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Hepatocyte growth factor (HGF) modulates GABAergic inhibition and seizure susceptibility

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Abstract

Disrupted ontogeny of forebrain inhibitory interneurons leads to neurological disorders, including epilepsy. Adult mice lacking the urokinase plasminogen activator receptor (*Plaur*) have decreased numbers of neocortical GABAergic interneurons and spontaneous seizures, attributed to a reduction of hepatocyte growth factor/scatter factor (HGF/SF). We report that by increasing endogenous HGF/SF concentration in the postnatal *Plaur* null mouse brain maintains the interneuron populations in the adult, reverses the seizure behavior and stabilizes the spontaneous electroencephalogram activity. The perinatal intervention provides a pathway to reverse potential birth defects and ameliorate seizures in the adult.

Keywords

epilepsy; autism; HGF/SF; Met; urokinase; GABA; interneuron; seizure; parvalbumin; EEG

Introduction

Excitatory neural activity in the mature cerebral cortex is modulated by local GABAergic interneurons. These neurons originate in the ventral telencephalon and migrate to populate the dorsal forebrain (Wonders and Anderson, 2006). Disruption of the GABAergic interneuron population during development results in improper circuit formation and seizures in humans and mice (Cobos, et al., 2005, Garbelli, et al., 2006, Powell, et al., 2003, Schwaller, et al., 2004). Hepatocyte growth factor/scatter factor (HGF/SF) is expressed in the prenatal forebrain

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and regulates neuronal migration (Achim, et al., 1997, Powell, et al., 2001). Latent HGF/SF is activated by serine proteases, including urokinase type plasminogen activator, uPA (Mars, et al., 1993). When uPA is bound to its receptor, uPAR (also known as Plaur, as the gene is *Plaur*), the protease activity is strongly accelerated (Ellis, et al., 1991). Loss of *Plaur* leads to the reduction of HGF/SF in the embryonic forebrain, interneuron deficits, and subsequent spontaneous seizures (Powell, et al., 2003, Powell, et al., 2001). In this report, endogenous postnatal supplementation of HGF/SF ameliorates the interneuron defects in the B6.129 – *Plaur*^{tm1/Mlg} mice (abbreviated *Plaur* in the report) and alters electrophysiological activity to approach normalcy.

Materials and methods

Animals

Experiments were conducted in accordance with IACUC approved protocols (University of Maryland School of Medicine) and the Policies on the Use of Animals and Humans in Neuroscience Research. The *HGF* mice were genotyped via PCR using the primer sets: 5'-ggCCATgAATTTgACCTCTATgAA-3' and 5'-TTCAACTTCTgAACACTgAggAAT-3' (370 bp) for *HGF*, and 5'-CCTCATCCTgggCCTggTTCTggTCT-3' and 5'-ggTTTTCCCCgCTgTggTCATCTgC-3' (200 bp) for *Serpin1* as a positive control. For genotyping *Plaur* mice, PCR was performed with primer sets: 5'-gATgATAgAgAgCTggAggTggTgAC-3' and 5'-CACCgggTCTgggCCTgTTgCAGAggT-3' (154 bp) for *Plaur*, 5'-ATTgAACAAgATggATTgCAC-3' and 5'-TTCgTCCAgATCATCCTgATCgAC-3' (500 bp) for the neomycin resistance gene. All mice were maintained as heterozygotes by crossing onto the C57Bl/6J (B6) background for more than 10 generations. Wildtype mice are denoted as WT. The results reported here were obtained from male littermates that were offspring from non-sibling matings.

Western Blot analysis

Samples of cerebral cortex were homogenized in sample buffer (10mM Tris-HCl (pH7.4), 2M NaCl, 1 mM PMSF, 1 mM EDTA, and 0.01% Tween 80). The supernatants of tissue homogenates were separated by a 35,000g centrifugation for 30 min at 4°C and their protein concentrations were determined via BCA assay (Pierce, Rockford, IL). 20 µg of protein per lane was fractionated by 9% SDS-PAGE gel and transferred to PVDF membranes (Immunobilon-P membrane; Millipore, Billerica, MA). The membranes blocked for 1h with 5% skim milk (Carnation), 0.05% Tween 20 (Sigma) in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.4) and subsequently incubated in anti-human HGF/SF primary antibodies (R & D systems, MAB294, 1:500) overnight at 4°C diluted in blocking buffer. After washes in 0.05% Tween-20 in PBS, membranes were incubated for 2h in donkey anti-mouse, horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, 1:2000), washed, and visualized by incubating in ECL (Super Signal West Pico Chemiluminescent substrate; Pierce). Films were scanned with a HP scanjet G4050 scanner to obtain digital images and labeled using Adobe Photoshop CS2.

HGF/SF Enzyme-Linked Immunosorbent Assay (ELISA)

The expression level of mouse and human HGF/SF was quantified using separate specific sandwich ELISAs (R&D Systems, Minneapolis, MN). The standard curve was linear from 0-5 ng/ml of HGF/SF. Tissue samples of somatosensory cortex were dissected from perinatal and adult mice, flash-frozen in liquid nitrogen and then processed according to the manufacturer's instructions. HGF/SF levels are presented as amount of HGF/SF in mg of total protein. The statistical significance among four different genotypes was examined using one-way ANOVA.

followed by Student-Newman-Keuls *post-hoc* analysis using Statistica (StatSoft, Inc, Tulsa, OK).

Immunohistochemistry

Mice were transcardially perfused with buffered 2% para-formaldehyde, 2% glutaraldehyde, and 0.2% picric acid fixative optimized for GABA and calcium binding protein markers. Vibratome sections (50 μ m) were cut and stained using routine laboratory protocols. Primary antibodies were used at the following dilutions: rat anti-GABA (1:1500, Protos Biotech, New York, NY), mouse anti-parvalbumin (1:2000, Sigma Chemical Co, St. Louis, MO), rabbit anti-calretinin (1:1500, Sigma), and rabbit anti-somatostatin (1:2500, Peninsula/Bachem, San Carlos, CA). Appropriate conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used at a 1:2500 dilution. Cresyl violet staining was performed to observe the gross anatomical structure in the cortex. The sections were mounted with DPX (Electron Microscopy Sciences, Hatfield, PA). Images were obtained using PowerPhase digital camera (Phase-one, Copenhagen, Denmark) or Fluoview v.5.0 software as part of a Confocal Olympus BX61 imaging system (Melville, NY).

Cell counting

The total numbers of cells were estimated using unbiased stereological methods (West, 1999). Immunoreactive cells were counted in 4 sections of the primary somatosensory cortex, based on anatomy (Paxinos and Franklin, 2001). For each area at each level, five dissectors (200 $\times$ 200 $\times$ 20 μ m³) were randomly chosen. All cell numbers in this paper are reported as mean $\pm$ standard error of the mean (s.e.m.). The optical fractionator method was implemented using a NeuroLucida morphometry system (software version 7; MicroBrightField Bioscience, MicroBrightField Inc. Williston, VT). Brains from at least three different mice were counted per age and genotype. The observer was blinded to the genotype of each brain during counting. Statistical significance among four different genotypes was examined using one-way ANOVA followed by Student-Newman-Keuls *post-hoc* analysis (Statistica, StatSoft Inc., Tulsa, OK).

Induced seizures

Pentylenetetrazol (PTZ; 50 mg/kg diluted in sterile 0.9% saline) was administered subcutaneously into adult mice. For each genotype, WT, *HGF*, *Plaur*, and *Plaur/HGF*, at least 9 male adult mice were tested. The mice were observed for 30 min and activity was recorded into mpaq files (Dazzle DVD Recorder, Pinnacle Systems, A division of Avid Technology, Inc., Mountain View, CA) and scored by at least two observers that were blinded to the mouse genotype. Behavioral responses were scored using the following scale: 0, no signs of motor seizure; 1, isolated twitches; 2, tonic-clonic convulsions; 3, tonic extension or death (Erickson, et al., 1996). The latency to the onset of the first seizure was recorded. The seizure severity and latency data were evaluated with one-way ANOVA with Student-Newman-Keuls *post-hoc* tests.

Electroencephalogram recordings

To record epidural activity in the orbitofrontal cortex, (AP: +2.1mm, ML: $\pm$ 1.3mm), hippocampus, (AP: -1.8, ML: 2.2), and occipital lobe (AP: -3.5, ML: $\pm$ 2.0) screws (Small Parts Inc, PN TX00-2-25) were inserted into pre-drilled burr holes in the anesthetized mouse. Stainless steel leads from a custom connector (Omnetics Connector Corp, Minneapolis, MN) were wrapped around the screws and affixed with conductive epoxy (Circuit Works, CW2400). The connector was then affixed to the skull with dental cement (Henry Schein, Melville, NY). Animals were given at least one week recovery prior to recording. Animals were awake, freely moving in their home cage and were continuously recorded for 2.5h of total, multiple day recordings. Data were recorded using a Neuralynx recording system, and raw data were

processed with a low pass filter (60 Hz) using Matlab (Natick, MA) followed by analysis using Offline Sorter (Plexon, Dallas, TX). Continuous data were viewed and scored for potential high amplitude spiking or seizure-like episodes. Each episode was defined by these criteria: 1. activity greater than 3 standard deviations from the mean amplitude for the recording session, 2. with duration longer than 30 seconds.

Behavioral testing

Light-dark avoidance - The light dark exploration test was conducted as previously described (Holmes, et al., 2002, Homanics, et al., 1999, Mathis, et al., 1994, Powell, et al., 2003). Mice were placed in the TruScan arena (Mouse *Truscan* Activity Arena; Coulbourn) as for the Open Field test, but with a partition dividing the arena into a light (maintained at ambient light) and dark (a dark box with only a small opening at floor level allowing for mouse entry) half. Mice were individually placed in the center of the light compartment and allowed to freely explore the arena for 10 min. The percentage of time spent in the light compartment was compared between genotypes using one-way ANOVA with Student-Neuman-Keuls posthoc analysis. Data is reported as the mean $\pm$ SEM, for groups of at least $n > 8$ male mice.

Results

Human HGF/SF (*HGF*) was expressed under the control of the mouse glial fibrillary acidic protein (*Gfap*) promoter (Fig. 1, S1). In the B6.129 – *Tg(Gfap-HGF)^{Ca}* mouse, (abbreviated as *HGF*), HGF/SF expression commences in astrocytes, at late embryonic and early postnatal ages. Postnatal cerebral cortical expression of HGF/SF is greater in the *HGF* mouse than in the WT littermate (Fig. 1c). In order to restore HGF/SF levels in the *Plaur* mouse, we mated the *Plaur* mouse strain with the *HGF* mouse to obtain the *Plaur/HGF* strain. Quantification of cerebral cortical levels of HGF/SF via ELISA demonstrated a main effect of genotype [$F(3,24) = 5.43$, $p = 0.005$, ANOVA]. The ELISA confirmed a deficit in *Plaur* null mice ($\sim 40\%$, $p = 0.03$ at birth and $p = 0.01$ in adult) and demonstrated that levels in the *Plaur/HGF* mice were similar to wildtype (WT) littermates ($p > 0.87$, Fig. 1d). In adult *HGF* mice, there was an 18% decrease in HGF/SF expression, although this was not significantly different from WT levels ($p = 0.15$). In summary, adult *Plaur* mice have an HGF/SF deficit in the cerebral cortex that can be restored upon overexpression with the *HGF* mouse.

Since the interneuron deficit in the *Plaur* mouse is attributed to decreased levels of HGF/SF, postnatal supplementation of HGF/SF in the *Plaur/HGF* mouse was predicted to ameliorate the interneuron deficit. Cresyl violet staining demonstrated that all mouse strains displayed grossly normal anatomy (Fig. 2a-d). However, immunohistochemical analysis for GABA demonstrated an effect of genotype [$F(3,14) = 7.543$, $p = 0.003$, ANOVA] with 50% fewer interneurons in somatosensory cortex of *Plaur* null mice (Fig. 2e, g, h, $p = 0.006$). GABAergic interneurons in the *HGF* mice were similar to WT (Fig. 2e, f, m, $p = 0.288$). The postnatal supplementation of HGF/SF in the *Plaur/HGF* mice led to an intermediate number of GABA cells, approximately 72% of WT ($p = 0.06$). In summary, postnatal addition of HGF/SF did not alter overall numbers of GABA-expressing cells, but increased the number of cells in the environment of the *Plaur* mutation.

The majority of the cortical interneuron population can be identified using neurochemical markers, parvalbumin, somatostatin, and calretinin, which represent non-overlapping groups (Kubota, et al., 1994). The mouse strains have significantly different numbers of parvalbumin-expressing (PV⁺) cells [$F(3,16) = 15.330$, $p < 0.0001$]. The numbers of PV⁺ were similar in the WT and *HGF* mice (Fig. 2i-j, m, $p = 0.499$), but the *Plaur* mice showed a 74% reduction in PV⁺ cells (Fig. 2i, k, m, $p < 0.0001$). The addition of HGF/SF in the *Plaur/HGF* mice prevented the loss of PV⁺ interneurons, and restored the PV⁺ neurons to 83% of WT numbers. The number of PV⁺ in *Plaur/HGF* mice was not significantly different from WT mice ($p =$

0.242). To demonstrate that the loss of PV in the *Plaur* mice represented a loss of cells and not simply a loss of detectable PV expression, the perineuronal nets were stained using Wisteria floribunda agglutinin (WFA) lectin (Haunso, et al., 2000). Fewer WFA⁺/PV⁺ cells were observed in *Plaur* mice, and no WFA⁺/PV⁻ cells were found (Fig. S2). Expression in the WT, *HGF*, and *Plaur/HGF* mice was similar. No significant differences were observed in somatostatin [$F(3, 16) = 0.239, p = 0.867$] or calretinin populations [$F(3, 15) = 0.717, p = 0.557$, Fig. 2m]. Thus, postnatal HGF/SF levels appear to specifically regulate the PV⁺ interneuron numbers.

Diminished inhibitory tone has been linked to epilepsy and seizure disorders, and many anti-epileptic drugs function by increasing GABA concentration. On rare occasions, *Plaur* mice display spontaneous generalized seizures while being handled (Powell, et al., 2003). No spontaneous seizures were observed in the *HGF* or *Plaur/HGF* mice during routine husbandry. Seizure susceptibility was evaluated using a single threshold injection of pentylenetetrazole (PTZ, 50 mg/kg) and scoring the behavioral responses (Fig. 3a). The response profiles of the mice differed with respect to genotype [$F(3, 38) = 8.888, p < 0.0001$]. Nearly all (83%) of *Plaur* mice displayed motor convulsions, compared with only 27% of WT, 10% of *HGF*, or 20% of *Plaur/HGF* mice. The latency to PTZ-induced seizures was measured in all groups (Fig. 3b), and only the *Plaur* mice demonstrated significantly reduced time until seizure onset (888 ± 115 s, $p = 0.002$) compared to WT mice. These data suggest that postnatal supplementation of HGF/SF altered seizure susceptibility and reduced the severity in the *Plaur/HGF* mice.

The electroencephalographic (EEG) activity was compared across mouse strains. WT mice showed consistent unremarkable baseline activity (Fig. 3c), whereas, recordings of the *Plaur* mice demonstrated abnormal baseline cortical activity, with sustained episodes of high amplitude spiking (Fig. 3c), in agreement with our previous report (Powell, et al., 2003). The *HGF* mice showed very rare low amplitude spiking. The altered cortical activation in *Plaur* mice was offset in the *Plaur/HGF* mice, which presented rare low amplitude spikes. Quantification of the episodes of spiking activity in the mice revealed an overall effect of genotype [$F(3, 16) = 10.373, p < 0.001$, Fig. 3c]. *Plaur* mice experience 4.3 ± 1.0 episodes/h which is significantly different from the *HGF* mice ($p = 0.002$) and *Plaur/HGF* mice ($p = 0.002$). No episodes were observed in the WT group. The *HGF* and *Plaur/HGF* mice were behaviorally indistinguishable from WT ($p > 0.07$). In summary, postnatal supplementation of HGF/SF to the *Plaur* mice led to restoration of PV⁺ interneurons and behavior that approached the WT phenotype.

Previously, *Plaur* mice were reported to have increased anxiety (Powell, et al., 2003). An entire cohort of adult male mice were tested for general open field activity (Fig. S3a) and no differences were found among genotypes [$F(3, 18) = 1.60, p = 0.22$]. On the light-dark avoidance test (Fig. 3e), there was an overall effect of genotype [$F(3, 38) = 15.27, p < 0.001$]. The *Plaur* mice spent significantly less time on the light side of the chamber ($p < 0.001$), an indication of increased anxiety. The *Plaur/HGF* mice displayed behavior that was significantly different from *Plaur* mice ($p < 0.001$), but indistinguishable from WT mice ($p = 0.74$). Similar results were obtained with another measure of anxiety, the elevated plus maze, with *Plaur* mice having decreased time in the open arms and *Plaur/HGF* responding the same as WT mice (Fig. S3b). These data indicate that the anxiety phenotype of the *Plaur* mice is remedied with the HGF/SF supplementation in the *Plaur/HGF* mice.

Discussion

These data suggest that the GABAergic interneuron deficit in the *Plaur* mouse is due in part to a postnatal reduction of HGF/SF, supporting multiple roles for HGF/SF in regulating neural

circuit formation and cell survival. In the *Plaur/HGF* mice, exogenous HGF/SF restores PV⁺ GABAergic interneurons in the parietal cortex to almost WT levels. The effect is specific for PV⁺ cells, as the numbers of SST⁺ and CR⁺ cells are unaffected either by the loss of *Plaur* or by increased HGF/SF expression. Restoration of the anatomy appears to dramatically reduce the seizure phenotype in the *Plaur* mice, as both induced and spontaneous susceptibilities are greatly decreased and statistically similar to WT mice.

The role of *Plaur* in the developing and adult brain is still being defined, but loss of *Plaur* leads to decreased cerebral cortical GABAergic interneurons, increased susceptibility to chemically induced seizures, and abnormal EEG recordings and spontaneous seizure activity (Powell, et al., 2003). In the rat status epilepticus model of epilepsy, *Plaur* mRNA and uPAR (the protein product of *Plaur*) were increased in the hippocampus, particularly in glial and PV-expressing cells (Lahtinen, et al., 2009). In humans, *Plaur* has also been reported to be upregulated after neuronal injury (Beschorner, et al., 2000) and in cases of neural degeneration, such as Alzheimer's disease (Walker, et al., 2002). Therefore, *Plaur* is likely involved with establishment and maintenance of neural circuitry.

The uPAR molecule has been associated with two distinct molecular pathways. As a receptor for uPA, uPAR regulates the activity of uPA in addition to plasmin and its substrates: extracellular matrix molecules, matrix metalloproteases, and some growth factors, including HGF/SF (Blasi and Carmeliet, 2002, Mars, et al., 1995). uPAR has been associated with multiple cellular receptors, including EGFR, β -catenin and Met (Guerrero, et al., 2004, Jo, et al., 2003, Monga, et al., 2002, Simon, et al., 2000). Loss of *Plaur* led to a reduction in embryonic levels of HGF/SF and Met as assessed by immunoblot (Powell, et al., 2001). In the present study, we show that HGF/SF levels in adult mice are reduced by 40% (Fig. 1d), and that postnatal supplementation, under the control of an astrocyte promoter can restore total HGF/SF levels. The increased HGF/SF levels were correlated with increased GABA⁺ and PV⁺ cells in the *Plaur/HGF* mice. However, the recovery was greatest in the PV⁺ neurons and only partial in the GABA⁺ cells. The estimates of cell numbers were based on immunohistochemistry, and many PV⁺ cells did not co-express GABA⁺, similar to the previous study (Powell, et al., 2003). The limitations of the immunohistochemistry technique may underestimate the number of GABA⁺ cells, especially those with low expression. Based on experience with the reagents, the PV⁺ immunoreactivity is more sensitive and that the PV⁺ expression represents functioning GABAergic interneurons. In summary, the increased numbers of PV⁺ and GABA⁺ cells in the *Plaur/HGF* mice represent a recovery of the loss in the *Plaur* mice.

Interactions between HGF/SF and uPAR occur during many stages of cerebral cortical ontogeny. During the fetal period, loss of *Plaur* reduces HGF/SF levels leading to decreased migration of subcortically derived interneurons into the dorsal telencephalon. At birth, few GABAergic interneurons were observed in the frontal and parietal cortices in locations rostral to bregma (Powell, et al., 2001). The addition of perinatal HGF/SF may have changed the migration defect into a delay. In the adult mouse, HGF/SF may act as a survival factor in the cerebral cortex (Sun, et al., 1999), with the diminished levels of HGF/SF in the *Plaur* mouse leading to neuronal cell death. In the presence of the exogenous HGF/SF in the *Plaur/HGF* mouse, the PV⁺ cell loss is prevented and the subsequent behavioral consequences are remediated.

The unique sensitivity of the PV⁺ population has several possible explanations. The PV⁺ population represents the fast-spiking interneurons that along with SST⁺ cells are reported to be susceptible to excitotoxic damage (Weiss, et al., 1990). The seizures in the *Plaur* mice may lead to increased glutamate concentrations, leading to loss of the PV⁺ cells. Alternatively, the loss of the uPAR receptor may have impaired responses to extracellular cues, including growth factors, required for maturation into PV⁺ interneurons or survival in the adult brain. It was

originally reported that perinatal *Plaur* mice lacked calbindin-expressing cells in frontal and parietal regions located rostral to bregma. A subsequent study showed no difference in calbindin-expressing cells at levels caudal to bregma (Eagleson, et al., 2005). The loss of PV⁺ cells in the absence of *Plaur* may be due to either loss of a gradient of maturation factors or the ability to respond to these factors. The exogenous HGF/SF provided in the *Plaur/HGF* mouse partially overcame the deficit in the *Plaur* mouse environment, suggesting that HGF/SF may be a maturation or survival factor for cerebral cortical PV⁺ interneurons.

The *Plaur* mouse is one of many mutants that display decreased interneuronal populations. Loss of brain derived neurotrophic factor (BDNF) or fibroblast growth factor 2 leads to interneuron deficits (Dono, et al., 1998, Jones, et al., 1994). A transgenic mouse lacking glial cell-line derived neurotrophic factor receptor (GFRalpha) specifically in neurons is also missing significant numbers of PV⁺ cells in the cerebral cortex (Canty, et al., 2009). In the case of *Plaur* mice, it is possible the supplementation with other growth factors may lead to a comparable outcome. Thus, the *Plaur* mouse may represent an example of a more general model of diminished growth factor responsiveness, with many avenues for ameliorating the phenotype.

The *Plaur* mice were reported to present spontaneous seizures (Powell, et al., 2003), attributed to loss of GABAergic PV⁺ interneurons (Eagleson, et al., 2005, Powell, et al., 2003). While the *Plaur* mice continue to display rare spontaneous seizures during handling or after the administration of anesthetic (data not shown), spontaneous seizure activity was not observed in the WT, *HGF* or *Plaur/HGF* mice. Maintaining the proper total number of PV⁺ cells, and ~75% of the GABAergic interneurons, greatly reduced the seizure phenotype.

These data support a role for HGF/SF in neural circuit formation and maintenance. The HGF/SF receptor Met is expressed during synaptogenesis (Judson, et al., 2009), and *in vitro*, HGF/SF regulates neuronal dendritic arbors (Gutierrez, et al., 2004, Lim and Walikonis, 2008). The *Plaur* and *HGF* mice represent loss and gain-of-function of HGF/SF expression phenotypes whose circuitry may be altered. The observed differences in seizure susceptibilities and EEG traces between WT, *HGF* and *Plaur* mice reflect altered connectivity. Along the same lines, presence of even a rare sustained spiking activity, although not statistically significant, suggests that the *HGF* and *Plaur/HGF* mice approach, but are not the same as, the WT mice.

A co-morbidity of increased anxiety (Powell, et al., 2003), as measured by the light-dark avoidance test and the elevated plus maze, was also reported in the *Plaur* mice. The behavior of the entire cohort demonstrates that after several generations on the B6 background, the *Plaur* mice still display increased anxiety. The *Plaur/HGF* mice were similar to WT and *HGF* mice, suggesting that retention of the GABA interneurons in the cerebral cortex was sufficient to prevent the co-morbidity. Additional long-term effects of these genetic manipulations on cognitive behaviors are currently being assessed.

The HGF/SF-Met signaling pair, and to a lesser extent uPA-uPAR, have been known for their roles in human cancer, including brain tumors (Abounader and Laterra, 2005, Arrieta, et al., 2002, Comoglio, et al., 1999). The postnatal overexpression of HGF/SF does not increase the overall number of GABAergic interneurons. In fact, the total HGF/SF levels in the *HGF* mouse were slightly decreased, reflecting downregulation of mouse HGF/SF, suggesting a regulatory mechanism of maintaining appropriate overall HGF/SF levels. Similarly, no signs of abnormal cellular growth were observed (data not shown). Instead, HGF/SF and Met appear to play a role in network maintenance, and current studies are revealing new roles for plasminogen associated molecules in controlling normal brain function. In addition to studies in injury and degeneration, diminished signaling of MET in the cerebral cortex has been reported in autism (Campbell, et al., 2007). Linkage analysis for *MET* and possibly *PLAUR* provide evidence for

a role in the HGF/SF in human disease (Campbell, et al., 2008). The use of the postnatal intervention to sustain HGF/SF-MET signaling offers a new pathway for clinical intervention for pediatric epilepsy and autism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Generation and initial characterization of the B6.129 – *Tg(Gfap-HGF)^{Ca}* mouse. (a) Construct for producing the transgenic mouse line. (b) Genotyping the B6.129 – *Tg(Gfap-HGF)^{Ca}* mice (abbreviated as *HGF*) using a PCR strategy with primers selective for human *HGF*. *HGF* is 250bp and *Serpin1* (200 bp) is used as positive control. (c) Western blot assay probing specifically for human HGF in protein extracts from cerebral cortex from postnatal day 14 WT and *HGF* mice. (d) ELISA assay for total HGF/SF demonstrating similar levels in WT and *HGF* mice. *Plaur* mice have decreased levels of HGF/SF, whereas the HGF/SF amount in the *Plaur/HGF* mice is similar to WT. The asterisk demonstrates statistical significance compared to WT, and the ampersand is used to compare to *Plaur* and *Plaur/HGF* mice, at the $p < 0.05$ level, as determined by ANOVA of at least 5 samples per genotype. The error bars indicate the s.e.m.

Figure 2.

Postnatal HGF/SF supplementation restores anatomical abnormalities. (a-d) Cresyl violet staining in adult WT (a), *HGF* (b), *Plaur* (c), and *Plaur/HGF* (d) mice. (e-h) Immunohistochemistry for expression of GABA in WT (e), *HGF* (f), *Plaur* (g), *Plaur/HGF* (h) adult mouse somatosensory cortex. (i-l) Immunohistochemistry for parvalbumin-expressing interneurons in WT (i), *HGF* (j), *Plaur* (k), and *Plaur/HGF* (l). Scale bar = 100 μ m. Roman numerals represent cortical layers (I-VI). (m) Stereological counts of immunoreactive cells are shown for. $n \geq 4$ mice, with error bars denoting the s.e.m. Asterisks indicate statistically significant differences in comparison to WT at the $p < 0.05$ level, and ampersands show differences between *Plaur* and *Plaur/HGF* mice, $p < 0.05$.

Figure 3.

Postnatal HGF/SF supplementation altered seizure phenotypes. (a) Percent of mice that exhibited specific behavioral responses to PTZ. A score of 0 indicates no signs of motor seizure, 1: isolated limb twitches, 2: tonic-clonic convulsion, and 3: tonic extension. The majority of *Plaur* mice demonstrated scores of 2 or 3, whereas the other groups exhibited few motor signs. Sample size is $n = 9 - 10$ mice per group. (b) Latency to demonstrate onset of seizure, as characterized by convulsions. The *Plaur* mice had the shortest latency, whereas the other groups were statistically similar. (c) Representative traces of spontaneous activity in a separate cohort of freely moving awake mice. Each trace is shown with dashed lines that represent the boundaries of 3 standard deviations of amplitude (threshold) for the >2h recording period.

Sustained periods of spiking activity were observed often in the *Plaur* mice. (d) The spiking activity was quantified by defining an episode as sustained spiking activity that exceeded threshold for >30 s. No episodes were found in WT mice. (e) Light-dark avoidance test for anxiety measures preference for the light side of the testing arena. The *Plaur* mice had decreased time on the light side, whereas the *Plaur/HGF* mice were similar to WT. The asterisk denotes statistical significance between the *Plaur* group and the other genotypes, ($p < 0.05$).