expression level (Fig. 1 *H–J*). Thus, the lack of KCC2 expression in Rett neurons significantly altered GABA function during early neuronal development. To ensure that the KCC2 deficit was not specific to the Q83X clone used, we further investigated KCC2 levels in different iPSC clones derived from the same patient (Q83X clone 6) and his father (WT83 clone 6). We found that similar to Q83X clone 1 (Fig. 1), KCC2 level was also significantly reduced in the Q83X Rett neurons derived from clone 6, and rescued by IGF1 treatment (Fig. 3 *E–H*). Accordingly, the E_{GABA} in Rett neurons derived from the new clone 6 of Q83X did not shift toward hyperpolarization like that in WT83 clone 6 after 2-mo of culture, but was rescued by IGF1 treatment (Fig. 3 *I–L*).

To further test whether our finding is consistent across different patients with Rett syndrome, we derived neurons from a different patient with Rett syndrome (a female carrying a different *MeCP2* mutation N126I). Compared with control neurons derived from a different human iPS cell line WT126, we found that N126I Rett neurons had no MeCP2 signal in the nucleus and a significant reduction of KCC2 expression level in the soma and dendrites (Fig. 4 *A* and *B*). The IGF1 treatment rescued the KCC2 deficit but not MeCP2 signal in the N126I Rett neurons (Fig. 4 *C* and *D*). Furthermore, gramicidin-perforated patch-clamp recordings revealed that, whereas E_{GABA} showed a normal shift from −48 mV to −68 mV in WT126 control neurons, there was no developmental shift of E_{GABA} in the N126I Rett neurons (Fig. 4 *E*, *F*, and *H*). IGF1 treatment also rescued the E_{GABA} deficit in N126I Rett neurons (Fig. 4 *G* and *H*). Therefore, KCC2 deficit and the consequent GABA functional alteration is a general feature associated with Rett neurons and can be rescued by IGF1 treatment.

To further investigate the mechanisms underlying MeCP2 regulation of KCC2, we used cultured mouse cortical neurons to

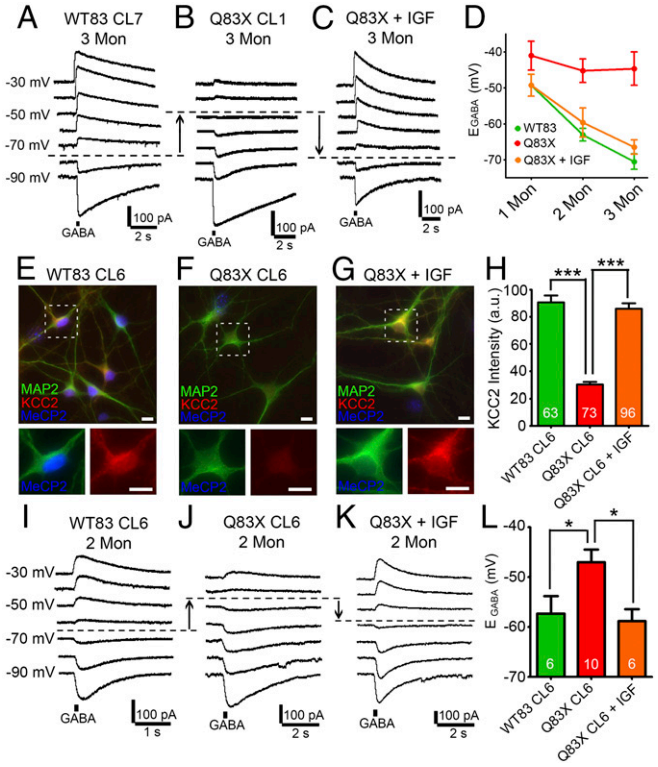

Fig. 3. Q83X Rett neurons from different clones show deficits in GABA functional switch. (*A–C*) Representative traces showing GABA-evoked currents under various holding potentials in 3-mo-old WT83 (*A*), Q83X (*B*), or Q83X neurons treated with IGF1 (*C*). Dashed lines indicate GABA reversal potential (E_{GABA}). (*D*) Quantified data illustrating the time courses of E_{GABA} changes during neuronal maturation. Note that Q83X neurons did not show typical E_{GABA} shift as that of WT83 neurons. (*E–G*) Representative micrographs showing KCC2 immunostaining in 2-mo-old WT83 CL6 (*E*), Q83X CL6 (*F*), or Q83X neurons treated with IGF1 (*G*). Enlarged views of neurons are presented below each micrograph. (Scale bars, 10 μm.) (*H*) Quantified results showing that KCC2 expression level is reduced in clone 6 Q83X Rett human neurons. (*I–K*) Representative GABA-evoked responses recorded from 2-mo-old neurons derived from a different pair of Q83X and WT83 clones. Dashed lines indicate GABA reversal potential (E_{GABA}). (*L*) Quantified data illustrating the E_{GABA} levels recorded at 2-mo time point. Note that Q83X clone 6 neurons did not show typical E_{GABA} hyperpolarizing shift as that of WT83 clone 6 neurons. Data are presented as mean ± SEM.

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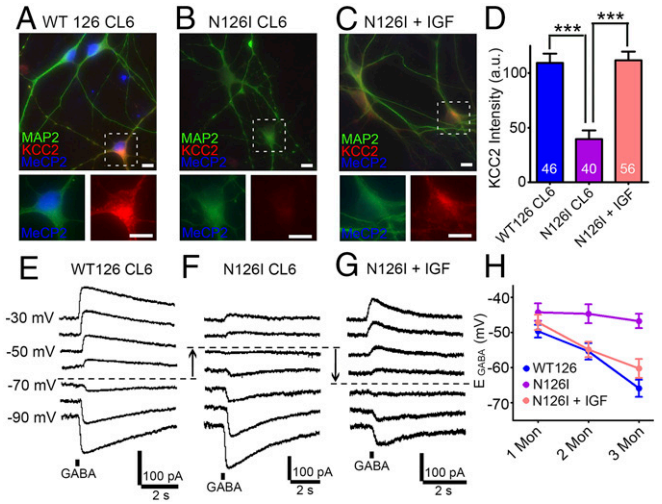

Fig. 4. Rett neurons derived from a different patient N126I also show deficits in KCC2 and GABA functional switch. (*A–C*) Representative micrographs showing KCC2 immunoreactivity (red) in WT126 neurons (clone 6, *A*), human neurons carrying a MeCP2 missense mutation N126I (clone 6, *B*), or N126I neurons treated with IGF1 (*C*) at 2-mo culture on astrocytes. Enlarged views of neurons are presented below each micrograph. (Scale bars, 10 μm.) (*D*) Quantification of the KCC2 expression level in WT126 CL6, N126I CL6, or N126I CL6 neurons treated with IGF1. (*E–G*) Representative traces showing GABA-evoked currents under various holding potentials in 3-mo-old WT126 (*E*), N126I (*F*), and N126I neurons treated with IGF1 (*G*). Dashed lines indicate E_{GABA}. (*H*) Quantified data illustrating the developmental changes of GABA reversal potential during neuronal maturation. Note that N126I neurons did not show typical E_{GABA} shift as that of WT126 neurons. Data are presented as mean ± SEM ***$P < 0.001$, one-way ANOVA with Bonferroni correction.

molecularly manipulate the MeCP2 level and monitor consequent KCC2 changes. We first compared both MeCP2 and KCC2 expression levels between mouse and human neurons during development. Interestingly, in both human and mouse neurons, MeCP2 and KCC2 showed highly correlative increase during neuronal maturation, although human neurons developed much slower than mouse neurons (Figs. S1 and S2). We then knocked down MeCP2 in mouse neurons and confirmed that KCC2 was consequently reduced and E_{GABA} shifted from −70 mV toward −50 mV (Fig. S3), consistent with our findings in human Rett neurons. Therefore, our experiments in mouse neurons essentially recapitulate the results in human Rett neurons that the absence of MeCP2 leads to a decrease of KCC2, which in turn causes alteration of GABA signaling.

MeCP2 is a global transcription regulator and binds with DNA in the nucleus, whereas KCC2 is a membrane transporter and also found in the cytoplasm of soma and dendrites. How does MeCP2 regulate KCC2? Previous studies have reported that MeCP2 can regulate the transcriptional repressor REST, a master regulator of neuronal gene expression (35, 36). Interestingly, KCC2 has been reported to be regulated by REST (37). We therefore hypothesized that MeCP2 might regulate KCC2 through REST. To test this hypothesis, we overexpressed REST in mouse neurons and found that KCC2 expression level was significantly reduced (Fig. 5 *A* and *B*). Interestingly, coexpression of MeCP2 with REST rescued the KCC2 deficit induced by REST alone (Fig. 5*C*), suggesting that MeCP2 suppressed the inhibitory effect of REST on KCC2 expression. In contrast, the IGF1 treatment failed to rescue the KCC2 deficit induced by REST overexpression (Fig. 5*D*), suggesting that transcriptional repression of KCC2 by REST is independent of IGF1 signaling. To further test the interactions among MeCP2, REST, and KCC2, we expressed a dominant negative mutant of REST (REST DN) in mouse neurons and found that the KCC2 expression level was not altered (Fig. 5*E*). Knockdown of MeCP2 induced a significant decrease of KCC2 expression in mouse neurons, as shown above (Fig. 5*F*). Interestingly, coexpressing REST DN with MeCP2 shRNA significantly rescued the KCC2 deficit induced by MeCP2 shRNA alone (Fig. 5*G*, quantified data shown in Fig. 5*H*). Consistent with the KCC2 changes, we found that overexpression of REST shifted E_{GABA} from −70 mV toward −50 mV, which was reversed by coexpression with MeCP2 (Fig. 5 *I–L*). These results demonstrate that MeCP2 regulates KCC2 through modulating REST activity.

Discussion

In this study, we demonstrate that MeCP2 regulates KCC2 expression through REST and ultimately controls GABA functions in neurons. Using human iPSC-derived neurons from different patients with Rett syndrome, we have discovered significant KCC2 deficit in Rett neurons, which hinders the normal GABA functional switch from excitation to inhibition. Because KCC2 is a late onset molecule during early brain development, our discovery may explain why Rett syndrome shows a delayed onset in developing infants.

Deficits in KCC2 expression have been linked to a number of human neuropsychiatric disorders. A disruption in KCC2 mRNA level has been reported in patients with schizophrenia (27, 28). Difference in the expression levels of specific KCC2 transcripts has been linked to schizophrenia and affective disorders (38). We have previously discovered a significant decrease of KCC2 expression induced by a neuroligin 2 mutation found in patients with schizophrenia (39, 40), suggesting a potential role of KCC2 in the pathogenesis of schizophrenia. Altered KCC2 expression has also been implied in stress (41). Recent studies found that inhibiting NKCC1, a chloride transporter with opposite function to KCC2, can be used to treat autism and fragile X syndrome

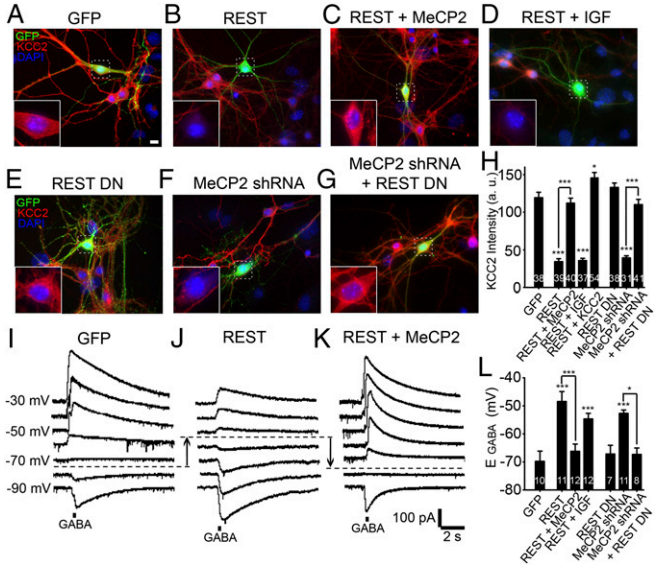

Fig. 5. MeCP2 regulates KCC2 through the transcriptional repressor REST. (*A–G*) Representative micrographs showing KCC2 immunoreactivity (red) in mouse cortical neurons after different manipulations of REST and MeCP2. (Scale bar, 10 μm.) (*H*) Quantified KCC2 staining intensity in various groups. Note that overexpression of REST significantly decreased the KCC2 expression level, which was rescued by coexpression of MeCP2. (*I–K*) Representative recording traces illustrating GABA-evoked currents under various holding potentials recorded in neurons transfected with GFP (*I*), REST (*J*), or (*K*) REST + MeCP2. (*L*) Quantified E_{GABA} changes after different manipulations of REST and MeCP2 expression levels in mouse neurons. Note that MeCP2 shRNA-induced E_{GABA} shift was rescued by coexpression with dominant negative mutant of REST (REST DN). Data are represented as mean ± SEM *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, determined by one-way ANOVA with Bonferroni correction.

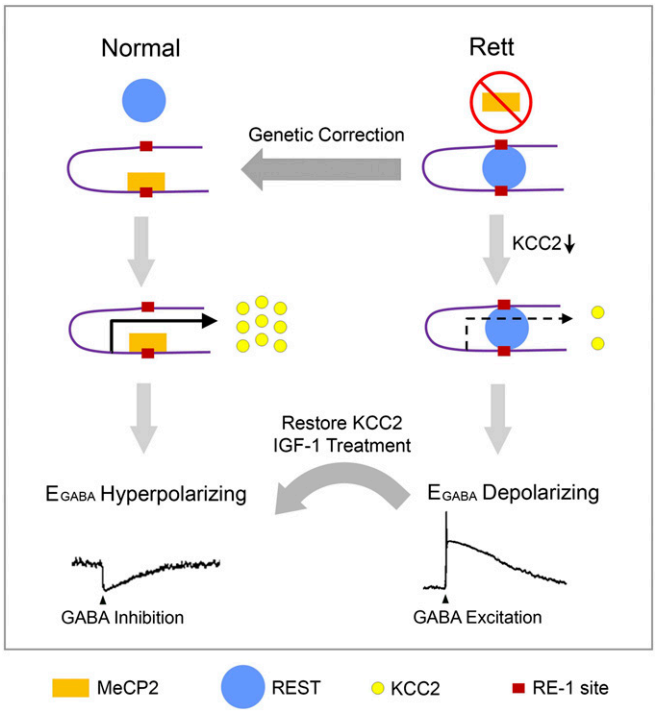

Fig. 6. A working model depicting the molecular mechanisms underlying KCC2 deficiency in Rett neurons and its functional consequence. The *KCC2* gene has two repressor element-1 sites (RE-1, red rectangle) flanking its transcription start site. REST (blue circle) binding to these two RE-1 sites inhibit the expression of KCC2 (yellow circle). In normal neurons, MeCP2 (orange rectangle) occupies RE-1 sites in the *KCC2* gene, thus preventing REST binding and inhibition of KCC2 expression. In Rett neurons that lack MeCP2, REST binds to the RE-1 sites and suppresses KCC2 expression. As a result of KCC2 deficiency, Rett neurons show a depolarizing response to GABA. The deficits in Rett neurons can be rescued by overexpression of MeCP2, KCC2, or IGF1 treatment.

(42, 43). NKCC1 also interacts with DISC1 to regulate the risk of schizophrenia (44). Our current study directly links KCC2 to Rett syndrome, suggesting that KCC2, together with NKCC1, may be a key factor(s) involved in a variety of neuropsychiatric disorders.

KCC2 expression is tightly regulated during brain development. Premature expression of KCC2 leads to deficits in neuronal maturation (45, 46), whereas lack of KCC2 causes massive brain developmental deficits and animal death shortly after birth (29). The developmental time course of KCC2 follows a general rule: KCC2 is late onset, gradually expressed in a caudal-to-rostral order (20, 32). In the neonatal mouse brain, the KCC2 expression level is very low in the cortical and hippocampal regions and gradually increases to adult level around 2 wk after birth (32). During human brain development, the KCC2 protein level at birth is only about 20% of the adult level. A significant increase in KCC2 expression takes place in the first postnatal year in humans (22, 23). In this study, we discovered that human Rett neurons derived from different patients with Rett syndrome showed a consistent KCC2 deficit and altered GABA functions. Therefore, KCC2 may be an important developmental marker for neuronal maturation. The late onset of KCC2 expression during human brain development coincides with the delayed onset of Rett syndrome in human patients. In fact, this may not be a simple coincidence, given the critical role of KCC2 in neuronal maturation and brain development (29). The neonatal brain may develop normally in its early stage until a time point where KCC2 starts to play an important role in switching GABA function from excitation to inhibition. However, in patients with Rett syndrome, the lack of MeCP2 leads to a deficit of KCC2, and therefore GABA function cannot properly switch into inhibition. As a result, the lack of KCC2 in Rett neurons causes brain development to stall. Consistent with our study in human Rett neurons, a delayed GABA functional switch has also been reported in mouse models of autism and fragile X syndrome (47, 48). Interestingly, we demonstrate that elevating KCC2 level can reverse the functional deficits caused by MeCP2 deficiency. Therefore, Rett syndrome is potentially treatable with appropriate drugs that can boost the function of KCC2.

MeCP2 regulates the expression of many neuronal genes (5, 6, 16). In this study, we discovered that KCC2 is a key downstream signaling molecule that determines the functional output of MeCP2. Interestingly, MeCP2 regulation of KCC2 is mediated by suppressing REST, a transcriptional repressor that inhibits neuronal genes (see Fig. 6 for our working model). In normal neurons, MeCP2 can bind to the RE-1 site within the *KCC2* promoter and prevents REST binding to *KCC2* promoter (37). In Rett neurons, where MeCP2 is deficient, REST can bind with an RE-1 site in the *KCC2* promoter region as well as an additional RE-1 site in the intronic region of the *KCC2* gene to suppress KCC2 expression (37). On the other hand, MeCP2 is known to regulate BDNF (16, 17), which in turn can regulate KCC2 (49). In this study, our data suggest that IGF1 may rescue KCC2 expression in Rett neurons, providing a potential mechanistic explanation for IGF1 treatment of Rett syndrome (13, 19, 50). Therefore, MeCP2 may regulate many downstream signaling molecules, with different signaling pathways that converge onto KCC2, a master regulator of GABA functions during brain development. In fact, restoring KCC2 is emerging as a valuable therapeutic approach (21, 51). Our study suggests that KCC2 may be a potential drug target for developing a therapy to treat Rett syndrome.

Materials and Methods

Maintenance and Differentiation of Human iPSC-Neuroprogenitor Cells. Wild-type neuroprogenitor cell (NPC) lines were derived from human iPSCs (WT126 clone 8 and WT33 clone 1) as described before (13). Q83X NPCs (clone 1 and 6) were derived from a male patient with Rett syndrome and WT83 control NPCs (clone 6 and 7) were derived from the unaffected father of the Q83X patient. The expansion of NPCs and neuronal differentiation are using methods developed in our laboratory recently (52). Mouse astroglial and neurons are primarily cultured using a protocol similar to the one previously described (53). For the pharmacological rescue experiment, IGF1 (Invitrogen) was added to culture medium at a concentration of 10 ng·ml^{-1}, 2 wk after neuronal differentiation. The generation and usage of iPSCs and their derived cells were approved by the institutional review boards at Salk Institute for Biological Studies and University of California San Diego, as well as Pennsylvania State University.

Electrophysiology. Electrophysiology recordings were performed using previously described standard protocol (54). To estimate GABA reversal potential (E_{GABA}) in neurons, we performed gramicidin-perforated patch-clamp recording to acquire GABA-evoked responses with intact intracellular Cl^- concentrations (33). Off-line data analyses of GABA reversal potential (E_{GABA}) were calculated using a linear regression equation of GABA responses recorded at different holding potentials.

Imaging. Immunostaining and fluorescent imaging experiments were performed on cultured cells using methods previously described (40, 55). A list of antibodies used in this study can be found in *SI Materials and Methods*.

For more materials and methods, please see *SI Materials and Methods*.

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