Brief Communications

Spatial and Temporal Requirements for huntingtin (*Htt*) in Neuronal Migration and Survival during Brain Development

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Huntington's disease (HD), caused by an expanded triplet repeat in the huntingtin (*Htt*) gene, results in extensive neuropathology, but study of the *Htt* gene in CNS development through gene knockout is problematic as the knockout leads to embryonic lethality in mice. Here, we report that the knockdown of *Htt* expression in neuroepithelial cells of neocortex results in disturbed cell migration, reduced proliferation, and increased cell death that is relatively specific to early neural development. In the cerebellum, however, *Htt* knockdown results in cell death but not perturbed migration. The cell death phenotype in cortex can be partially reversed with co-knockdown of *Casp9*, indicating that mitochondria-mediated cell apoptotic processes are involved in the neuronal death. The timing of knockdown during early development is also an important variable. These results indicate a spatial and temporal requirement for *Htt* expression in neural development. Although it is uncertain whether the loss of wild-type huntingtin function contributes to pathogenesis in Huntington's disease, these results clearly contraindicate the use of nonspecific knockdown of *Htt* as a therapeutic measure in HD, particularly *in utero*.

Introduction

An expanded CAG repeat in the huntingtin (*Htt*) gene, which is ubiquitously expressed, is causal for marked neurodegeneration in the striatum and cortex and elsewhere in the brain of mid-aged humans, and leads to death in an uncompromising fashion. The role that the protein derived from the normal *Htt* allele plays in neural development has not been delineated. Using *Htt* knockout mice, it has been found that *Htt* is required for early embryonic development (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995). Embryonic stem cells with the *Htt* deletion, however, when injected into the blastocyst, can participate in embryonic development, and chimeric mice with null and wild-type cells survive (Dragatsis et al., 1998). We found that $Htt^{-/-}$ cells in such chimeras can readily survive in certain regions of the brain, but are greatly underrepresented in cortical and striatal areas, the major targets of Huntington's disease (HD) (Reiner et al., 2001). In embryonic day 12.5 (E12.5) blastocyst injection chimeras, we found that $Htt^{-/-}$ neuroblasts are as abundant in cortex, striatum, and thalamus as they are in mid-brain and hindbrain regions (Reiner et al., 2003) However, the mutant cells show evidence of pathology (i.e., apparent cell death) at this early stage in development. These studies suggested that *Htt* is needed specifically for development of cortical and striatal neurons.

In this study, to determine the temporal and spatial requirement of *Htt* in neuronal development, we used shRNA to knock down its expression at different time points early in development. We examined two regions of the brain, one where our previous data indicated a critical role for *Htt* (i.e., cortex), and one where we found that *Htt*-null cells seemed to have uncompromised survival (i.e., cerebellum). Our results revealed that *Htt* plays a critical role for neuron survival, proliferation, and neuronal migration in early cortical development; and for survival but not migration in cerebellar development.

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Materials and Methods

Mice and in utero *transfection*

Htt shRNA and EGFP (the shRNA experimental condition) or unrelated control shRNA and EGFP plasmids (the control condition) were cointroduced into the neural epithelium of the lateral or fourth ventricle of timed-pregnant ICR mice (originally from Charles River) using an *in utero* electroporation method described previously (Tabata and Nakajima, 2001). The use of mice was in accord with Society for Neuroscience guidelines and Institutional Animal Care and Use Committee-approved protocols.

Design of shRNA

The mU6pro vector with a mU6 promoter was a kind gift from Drs. Jenn-Yah Yu and Dave Turner (Yu et al., 2002). Four sequences of the mouse *Htt* and three sequences of the mouse *Casp9* were designed by using GenScript (www.genscript.com/ssl-bin/app/sirna, Htt-204: 5′-gaaggaactctcagccaccaa-3′; Htt-2502: 5′-gcctctgaagaacagctccta-3′; Htt-4908: 5′-gttcatcactccaagcacaat-3′; Htt-5596: 5′-ggaatgtgcaatagagaaata-3′;

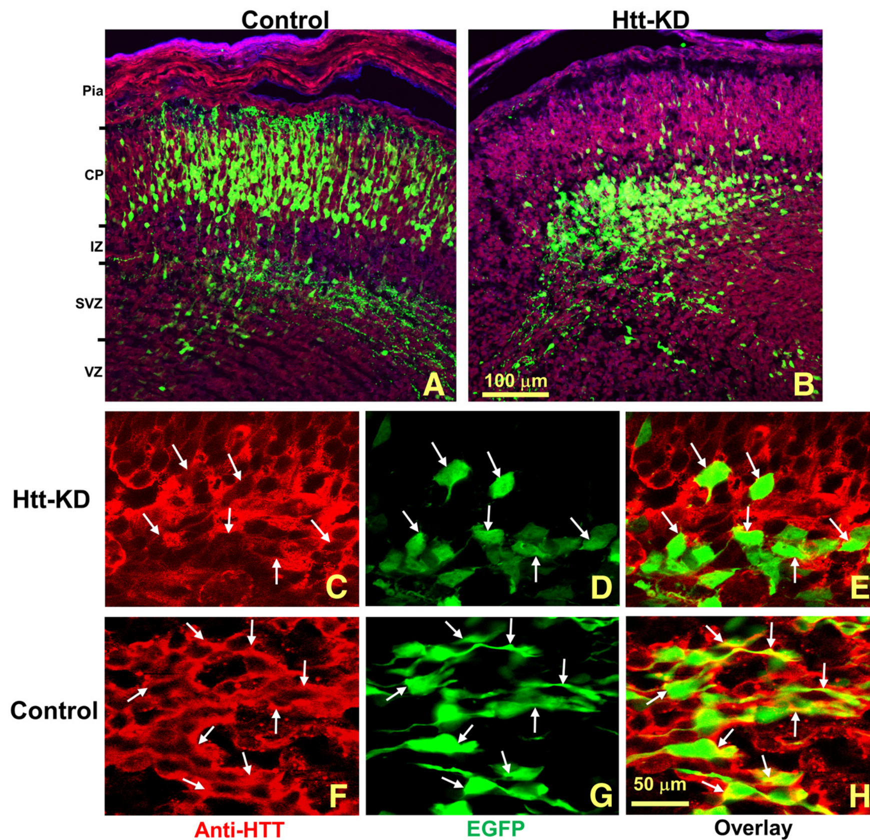

Figure 1. *Htt* shRNA disrupts the migration of neurons in the developing cortex. ***A***, The normal development of cortical neurons is shown at E15.5 following transfection with EGFP at E12.5. More than 80% of the EGFP-labeled cells have migrated from the VZ to the CP. ***B***, Transfection of *Htt* shRNA inhibited the number of cells that successfully migrated to the CP. Pia, Pia mater. ***C–H***, *Htt* shRNA significantly reduced *Htt* immunostaining in transfected cells (***C–E***); anti-HTT immunostaining is not changed in the control (***F–H***). Note that yellow, indicating costaining with EGFP and HTT, is apparent in the control (***H***) but is not observed in the *Htt* shRNA-transfected group (***E***); white arrows indicate individual transfected cells. Scale bars: ***A***, ***B***, 200 μm; ***C–H***, 50 μm.

Casp9-124: 5′-gatattcagcaggcaggatct-3′; Casp9-469: 5′-gaggttctcagaccagaaaca-3′; Casp9-554: 5′-gacatgcagatatggcataca-3′). An unrelated shRNA made from the *Mcm5* gene (Mcm5-86: 5′-gcaactgcagaggcgattcaa-3′) that is expressed in the neuroepithelium of cortex and cerebellum was used as a control.

Processing of embryos for histology

Embryos were killed 2–3 d following electroporation, and the fetal brains were immersion fixed in 4% paraformaldehyde in 0.1 M PBS for 6 h at 4°C. To study cell proliferation, BrdU (50 μg/g body weight, Sigma) was injected intraperitoneally 1 h before embryos were killed. Brains were cryoprotected, sectioned (14 μm), and mounted on slides. To demonstrate cell bodies, propidium iodine or Toto3 (Invitrogen) was used. To detect the HTT protein, the sections were preincubated with 1× PBS containing 0.2% Triton-100 (PBS-T) and 5% normal goat serum for 2 h at room temperature, and incubated with the anti-HTT antibody (1:400; MAB2166, Millipore Bioscience Research Reagents) overnight. The rinsed sections were then incubated with goat-anti-mouse antibody conjugated with Alexa Fluor 595 for 1.5 h at room temperature. Tissue was coverslipped and fluorescence preserved with FluorSave (Calbiochem). For BrdU staining, sections were treated with 1 M HCl for 30 min at 37°C to denature DNA, rinsed three times with 0.1 M PBS, and immunostained as described above with the BrdU antibody (1:200; catalog #ab6326, Abcam). For caspase immunostaining, sections were antigen retrieved in 0.01 M NaOAC buffer, pH 5.8, in a microwave oven for 15 min and washed in PBS-T buffer three times. The sections were incubated with anti-cleaved CASP3 (anti-cCASP3, 1:400; catalog #9664, Cell Signaling Technology) or CASP9 antibody (anti-CASP9, 1:1000; catalog #M054-3, MBL).

Quantitative analyses

Effect of shRNA on protein expression. Immunofluorescent images obtained with a confocal microscope (60×) were analyzed using "Analysis" 3.0 software (Soft Imaging System Corp.). An elliptical window was extended to cover each identified transfected or control cell, and optical density was measured. Data were obtained from four fetuses with a minimum of 40 cells tallied in each group.

Assessing cellular phenotypes of migration and death. As plasmid injection and transfection are inherently variable, only samples that showed robust labeling were processed for quantification. Furthermore, as the quantification was made from digital images, only sections that yielded high-quality images were used for analysis. At least two digital images were counted for each embryo. Three to 10 fetuses were analyzed for each manipulation and time point. To assess cellular migration, the number of EGFP-positive cell bodies was counted in the cortical plate (CP) compared with the combined number of cells in the intermediate zone (IZ), subventricular zone (SVZ), and ventricular zone (VZ). To examine cell death or proliferation, $EGFP^+$ cells immunopositive for anti-cleaved CASP3 or anti-BrdU were counted, and the percentage of apoptotic or proliferating cells (mean ± SEM) was determined by calculating the ratio of double-positive cells over all transfected cells in the confocal images.

Statistical analysis

For statistical analysis, Student's *t* test was performed for pairwise comparison between *Htt* shRNA knockdown and a control group, or *Htt* shRNA and *Htt* shRNA+*Casp9* shRNA group.

Results

Htt shRNA abolishes normal cell migration from the VZ to the CP of the developing cerebral cortex

The E12.5 time point was assessed in control and *Htt*-shRNA-transfected embryos as this was the time when we observed apoptotic *Htt*-null cells in cortex (Reiner et al., 2003). In control E12.5 embryos, with 72 h of survival after EGFP plasmid was injected into the lateral ventricle, 82% of all EGFP-labeled cells had migrated to the CP, and many axons were present in the IZ (Fig. 1*A*). Four *Htt* shRNA constructs were separately injected to determine their effect upon cortical development. In all experimental cases, transfected cells were able to leave the VZ/SVZ region but then demonstrated variable pathologies in progressing toward the CP. Construct Htt-5596 had the most profound effect on cell migration, resulting in 90 ± 1% of the transfected cells remaining deep in the IZ and SVZ ($p < 0.001$; Fig. 1*B*). Even the few cells that entered the CP could only migrate into the deep CP region. As the Htt-5596 construct yielded the greatest developmental defect, this construct was used in all subsequent experiments.

Brains transfected with EGFP at E12.5 and harvested 48 h later contained cells in cortex with moderate to strong anti-HTT immunostaining in the cytosol and processes (Fig. 1*F*). There was no obvious change in the HTT immunofluorescent signal between EGFP-labeled and unlabeled cells in control embryos (Fig. 1*F–H*). In the shRNA-transfected cortex (Htt-5596), EGFP-

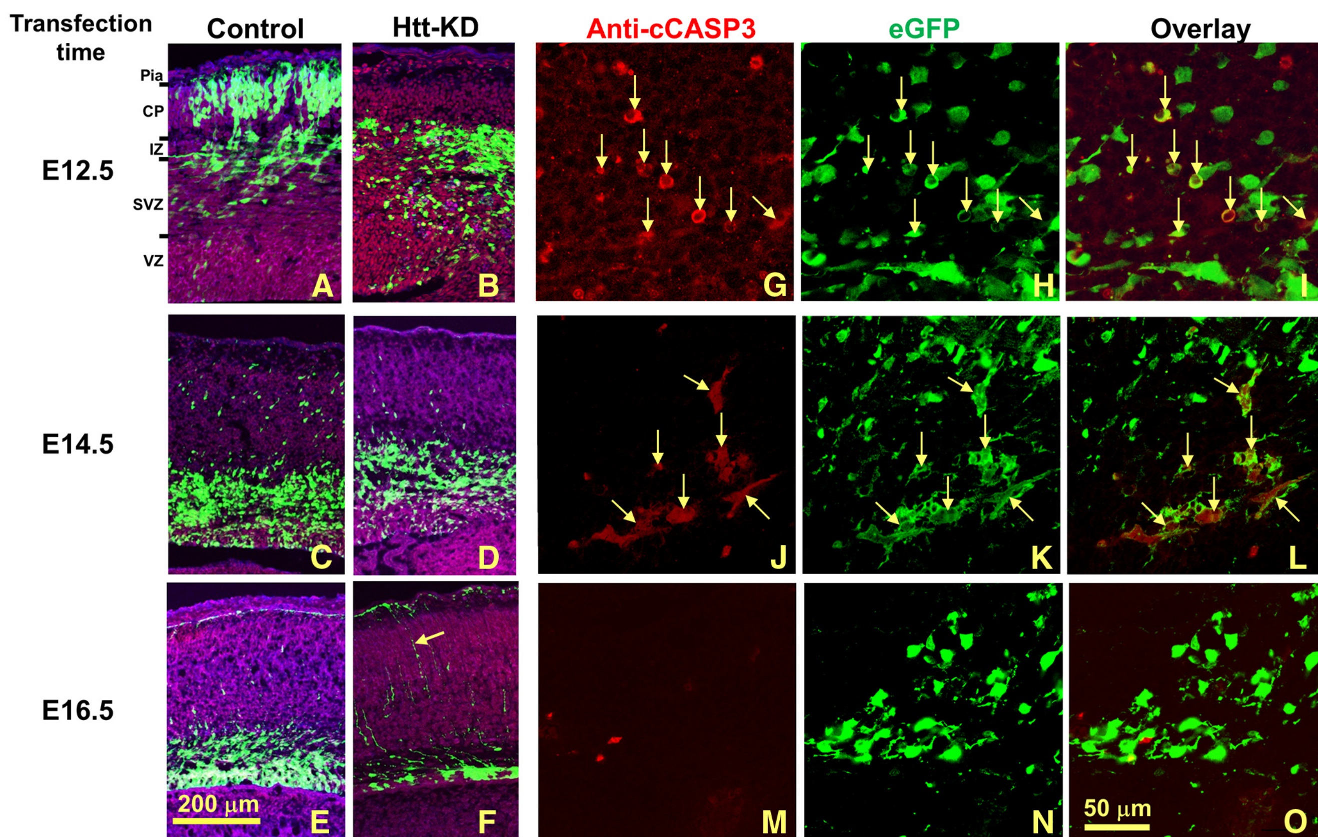

Figure 2. The effect of *Htt* shRNA on cell migration and survival is time dependent in the developing cerebral cortex. ***A–O***, *Htt* shRNA transfection was performed at E12.5 (***A***, ***B***, ***G–I***), E14.5 (***C***, ***D***, ***J–L***), and E16.5 (***E***, ***F***, ***M–O***) with 72 h survival. For E12.5 transfection, while many EGFP-labeled control cells migrate to the CP (***A***), *Htt* shRNA-transfected cells remain in the IZ (***B***). For E14.5 and E16.5 transfection, control transfected cells have left the VZ and largely reside in the IZ, with some cells present in the CP (***C***, ***E***). Following *Htt* shRNA at these time points, most EGFP-labeled cells still remain in the VZ or the nearby SVZ (***D***, ***F***). Pia, Pia mater. ***G–O***, Higher-magnification confocal images show that cleaved CASP3-positive cells were also *Htt* shRNA positive and were easily detectible in the E12.5 condition (***G–I***, arrows). The apoptotic cells were greatly reduced at E14.5 (***J–L***, arrows), and only a few were observed at E16.5 transfection (***M–O***). Red, Anti-cleaved CASP3 immunostaining (anti-cCASP3); green, EGFP. Scale bars: (in ***E***) ***A–F***, 200 μm; (in ***O***) ***G–O***, 50 μm.

negative cells had similar HTT expression to that from control embryos. However, adjacent EGFP-positive cells were only weakly immunostained for HTT (Fig. 1*C*). The intensity of the fluorescent staining revealed an average 51% reduction in immunofluorescent signal in transfected cells for the whole cell body ($n = 43$ cells, $p < 0.001$). If only cytosol expression (whole cell body minus nucleus area) was considered, where HTT was primarily localized, the average reduction was 75% of control HTT levels ($n = 43$ cells, $p < 0.001$), with only a limited overlap between immunofluorescent HTT and EGFP signals (Fig. 1*C–E*).

Knockdown of *Htt* leads to a time-dependent defect in cell migration and apoptosis in the developing cortex

When there is a prolific movement of cortical neuroblasts from the ventricular zone to the cortical plate, the temporal window is limited (Takahashi et al., 1996). In control tissue, this age-dependent process was clearly visible (Fig. 2*A*,*C*,*E*). When the control shRNA construct was introduced into E12.5 fetal neuroepithelia, 85 ± 1% of the labeled cells migrated into the CP at 72 h of survival ($n = 8$; Fig. 3*A*). In contrast, only 24 ± 3.5% of the transfected cells migrated into the CP when fetuses were injected on E14.5 with 72 h of survival ($n = 3$; Fig. 2*C*), and migration was further reduced with control injection at E16.5 (survival for 72 h; $n = 4$), with only 4 ± 1% of EGFP-labeled cells reaching the CP (Fig. 2*E*).

When the *Htt* shRNA was introduced into neuroepithelial cells of E12.5 embryos and examined 72 h later ($n = 9$), cell migration was greatly reduced in the transfected cells, with only 10 ± 1% making their way to the CP compared with 85 ± 1% in the control ($p < 0.001$; Fig. 2*B*). With *Htt* shRNA introduced into E14.5 and 16.5 fetuses and examined 72 h later ($n = 11$ and 4, respectively), the percentage of neurons that successfully migrated was also diminished [9 ± 1% and 1% compared with 24 ± 3.5% ($p < 0.05$) and 4 ± 1% ($p < 0.05$) in the control groups, respectively; Fig. 2*D*,*F*]. In addition to its disruptive effect on cell migration into the developing CP, many Htt-5596-transfected cells along the migratory pathway from the VZ to the IZ were also seen with condensed chromatin at 48 h ($n = 14$) or 72 h ($n = 37$). Using an anti-cleaved CASP3 antibody to mark cells that have entered the cell death pathway, we found numerous Htt-5596 shRNA-transfected cells that were also cleaved CASP3 positive (Fig. 2*G–I*, arrows); 38.2 ± 1.4% of the E12.5 transfected cells were costained with the antibody to cleaved CASP3 compared with 4.4 ± 1% in the control ($p < 0.001$). At E14.5 and E16.5 transfections, 35.7 ± 1.2% and 6.6 ± 0.5% of *Htt* shRNA-transfected cells were immunopositive for cleaved CASP3, whereas in the control group there were very few anti-cleaved CASP3-positive cells (1 and 0.5% respectively, $p < 0.001$ for both *Htt* shRNA vs control; Fig. 2*J–L*,*M–O*).

Dissociation of cell death and arrested migratory phenotypes: examination of the developing cerebellum

In the *Htt*-null chimera, *Htt*-null cells are well represented in the cerebellum, suggesting that *Htt* is not required for cells to develop

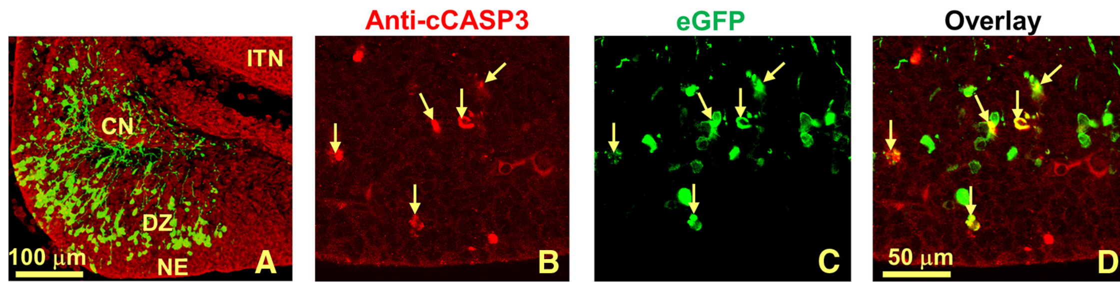

Figure 3. *Htt* shRNA does not impact cell migration but leads to cell death in the developing cerebellum. *Htt* shRNA was introduced into the cerebellar neuroepithelium at E12.5 and examined 48 h later. ***A***, As in controls (data not shown), abundant numbers of transfected cells have left the neuroepithelium and traveled toward the region of the differentiating zone. EGFP-labeled cells have well formed processes and surround the cerebellar nuclei. ***B–D***, Higher-magnification confocal images of anti-cleaved CASP3 immunostaining (***B***), EGFP (***C***), and costaining (***D***) that show cell death in transfected neurons. CN, Cerebellar nuclei; ITN, inferior tectal neuroepithelium; NE, cerebellum neuroepithelium; DZ, differentiating zone. Scale bars: ***A***, 100 μm; ***B–D***, 50 μm.

in this region of the brain (Reiner et al., 2001). Thus, we transfected cells in the cerebellar anlagen with *Htt* shRNA at E12.5, and allowed 48 h survival ($n = 3$). Migration appeared normal in both the migrating cells of the neuroepithelium (Fig. 3*A*) as well as in the external germinal layer in the E14.5 transfected cerebellum (data not shown). As in the control, there were many transfected cells with long leading processes characteristic of migrating cells in the cerebellum. However, when cleaved CASP3 was examined in these cerebella, immunostained cells were found in the VZ of the developing cerebellum (Fig. 3*B–D*, arrows). Thus, while knockdown of *Htt* in the cerebellum did not affect migration, it did result in abundant cell death (39 ± 3.5% of transfected cells compared with 5 ± 1% in the control group, $p < 0.05$).

Caspase 9 shRNA significantly reduces the *Htt* shRNA-caused cell apoptosis

To examine whether blockade of the apoptotic pathway can reverse the cell death caused by *Htt* knockdown in the cortex, we cotransfected *Casp9* shRNA plasmids together with the *Htt* shRNA at age E12.5, and allowed a survival of 72 h, as previously done. When the *Casp9* shRNA (Casp9–469) was cotransfected with *Htt* shRNA ($n = 10$), there was an average 43.6% reduction in fluorescent signal upon immunostaining for CASP9 ($n = 45$ cells, $p < 0.001$), thus showing its efficacy. The morphology of cotransfected cells was greatly improved, and 26 ± 1% of the cotransfected cells successfully migrated to the CP compared with only 10 ± 1% in the *Htt* shRNA-only group ($p < 0.05$; Fig. 4*A*). Anti-cleaved CASP3 immunostaining of this tissue revealed decreased numbers of apoptotic cells (28.8 ± 1% of transfected cells compared with 38.2 ± 1.4% in the *Htt* shRNA-only group, $p < 0.001$; Fig. 4*C*,*D*). Thus, the *Casp9* shRNA improved cell morphology, limited the number of apoptotic cells caused by *Htt* knockdown, and partially rescued the migration defect in the developing cerebral cortex.

In light of the recent discovery that *Htt* is required for mitotic spindle orientation and neurogenesis (Godin et al., 2010), we examined the effect of shRNA knockdown of *Htt* on mitosis in cortical development with BrdU delivered 1 h before embryo harvesting with transfection at E12.5 with 72 h survival. The percentage of transfected cells (EGFP$^+$) that were also BrdU$^+$ was calculated in control, *Htt* shRNA-transfected, and *Htt* and *Casp9* shRNA-cotransfected groups. Compared with 33.3 ± 1% of BrdU$^+$-transfected cells in the control group ($n = 3$; Fig. 4*E*), only 8.9 ± 1% of the transfected cells were BrdU$^+$ in the *Htt* shRNA group ($n = 5$, $p < 0.001$, Fig. 4*F*), and 20.9 ± 1% transfected cells were BrdU$^+$ in the *Htt* and *Casp9* shRNA-cotransfected group ($n = 7$, $p < 0.001$; Fig. 4*G*,*H*). Therefore, shRNA knockdown of *Htt* had an adverse effect on mitosis, and cotransfection with *Casp9* shRNA was able to partially rescue this defect.

Discussion

A host of developmental problems has been identified with the early deletion of *Htt*, with no clear resolution as to the role of *Htt* in neural development. Evidence for its importance in neural development, especially in the forebrain, has come from several lines of research. First, *Htt* has been found to be expressed very early in the human (19–21 week fetus) and mouse forebrain (as early as E10) (Dure et al., 1994; Bhide et al., 1996). Second, White et al. (1997) made and analyzed a hypomorphic knock-in allele of the *Htt* gene (Htt^{neoQ50}) that resulted in profound early neural defects. The *neo* insert produced a greatly attenuated expression of *Htt*, and mice homozygous for this allele showed perinatal lethality with ectopic masses of proliferating cells in the SVZ of the forebrain. Additionally, there were major disturbances in overall brain organization. Third, when the embryonic lethal effect of the $Htt^{-/-}$ allele was bypassed using blastocyst injection chimeras, we found that there was a striking deletion of neurons in forebrain regions (Reiner et al., 2001) and that this absence of neurons was likely due to an early apoptosis of *Htt*-null cells in the E12.5 embryo (Reiner et al., 2003). The chimeric setting offers one way, albeit random, to look at the role of the *Htt* gene in development. Another approach is to use *in utero* transfection of *Htt* shRNA in the developing brain with resolution in both time and space. In this manner, we were able to identify three elements of neuronal development that are dependent upon normal *Htt* expression: cell migration, cell proliferation, and survival.

Htt knockdown disrupts normal cell migration in a region-specific manner

The migration of cells from the neuroepithelium to a final destination is a critical step in CNS development. In this report, we show that *Htt* knockdown leads to region-specific disruption of neuronal migration. Knockdown of *Htt* in neurons of the neuroepithelium at E12.5 by shRNA impairs normal cell migration in cerebral cortex, but not in cerebellum. We observed the accumulation of transfected cells in the IZ of developing cortex as early as 48 h after transfection. As far as we know, this is the first report of *Htt*'s involvement in neuronal migration, and the mechanism of regional specificity is currently unknown. The recent report by Godin et al. (2010) found that siRNA knockdown of *Htt* had no effect on neuronal migration. There are two main differences between the current report and the work of Godin et al. (2010) that could explain these discordant results. First, the time of

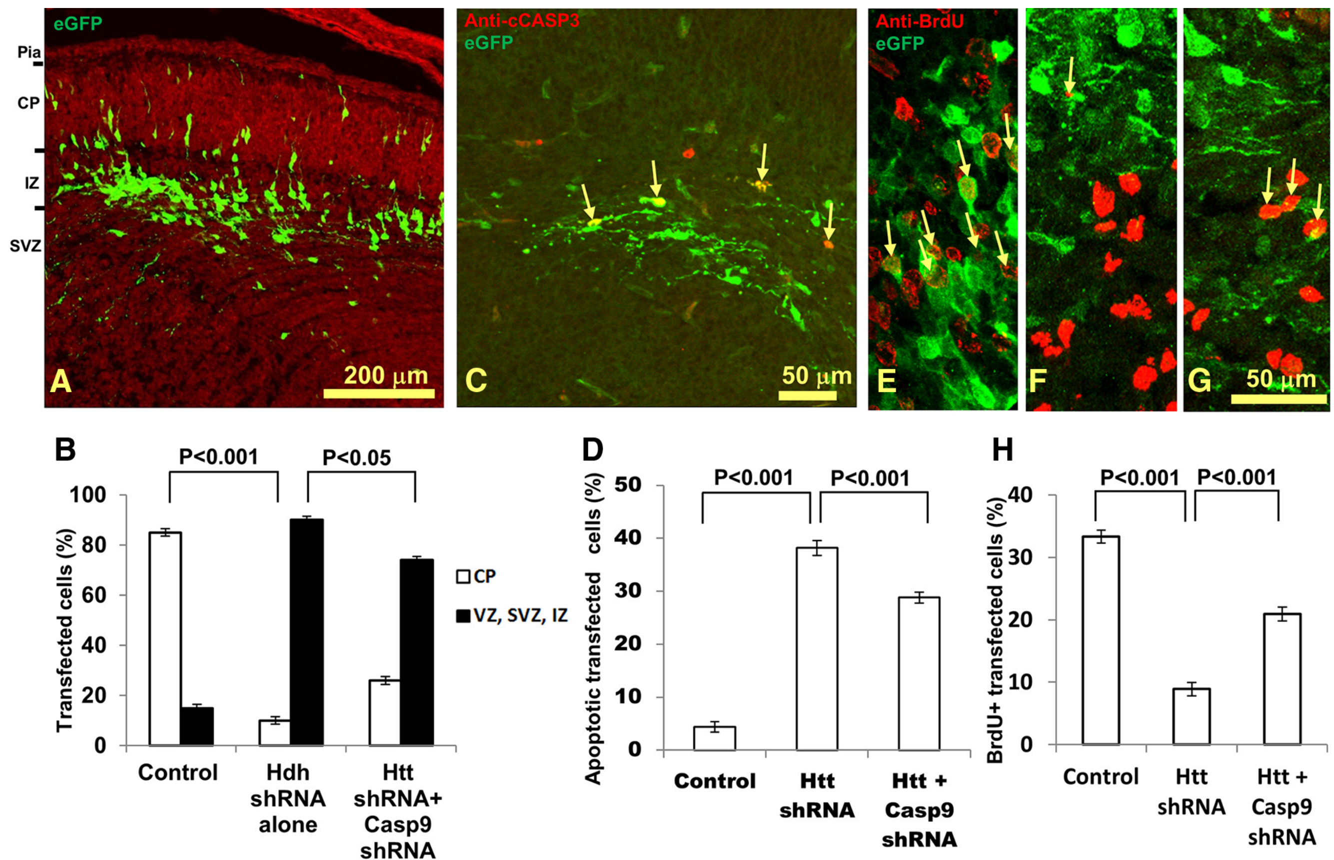

Figure 4. Cotransfection of *Casp9* shRNA partially rescues the deleterious effects of *Htt* shRNA on neuronal survival and proliferation. ***A***, ***B***, *Casp9* shRNA was cotransfected with *Htt* shRNA at E12.5 (with 72 h survival). The cotransfection of the *Casp9* shRNA greatly improved the morphology of the transfected cells (***A***) compared with the *Htt* shRNA transfection alone (see Figs. 1*B*, 2*B*). Pia, Pia mater. Scale bar, 200 μm. ***B***, Percentage of transfected cells reaching CP and those remaining cells in VZ, SVZ, and IZ for control, *Htt* shRNA, and *Htt* and *Casp9* shRNA-cotransfected groups. ***C***, ***D***, Anti-cleaved CASP3 immunostaining revealed that cotransfection of *Casp9* shRNA with *Htt* shRNA decreased the percentage of transfected cells undergoing apoptosis (***C***). Arrows indicate the colocalization of anti-cleaved CASP3 immunostaining (red) and EGFP (green). ***D***, Percentage of apoptotic cells over all transfected cells for control, *Htt* shRNA, and *Htt* and *Casp9*-cotransfected groups. ***E–G***, Knockdown of *Htt* results in decreases of BrdU-positive cells, and cotransfection with *Casp9* shRNA rescues the disruptive effect of *Htt* on mitosis. Arrows indicate the colocalization of anti-BrdU immunostaining (red) and EGFP (green). Control (***E***), *Htt* shRNA (***F***), and *Htt* and *Casp9* shRNA (***G***) cotransfection. ***H***, Percentage of BrdU-double-labeled cells over all transfected cells for control, *Htt* shRNA, and *Htt* and *Casp9* shRNA-cotransfected groups. Scale bar, 50 μm. Error bars in ***B***, ***D***, and ***H*** represent SEM.

transfection is different. While our data include knockdown at three time points, E12.5, E14.5 and E16.5, Godin et al. (2010) only examined knockdown at E14.5. We find that the disrupted migratory phenotype with *Htt* shRNA knockdown is most obvious at E12.5. By contrast, at E14.5 the normal egress of cells from the neuroepithelium has diminished such that it would have been difficult to observe the relatively minor abnormality in migration that we observed at that time point. Second, shRNA target sequences are different. We tested four constructs, and used the one that gave the most prominent phenotype (Fig. 1), while Godin et al. (2010) reported the use of only two *Htt* siRNA constructs that may have been less efficient than the optimal construct we identified in yielding deficits in cell migration.

Neuroprotective activity of *Htt* and its implication for therapeutic use of *Htt* RNAi

Wild-type *Htt* has been documented to provide a positive effect on cell survival and can mitigate the effects of the mutant *Htt* (Rigamonti et al., 2000; Reiner et al., 2003). The absence of the HTT protein can have profound consequences on the ability of neurons to successfully colonize cortex and striatum. We previously reported that chimeric mice composed of a mixture of wild-type and $Htt^{-/-}$ cells ($Htt^{+/+}$ and $Htt^{-/-}$) showed that forebrain neurons needed *Htt* for normal cortical and striatal development, and neurons throughout the brain needed *Htt* for the long-term health of the animal (Reiner et al., 2001). Furthermore, Dragatsis et al. (2000), using conditional inactivation of *Htt*, have shown that loss of *Htt* late in development or shortly after birth can lead to subsequent degeneration of cortical and striatal neurons. $Htt^{-/-}$ neurons in culture have been shown to be especially vulnerable to the effects of serum deprivation (Rigamonti et al., 2000), which suggests the possibility that *Htt* plays a role in enabling at least some types of neurons to withstand stressful events. This neuroprotective role of *Htt* may be more important for cortical and striatal neurons, which are most vulnerable in HD.

In this report, with shRNA knockdown we demonstrate that the *Htt* gene product is a critical protein in early cortical and cerebellar development for cell survival. Knockdown of *Htt* expression leads to caspase-mediated cell apoptosis both in cortex and cerebellum. The application of *Casp9* shRNA to block the mitochondria-mediated apoptotic pathway reduced cell apoptosis, improved cell morphology and mitosis, and rescued the migration of some cells in cortex. Interestingly, much of the cell apoptosis was still present in the *Casp9* shRNA-cotransfected tissues. This can be due to two possibilities. First, the *Casp9* shRNA may not have completely knocked down the *Casp9* message in all transfected neurons, resulting in some apoptotic cells and in-

complete rescue of migration defect in the *Casp9* shRNA-cotransfected fetus tissues. The second possibility is that the *Casp9* shRNA in our experiment may target only one of multiple apoptotic pathways engaged by *Htt* knockdown (Graham et al., 2006).

A link between abnormal neurogenesis and *Htt* knockdown is suggested by the findings of White et al. (1997) that reduced expression of *Htt* was associated with reduced neurogenesis and aberrant brain development. In this report, we show that *Htt* shRNA knockdown reduced the number of cells undergoing mitosis in the SVZ/VZ and cotransfection with *Casp9* shRNA partially reversed the disruption. This is also in line with the recent report by Godin et al. (2010), who showed that loss of *Htt* expression increased neuronal differentiation, and decreased proliferation and maintenance of cortical progenitors in the VZ.

Finally, we show that there is a restricted temporal window when *Htt* is critical to cortical development. The deleterious effect of *Htt* shRNA is most pronounced at E12.5 and is attenuated during later development, pointing to a critical time when *Htt* is needed in developing neurons. Furthermore, our results point toward a cell-autonomous need for *Htt* expression in cortical development. The phenotypic abnormalities of impaired migration and cell death were only found in transfected cells, while neighboring nontransfected cells showed no signs of a defect in cell migration or apoptosis, which is consistent with our previous work on wild-type and $Htt^{-/-}$ chimeric mice (Reiner et al., 2001). These findings are important for understanding the role of *Htt* in normal neural development, and for strategies for *Htt* gene knockdown or elimination as a cure for HD.

Notes

Supplemental material for this article is available at http://grits.dglab.org/Downloads/Tong_2011_Supplemental_Figures_and_Tables.pdf. The PDF file contains two supplemental figures regarding Htt shRNA and Casp9 shRNA constructs and eight supplemental tables that summarize the results. This material has not been peer reviewed.

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