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Relative expression of hepatocyte growth factor in cerebrospinal fluid during mouse embryonic development and early postnatal stage; A Western Blot analysis

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ABSTRACT

Hepatocyte growth factor (HGF), is a growth factor which promotes the survival and migration of immature neurons. HGF is widely expressed in the developing brain. In early stages of embryogenesis, cells within the ependymal lining of neural tube are thought to secrete cerebrospinal fluid (CSF). As the neural tube closes, the choroids plexuses secrete proteins, including growth factors into the CSF. In this study, CSF was aspirated from the cisterna magna of embryonic mice and the relative HGF expression was measured on gestational days 12 to 21 (E12-E21) and postnatal days 1-10 (P1-P10) by Western Blot. The relative CSF HGF expression increased from E12 to E18 and decreased from E19 until birth. After birth there was a rapid increase in HGF expression until day P2, and thereafter the levels decreased from day P4 to day P9. Days E16-E18 and P1-P3 coincide with the onset of neurons and glial migration in the cerebral cortex, respectively. Since CSF is in contact with the cerebral cortical germinal epithelium, changes in the HGF expression may reflect neuroepithelial cell migration. It is concluded that HGF might be involved in cerebral cortical development and it is a constant component of CSF during mouse embryonic development and early postnatal stages.

Keywords: hepatocyte growth factor, expression, cerebrospinal fluid, development, mouse.

INTRODUCTION

During development, the laminated structure of cerebral cortex is organized by proliferative, morphogenetic and migratory processes [1]. There has been considerable recent progress in understanding the processes involved in cerebral cortex development and many of the molecular

mechanisms involved are becoming clear [2]. The generation of differentiated neurons and glial cells from proliferating neural progenitor or stem cells is a complex process involving interplay between intrinsic cellular programmes and extrinsic cues such as growth factors [3].

During early embryonic development, the neural tube is formed by an epithelial wall, the neuroepithelium, surrounding a cavity, which is filled with the primitive cerebrospinal fluid (CSF) called neural tube fluid (NTF), which is secreted by the brain tissue [4]. Later in development, the choroids plexuses secrete CSF. CSF passes from the lateral ventricles into the third ventricle via the foramina of Monro and then into the fourth ventricle through the aqueduct of Sylvius. During early development of the nervous system, CSF passes down the central canal of the spinal cord. The CSF breaks out of the ventricular system into the basal cisterns through the foramina of Lushka and Magendi in the fourth ventricle formed through the programmed death of cells in those locations. CSF passes out of the brain from the fourth ventricle entering the cisterna magna before passing through the subarachnoid space, circulating around the brain and spinal cord until it exits into the sagittal sinus and facial lymphatics [5].

CSF contains concentrations of growth factors that change during different stages of embryonic development. A number of studies have identified CSF as a carrier of important cytokines such as transforming growth factor-ß (TGF-ß), nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), insulin-like growth factor (IGF) and hepatocyte growth factor (HGF) [6-8]. Thus, the CSF pathway can be regarded as a one-way flowing river carrying signals [5].

HGF is a glycoprotein which influences the growth, motility and morphogenesis of various epithelial cells and functions as a trophic factor for organ regeneration [9]. Expression of the mRNA for HGF has been seen in rat brain, and neuronal expression of HGF in mammalian brain tissues has been reported [10; 11]. HGF is a disulfide-linked heterodimeric polypeptide. Both chains are derived from a single chain precursor (pro-HGF), which is cleaved to yield a mature two-chain HGF. The mature HGF binds to c-Met receptor with high affinity, triggering its kinase activity and a biological response in target cells [12].

Astrocytes, neurons, microglia and oligodendrocytes all have been reported to express HGF [13]. It has been shown that HGF is involved in the development of cortical neurons [14]. There are reports that HGF/c-Met signaling plays an essential role in the development and maintenance of the nervous system. HGF participates in early neural tube development and supports the survival of motoneurons [15]. HGF and its receptor are expressed within developing cerebral cortex [11]. In this study we studied the relative HGF expression in the CSF from mouse embryos by Western Blot.

MATERIALS AND METHODS

Animals

Balb/c mice were used in this study. Timed pregnant Balb/c mice were purchased from Pastur inistitute, Tehran, Iran. They were maintained on 12:12 h light dark cycle beginning at 8.00 a.m. They were kept at a constant temperature in mouse boxes with unrestricted access to food and water. The colony was maintained through random pair mating. Timed mating was carried out by

placing a male and female together in a box and checking for the presence of a vaginal plug. The presence of a plug was taken to indicate successful mating and the time was taken as gestational day 0 (E0) and the day of birth was designated postnatal day 0 (P0). All animal procedures were carried out in accordance with the Animals (Scientific Procedure) Act, 1986.

CSF samples

The CSF was aspirated from mouse embryos from day-12 to day-21 (full gestation) (E12–E21) and P1 to P9 under a microscope using a 20 μL pulled-tip glass microcapillary pipette (Drummond Scientific Company, Broomall, PA, USA). The needle was held steadily inside the cisterna magnum to avoid contact with the neuroepithelial wall and the CSF was slowly aspirated. It was technically almost impossible to obtain CSF from embryonic mice earlier than E12. CSF samples were centrifuged at 15 000 revolutions per minute (rpm) at 4 °C for 10 min to remove any contaminating cells. The samples when examined microscopically showed no visible signs of contamination with neuroepithelial cells or red blood cells. The supernatant was frozen immediately and stored at -70 °C. Twenty-two samples from each embryonic time-point were analyzed for concentrations of HGF.

Protein analysis: *Total protein concentration and Western Blot*

For Western Blot analysis, aliquots of CSF were mixed with a sample buffer containing 3.2% sodium docecyl sulfate (SDS), 15% glycerol, 2.8 M betamercaptoethanol and 0.0015% bromophenol blue. Samples were applied to a 5% to 20% gradient for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad Laboratories) according to the Laemmly method and the proteins obtained were transferred to nitrocellulose sheets, pore size 0.45 μm (Bio-Rad Laboratories). After incubation for 2 hours at room temperature in the blocking solution [phosphate buffered saline (PBS) containing 5% skimmed milk], the nitrocellulose sheets were exposed overnight at 4 °C to anti-HGF monoclonal antibody (Santa Cruz Biotechnology) and identified with a peroxidase-labeled mouse immunoglobulin M (IgM) (PK 4010 Vectastain Avidin Biotin complex kit; Vector Laboratories). The peroxidase activity was detected with diaminobenzidine (0.5 mg/mL in PBS with 0.02% hydrogen peroxide).

RESULTS

In this study the relative expression of HGF in the CSF from days E12 to E21 and P1 to P9 was analyzed by Western Blot. A Western Blot analysis using anti-HGF antibody as a probe confirmed the presence of HGF in all CSF samples from days E12 to E21 and P1 to P9 (Figure 1). In order to obtain semi-quantitative estimates of the relative amounts of the HGF protein band, an image analyzer was used to determine the intensities of the gels from repeated experiments (n = 22 for each time point). This showed that the expression of HGF increased from days E12 to E17. There was a rapid increase in HGF concentration on day E18 and then the HGF levels decreased from days E19 to E21. We have also analyzed the CSF HGF expression in the postnatal days 1 to 9 (P1-P9). After birth there was a rapid increase in HGF expression until day P. There was a peak of HGF expression in the CSF on P3, and thereafter the expression decreased from day P4 to day P9 (Figure 2). HGF appears to be a constant component of mouse CSF during embryonic development. It is also shown that the expression of HGF in the CSF changes during the embryonic development and early postnatal stages.

DISCUSSION

To our knowledge, this is the first study of HGF expression in the CSF from mouse embryos by Western Blot. Previous studies focused on the roles of HGF in the developing brain, and revealed it to be a neural inducer during embryonic development, an neurotrophic and neuroprotective survival factor [16; 17]. HGF and its receptor, c-Met, have been found to be present in specific subpopulations of hippocampal neurons, cortex and cerebellum of both developing and adult mammalian brains [11; 18]]. Changes in HGF and c-Met expression in the developing rat cerebral cortex has been demonstrated, which suggests a role for each in this process. During embryonic development, the expression of c-Met and HGF RNAs gradually increased and peaked at E18 when cortical neurons actively differentiate and neuroepithelial cells cease to proliferate [14]. Newly generated neurons from the germinal epithelium migrate to their destination, either radially or tangentially. Localization of c-Met immunoreactivity in intermediate zone (IZ) postmitotic, migrating cortical neurons and the *in vitro* motogenic activity of HGF both suggest a role for HGF in neuronal migration [14]. HGF is a potent scatter factor, since it enhances the motility of many classes of epithelial cells [19]. It has been shown that HGF may play an important role in the tangential migration of interneurons from the ganglionic eminence to the cerebral cortex [20]. It has been demonstrated that many neurons from the E18 cerebral cortex explants as well as E18 ventricular zone explants migrated in response to exogeneously applied HGF mainly from the ventricular side [14].

HGF has also been shown to promote the survival of several neuron types [13]. HGF functions as a neurotrophic factor promoting the survival of cerebral cortex neurons. HGF may also function as a neurite outgrowth promoting factor for cortical neurons [14]. These results indicate the involvement of HGF in cerebral cortical development.

HGF is expressed in various somatic cell types. HGF and its receptor are expressed not only in neurons but also in nonneuronal cells within the nervous system including microglia, oligodendrocytes, astrocytes and shwann cells [21; 22]. It has been shown that HGF is normally expressed by ependymal cells and choroids plexuses enabling growth factors to diffuse to their sites of activity [23].Because molecules in the CSF can enter the brain tissue [24], choroids plexus-derived HGF in the CSF possibly act on brain tissue [25]. After growth factors and other neurotrophic factors are secreted into the ventricles, they are conveyed by CSF bulk flow to various regions of the brain, placing many neurons in contact with the products and secretions of the choroids epithelial cells [26]. HGF is secreted by the fetal choroids plexus to provide trophic support for developing brain. Several molecules have been implicated in the direct migration of central nervous system neurons [23]. Neurogenesis may be mediated by several different signaling molecules, which include IGF-1, BDNF, NGF and HGF. HGF mediates neurotrophic functions during neurogenesis and promoting survival and/or migration of central nervous system neurons [27].

The results from this study showed that the expression of HGF increases from day E12 to E18. Days E16 to E18 coincide with the onset of neuron differentiation, migration and organization of the cytoarchitecture of the developing cortex [14; 28]. As CSF is in contact with cerebral cortical germinal epithelium, changes in the expression of CSF HGF could affect neuroepithelial cell migration, proliferation and survival.

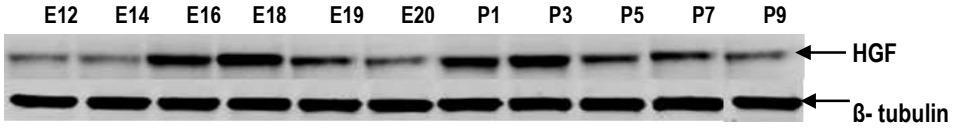

Figure 1. Western blot showing expression of hepatocyte growth factor in the cerebrospinal fluid (CSF) from days E12 to E20 and P1 to P9. β- Tubulin expression was used as a protein loading control. In each of the time points the number of animals investigated was n= 22

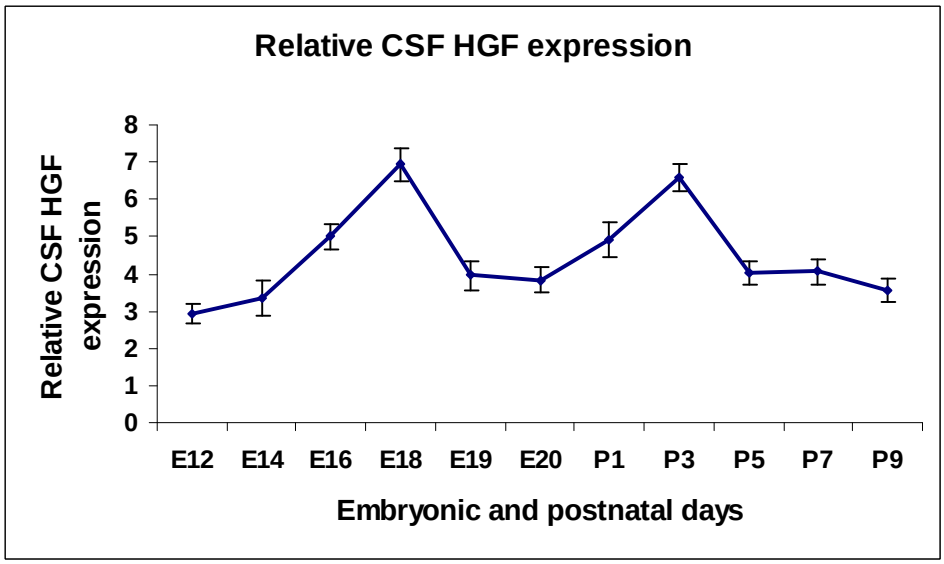

Figure 2. Relative expression of hepatocyte growth factor (HGF) in the cerebrospinal fluid (CSF) from embryonic days E12 to E21 and postnatal days P1 to P9. An image analyzer was used to determine the intensities of the band in the respective lanes in the gels. Results shown are mean ± standard error of the mean.

CONCLUSION

It is thus concluded that HGF is not only a constant component of embryonic mouse CSF and early postnatal stage, but it might also be involved in cerebral cortical development.

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