Hepatocyte Growth Factor (HGF): Neurotrophic Functions and Therapeutic Implications for Neuronal Injury/Diseases

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Abstract: Hepatocyte growth factor (HGF), which was originally identified and molecularly cloned as a potent mitogen for primary hepatocytes, exhibits multiple biological effects, such as mitogenic, motogenic, morphogenic, and anti-apoptotic activities, in the liver and other organs throughout the body by binding to the c-Met/HGF receptor tyrosine kinase (c-Met). In addition to hepatotropic activities, HGF and c-Met are expressed in both developing and adult mature brains and nerves, and plays functional roles in the central as well as peripheral nervous systems. A large number of studies have accumulated evidence showing that HGF is a multipotent growth factor that functions as a novel neurotrophic factor for a variety of neurons, including the hippocampal, cerebral cortical, midbrain dopaminergic, motor, sensory, sympathetic, parasympathetic and cerebellar granule neurons in vitro. In vivo, HGF exerts neuroprotective effects in the animal model of cerebrovascular diseases, spinal cord injury, neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), and neuroimmune diseases, preventing neuronal cell death and functioning on glial, vascular and immune cells. The multiple activities of HGF, in addition to highly potent neurotrophic activities, suggest that HGF is a potential therapeutic agent for the treatment of various diseases of the nervous system. Furthermore, the anxiolytic activity of HGF and an association of c-met with autism, as well as neuropsychiatric and schizophrenia, have been reported, suggesting a role for HGF in emotional and psychiatric status. This review describes the role of HGF in the nervous systems during development and focuses on the therapeutic potential of HGF for a variety of neurological, neuroimmunological and psychiatric diseases among adults.

Keywords: HGF, c-Met, neurotrophic, amyotrophic lateral sclerosis (ALS), spinal cord injury (SCI), autophagy.

1. INTRODUCTION

Neurological diseases are disorders of brain, spinal cord and peripheral nerves throughout the body, which evoke abnormality of neurological functions such as the inability to speak, decreased sensation, loss of balance, weakness, mental function problems, visual changes, abnormal reflexes, and walking problems. These diseases are caused by faulty genes, problems with the way the nervous system develops, degeneration of neuronal cells, diseases of the blood vessels that supply the brain, injuries to the spinal cord and brain, seizure disorders, cancer, and infections. Although neurological disorders have been recognized as important diseases, their treatment with available medications has many limitations.

The diseases are characterized by dysregulation/disruption of a variety of neurological functions, such as the survival, proliferation, differentiation, maturation, and activity-dependent modulation of neurons during development and in adults. The factors affecting these functions include neurotrophic factors such as neurotrophins (NGF, BDNF, NT-3 and NT-4) [1-4], the glial cell line-derived neurotrophic factor (GDNF) family (GDNF, Nurturin, Persenphin and Artemin) [5], and the ciliary neurotrophic factor (CNTF)/ interleukin (IL)-6 family (CNTF, LIF, cardiotoxin and IL-6) [6, 7]. However, neurological, neuroimmunological and psychiatric diseases are not simply dependent on the disruption of neuronal functions, but are sometimes dependent on extraneuronal activities by glial, vascular and immunological cells. For example, multiple sclerosis (MS) and animal models of inflammatory demyelination are characterized by a complex interplay between degenerative and regenerative processes, many of which are regulated and mediated by various types of cells, including glial cells. Cellular communication between neurons, glial cells and immune cells is critical and is controlled to a large extent by the activity of cytokines/growth factors. Therefore, in addition to the classical neurotrophic factors, mounting evidence suggests that multipotent growth factors with neurotrophic activity play a pivotal role in the nervous system, during development and for maintenance in adults, and may have advantages for the treatments of individuals with diseases of the nervous system. In addition to direct neurotrophic functions in neurons, such extraneuronal potential of these factors may be important in the development of therapeutic agents against neurological, neuroimmunological and psychiatric diseases.

Hepatocyte growth factor (HGF) was initially identified as a mitogen for primary hepatocytes and was molecularly...
Hepatocyte Growth Factor (HGF): Neurotrophic Functions

In addition to classical neurotrophic factors, hepatocyte growth factor (HGF) has been implicated in neurotrophic activities with various extraneuronal functions, including angiogenic activity as well as functions in glial and immune cells, via binding to its specific receptor the c-Met/HGF receptor (c-Met) [8-10]. Studies of c-met using knock-out/in mice strategies and anti-HGF treatment revealed that the HGF-c-Met system is crucial for the development of motor, sensory, sympathetic, cerebellar, and cerebral cortical development, as well as oligodendrocyte development in vivo [11, 12]. Here, recent findings of a wide variety of biological functions of HGF in the nervous systems are described with a special focus on potential therapeutic effects on neurological, neuroimmunological and psychiatric pathologies, as a highly potent neurotrophic factor with pleiotrophic activities on various types of cells, which is crucial for neuroregenerative medicine.

2. IDENTIFICATION OF HGF AS A NEUROTROPHIC FACTOR

2.1. Activities of HGF on Various Cells in the Nervous System In Vitro

In addition to the role of HGF in the regeneration and protection of a variety of organs, including liver, kidney and lung, it has the ability to exert multipotent activities under pathophysiological conditions [8, 13]. In the nervous system, for example, c-Met/HGF signaling plays an essential role in development and maintenance [8, 11, 14]. In brief, c-Met is widely expressed in various regions of both developing and adult brains in vivo, including neurons of the cerebral cortex [15-19], hippocampus [15-18, 20], cerebellum [15-17], brainstem motor nucleus [16, 21], retina [22] and sensory ganglia [23, 24], and the spinal cord [25, 26], as well as non-neuronal cells such as reactive astrocytes [25, 26], oligodendrocytes progenitors [12], oligodendrocytes [12, 27], and microglia [28, 29]. Combined with the expression and regulation of HGF during development and in adult nervous systems, these findings suggest a functional coupling of HGF and c-Met in both the central and peripheral nervous systems.

Consistent with the expression profiles, in 1995, Honda et al. used primary cultured rat hippocampal neurons to find the first indication that HGF functioned as a neurotrophic factor [16]. The Honda study found that HGF dose-dependently increased the numbers of surviving hippocampal neurons after serum starvation [16]. Combined with the notion that HGF and c-Met are expressed in various regions of the nervous system during development and in adults, these results led to the recognition that HGF also functions as a neurotrophic factor for other types of neurons.

In neurons, HGF also promotes not only survival but also neurite expression and branching. For example, HGF enhances survival, promotes neurite outgrowth, and stimulates dendrite growth in a variety of neurons such as hippocampal, midbrain dopaminergic, cerebral cortical, motor, sensory, sympathetic, parasympathetic, and cerebral granular neurons. HGF also guides axons to targets in vitro, as summarized in Table 1 [8, 16, 19, 30, 31]. HGF promotes cellular migration

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampal neurons</td>
<td>Survival, dendritic maturation, differentiation, modulation of NMDA receptor and PSD-95 localization</td>
<td>[16, 20, 32, 33, 96]</td>
</tr>
<tr>
<td>Midbrain dopaminergic neurons</td>
<td>Neurite extension, increased Tyrosine Hydroxylase (TH) activity</td>
<td>[31]</td>
</tr>
<tr>
<td>Cerebral cortical neurons</td>
<td>Survival, neurite extension, migration, numbers of dendritic arbors</td>
<td>[19, 30, 97]</td>
</tr>
<tr>
<td>Cerebellar granular neurons</td>
<td>Survival</td>
<td>[98, 99]</td>
</tr>
<tr>
<td>Thalamic neurons</td>
<td>Neurite extension</td>
<td>[100]</td>
</tr>
<tr>
<td>Motor neurons</td>
<td>Survival, neurite extension (chemoattractant)</td>
<td>[68-70]</td>
</tr>
<tr>
<td>Sensory neurons</td>
<td>Survival, neurite extension, migration</td>
<td>[23, 24]</td>
</tr>
<tr>
<td>Sympathetic neurons</td>
<td>Survival (postnatal), neurite extension, branching</td>
<td>[101-103]</td>
</tr>
<tr>
<td>Sympathetic neuroblasts</td>
<td>Survival, differentiation (but not proliferation)</td>
<td>[101]</td>
</tr>
<tr>
<td>Parasympathetic neurons</td>
<td>Survival</td>
<td>[104]</td>
</tr>
<tr>
<td>Olfactory interneuron precursors</td>
<td>Migration by Met-Grb2 coupling (slice culture)</td>
<td>[105]</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>Migration, EAAT2/GLT-1 expression</td>
<td>[25, 106]</td>
</tr>
<tr>
<td>Schwann cells</td>
<td>Proliferation (mitogenic)</td>
<td>[107]</td>
</tr>
<tr>
<td>Oligodendrocyte progenitor cells</td>
<td>Proliferation, migration</td>
<td>[34]</td>
</tr>
<tr>
<td>Olfactory ensathing cells (OEC)</td>
<td>Proliferation (mitogenic)</td>
<td>[108]</td>
</tr>
<tr>
<td>SVZ neural stem-like cells</td>
<td>Growth and self-renewal</td>
<td>[109]</td>
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</table>
and the synthesis of neurotransmitter synthesizing enzymes, such as Tyrosine Hydroxylase (TH) [31]. Furthermore, HGF modifies the localization of PSD-95 clusters and NMDA receptors in primary hippocampal neurons [32, 33].

In non-neuronal cells, HGF promotes chemokinesis [34] and modulates the expression levels of glial specific glutamate transporter EAAT2/GLT-1 of primary astrocytes [25]. In addition, HGF promotes the proliferation of oligodendrocyte progenitor cells (OPCs) in vitro [34] and in vivo [12], and modulates differentiation into oligodendrocytes [12]. Furthermore, HGF modulates cytokine production in immune cells [35].

This accumulating evidence indicates that HGF is a multipotent growth factor with highly potent neurotrophic activity. In comparison with classical neurotrophic factors, the extraneuronal activity of HGF, in addition to its neurotrophic activity, may increase the therapeutic advantage of HGF for the treatment of several neurological diseases. Many researchers have reported the successful application of HGF in various animal models of neurological conditions, including cerebrovascular, neurodegenerative and psychiatric diseases.

3. DEVELOPMENTAL ROLES OF THE HGF-C-MET SYSTEM IN THE NERVOUS SYSTEMS

Knock-out/in studies as well as stereotaxic injections of HGF and anti-HGF antibody into the striatum reveal the critical role of HGF in the nervous systems, as summarized in Table 2. HGF plays important roles in motor (including muscles), sensory, sympathetic, parasympathetic, and cortical neuronal development [11] & Table 2. In addition to the neuronal development, intrastrial injections of HGF or anti-HGF IgG demonstrated the critical role of HGF in the proliferation of oligodendrocyte progenitor cells and their differentiation into oligodendrocytes [12]. HGF is thus implicated in neuronal as well as glial development, in an orchestrated manner [12].

4. MUTATION(S) OF HGF-C-MET ASSOCIATED WITH NEURAL DISEASES/SYMPTOMS

Recent studies have demonstrated the involvement of mutation(s) in the HGF and c-Met genes in several disorders, such as autism, schizophrenia and nonsyndromic hearing loss, as summarized in Table 3.

4.1. Autism

The association of mutation in the Met with autism was first described in 2006 by Campbell et al. [36]. They showed the genetic association of a common C allele in the promoter region of the MET gene in 204 families with autism. The allelic association at this MET variant was confirmed in a replication sample of 539 families with autism and in a combined sample. They also showed that MET protein levels were significantly decreased in autism spectrum disorder (ASD) cases, compared with control subjects. This was accompanied in ASD brains by increased messenger RNA expression of proteins involved in regulating MET signaling activity. Analyses of the coexpression of MET and HGF demonstrated a positive correlation in control subjects that was disrupted in ASD cases [37]. Their most recent study supported the association of the MET promoter variant rs1858830 C allele with ASD, implying a promoter mutation in ASD [38].

Sousa et al. reported new mutation(s) in autism, in both single locus and haplotype approaches, with a single nucleotide polymorphism in intron 1 (rs38845) and with one intronic haplotype (AAGTG), in 325 multiplex International Molecular Genetics of Autism Consortium (IMGSAC) families and 10 IMGSAC trios. Although another study with an independent sample of 82 Italian trios failed to replicate these results, the association itself was confirmed by a case-control analysis performed using the Italian cohort (P<0.02) [39].

4.2. Schizophrenia and General Cognitive Ability

Comparison of 21 Single Nucleotide Polymorphisms (SNPs) in the MET with schizophrenia status in 173 Caucasian patients and 137 controls revealed an association between genetic variation in the MET and schizophrenia [40]. It was also reported that genetic variations of the MET are associated with general cognitive ability [40]. These findings

<table>
<thead>
<tr>
<th>Target System</th>
<th>Function</th>
<th>References</th>
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<tbody>
<tr>
<td>Motor nervous system</td>
<td>Survival, Neurite extension, migration</td>
<td>[110, 111]</td>
</tr>
<tr>
<td>Sensory nervous system</td>
<td>Survival, axonal growth</td>
<td>[23]</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td>Survival of sympathetic neuroblasts</td>
<td>[101]</td>
</tr>
<tr>
<td>Cortical interneuron</td>
<td>Migration</td>
<td>[112, 113]</td>
</tr>
<tr>
<td>Cerebellar development</td>
<td>Proliferation of granule precursors in vivo was 25% lower than in controls. Behavioral tests indicated a balance impairment in Met(grb2/grb2neo-) miceMet signaling knockdown disrupts specification of VZ-derived cell types, and also reduces granule cell numbers, due to an early effect on cerebellar proliferation and/or as an indirect consequence of the loss of signals from VZ-derived cells later in development. These patterning defects preclude the analysis of cerebellar neuronal migration, but it was found that Met signaling is necessary for migration of hindbrain facial motor neurons.</td>
<td>[111, 114]</td>
</tr>
<tr>
<td>Forebrain development</td>
<td>Motogen and guidance signal for gonadotropin hormone-releasing hormone-1 neuronal migration.</td>
<td>[115]</td>
</tr>
<tr>
<td>Oligodendrocyte development</td>
<td>Modification of oligodendrocyte progenitor cell proliferation and its differentiation</td>
<td>[12]</td>
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5. ROLE OF HGF IN ANXIETY-RELATED BEHAVIOR

When HGF was infused at a constant rate into the cerebrospinal fluid [53], many studies have demonstrated that HGF has the ability to act as a potent cerebroprotective agent for functional recovery after ischemic brain injuries, by preventing several pathophysiological alterations caused by brain ischemia, as follows Table 4 and Fig. (1).

6. POTENTIAL IMPLICATIONS OF HEPATOCYTE GROWTH FACTOR FOR DISEASE MODELS IN THE NERVOUS SYSTEM

6.1. Ischemic Diseases in the Nervous System (Cerebrovascular Diseases)

Ischemic stroke in the brain (brain ischemia) is associated with cerebrovascular disease characterized by a sudden loss of function due to the loss of blood supply to an area of the brain that controls that function. The biochemical changes of brain ischemia result in neuronal cell death and endothelial cell damage. Brain ischemic neuronal cell death can cause dysfunction of the central nervous system such as permanent paralysis, loss of vision, and impairments of speech, cognition or learning and memory. Furthermore, endothelial cell damage can cause disruption of the blood-brain barrier (BBB), resulting in the extravasation of plasma components and the formation of edema (ischemic cerebral edema), resulting in secondary damage to neurons [46]. Gliosis may modulate the progression of the disease.

The most popular classes of drugs used to prevent or treat stroke are anti-thrombotics and thrombolytics. However, there is no specific treatment to prevent neuronal and endothelial cell death and improve functional recovery after ischemic stroke.

Many studies have demonstrated that HGF has the ability to act as a potent cerebroprotective agent for functional recovery after ischemic brain injuries, by preventing several pathophysiological alterations caused by brain ischemia, as follows Table 4 and Fig. (1).

6.1.1. Prevention of neuronal cell death

Continuous post-ischemic administration of recombinant human HGF protein (rhHGF) effectively prevented the delayed neuronal cell death in the hippocampus, which is one of the brain regions most vulnerable to ischemia [47-52]. Gene transfer of HGF into the subarachnoid space using Hemagglutinating virus of Japan (HVJ)-Liposome prevented delayed neuronal cell death in the hippocampus by increasing HGF protein in the cerebrospinal fluid [53].

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### Table 3. Mutations of the HGF and c-Met Genes in Mental/Psychiatric Diseases/Status

<table>
<thead>
<tr>
<th>(I) Autism</th>
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<tbody>
<tr>
<td>a. c-met</td>
<td>[36]</td>
<td>mutation in promoter region of c-met</td>
</tr>
<tr>
<td>b. c-met</td>
<td>[37, 116]</td>
<td>mutation in promoter region of c-met</td>
</tr>
<tr>
<td>c. c-met</td>
<td>[38]</td>
<td>mutation in promoter region of c-met</td>
</tr>
<tr>
<td>d. c-met</td>
<td>[39]</td>
<td>mutation in promoter region of c-met</td>
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<tr>
<th>(II) Schizophrenia and General Cognitive Ability</th>
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<tbody>
<tr>
<td>a. c-met</td>
</tr>
<tr>
<td>b. c-met</td>
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<table>
<thead>
<tr>
<th>(III) Nonsyndromic hearing loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. HGF</td>
</tr>
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</table>
Table 4. Therapeutic Potential of HGF for Neurological Diseases in the Animal Model

<table>
<thead>
<tr>
<th>Neurological Disorders</th>
<th>Application</th>
<th>Molecular Mechanisms</th>
<th>References</th>
</tr>
</thead>
</table>
| Ischemic brain injury            | Recombinant human HGF (rhHGF) protein                                       | Prevention of neuronal cell death  
  - Bcl-2 protein ↑  
  - Bcl-xL protein ↑  
  - Bax translocation from cytoplasm to nucleus ↓  
  - Caspase-3 activation ↓  
  - AIF translocation ↓  
  (via preventing PARP formation and p53 expression)  
  Prevention of endothelial cell death  
  - Bcl-2 protein ↑  
  - Tight-junction proteins (occluding/ZO-1) ↑  
  - Modulation of apoptosis and autophagy                                                                                                           | [47-51, 58, 60, 63] |
|                                  | Spongel soaked with rhHGF protein                                           |                                                                                                                                                                                                                       |            |
|                                  | HGF gene (HVJ-Liposome)                                                    | Angiogenesis ↑  
  Cerebral blood flow ↑                                                                                                                                                                                              | [53, 117]  |
|                                  | HGF gene (HVJ-Envelope)                                                    | Neurite extension ↑  
  Synapses ↑  
  Inhibition of gliosis (astrocytosis and glial scar formation)                                                                                                                                                     | [64]       |
|                                  | Transplantation of bone marrow mesenchymal cells (MSCs) with ex vivo HGF gene | Anti-apoptosis                                                                                                                                                                                                       | [118]      |
|                                  | transfer using HSV-1                                                        |                                                                                                                                                                                                                       |            |
|                                  | Alzheimer’s disease             | HGF gene (naked plasmid)                                                   | Recovery of vessel density  
  Blood flow ↑  
  BDNF protein ↑                                                                                                                                                                                                     | [80]       |
|                                  | Parkinson’s disease             | HGF gene (naked plasmid)                                                   | Prevention of neuronal cell death                                                                                                                      | [86]       |
|                                  | Amyotrophic lateral sclerosis (ALS)                                         | HGF gene (transgenic mouse)                                                 | Prevention of motor neuronal cell death  
  Caspase-1, -3 and -9 activation ↓  
  XIAP protein ↑  
  iNOS protein ↓  
  EAAT2 protein ↑ in astrocytes  
  Inhibition of gliosis (astrocytosis and microgliosis)                                                                                                         | [21, 25, 77] |
|                                  |                                  | rhHGF protein (intrathecal administration)                                 |                                                                                                                                                                                                                       |            |
|                                  | Spinal cord injury (SCI)                                                   | HGF gene (HSV-1 viral vector)                                               | Prevention of motor neuronal cell death  
  Prevention of oligodendrocyte death  
  Caspase-3 activation ↓                                                                                                                                                                                          | [27]       |
|                                  | Multiple sclerosis (MS)                                                    | HGF gene (Transgenic mouse)                                                 | Modulation of neuroimmune system                                                                                                                       | [35]       |
|                                  | Seizure                                                                      | HGF gene (Transgenic mouse) rhHGF protein                                  | Modulation of GABAergic inhibition and seizure susceptibility                                                                                           | [91]       |
|                                  | Hydrocephalus                   | rhHGF protein                                                              | TGFbeta inhibition  
  Anti-fibrosis                                                                                                                                                                                                       | [90]       |
|                                  | Retinal injury                  | rhHGF protein                                                              | Anti-apoptosis  
  Neuroprotection of the Photoreceptor and retinal pigment epithelium (RPE)                                                                              | [92, 119]  |
|                                  | Hearing impairment              | rhHGF protein (gelatin hydrogels)                                          | Neuroprotection of outer hair cells (OHC)                                                                                                               | [120]      |
|                                  |                                | HGF gene (HVJ-E)                                                           | Prevention of loss of hair cells (HC)  
  Apoptosis of spinal ganglion cells                                                                                                                       | [93]       |
|                                  | Peripheral nerve injury and neuropathy                                      | Recombinant rat HGF protein                                                 |                                                                                                                                                                                                                       | [71-73, 121-124] |
|                                  |                                | HGF gene (adenoviral vector)                                               |                                                                                                                                                                                                                       |            |
|                                  | Neuropathic pain                | HGF gene (HVJ)                                                            |                                                                                                                                                                                                                       | [94]       |
Fig. (1). Pleiotrophic functions of HGF during development and in the animal model of neurological and neuroimmunological diseases. HGF acts on neuronal cells to attenuate neuronal cell death by preventing caspase-dependent and -independent apoptosis and by inhibiting gliosis (microgliosis and astrocytosis), and by acting on glial cells, such as astrocytes, to retain (or even increase) the levels of glial-specific glutamate transporter 1 (GLT-1)/excitatory amino acid transporter 2 (EAAT2), which might be favorable to reduce glutamatergic neurotoxicity. The possibility that HGF directly functions on microglia cannot be excluded, since c-Met is expressed in the subpopulation of microglia in ALS model mice and in primary culture (Ohy-a-Shimada and Funakoshi, unpublished results). HGF also attenuates down-regulation of tight junctional proteins (occludin and ZO-1) and promotes angiogenesis that is beneficial for ischemic conditions. Under development and demyelinating conditions, such as MS, HGF may promote OPC (reservoir cells of oligodendrocytes) proliferation and migration into demyelinated regions and modulate remyelination. Further, HGF modulates immune cell functions, such as tolerization of DCs. This figure is a modified version of Fig. (1) of [95].
Overexpression of HGF using HVJ envelope vector in the brain enhanced the neurite extension, increased synapses and prevented gliosis and glial scar formation, which inhibits neuronal survival or regeneration in the peri-infarct region of the neocortex [64].

The molecular mechanisms by which HGF prevents neuronal cell death are the inhibition of apoptosis through upregulation of the production of anti-apoptotic Bcl-2 [48, 49] and Bcl-xL proteins, stimulation of increased extracellular signal-regulated kinase phosphorylation [50, 54], and blockage of Bax translocation from the cytoplasm to the nucleus [53]. Furthermore, HGF also plays a role in the prevention of primary oxidative DNA damage after ischemia via an independent pathway to the caspase. In brief, HGF prevents primary oxidative DNA damage after ischemia by attenuating the activation of poly(ADP-ribose) polymerase (PARP) and the expression of p53, which prevents an increase in the expression of apoptosis-inducing factor protein (AIF) in the nucleus [51]. AIF has been shown to act as a main molecular effector of caspase-independent apoptosis-like program cell death via mitochondrial release and nuclear translocation, which leads to characteristic non-nucleosomal secondary DNA damage (DNA fragmentation) [55, 56]. Furthermore, recent findings reveal that HGF modulates not only apoptosis but also autophagy after transient middle cerebral artery occlusion in rats [57]. This evidence suggests that HGF exerts neuroprotective effects against brain ischemic neuronal cell death by preventing caspase-dependent and -independent cell death signals.

6.1.2. Reduction of Infarction Volume

The infarction volume was significantly reduced by the administration of rhHGF into the ventricle, by Spongel, or gene transfer of HGF with HVJ envelop vector into the cerebrospinal fluid via the cisterna magna or transplantation of bone marrow stromal cells (MSC) with expression of the HGF gene by ex vivo gene transfer using HSV-1 [47-49, 57-59].

6.1.2. Prevention of Disruption of the Blood-Brain Barrier (BBB)

Treatment with rhHGF attenuated the disruption of the BBB after microsphere embolism-induced sustained cerebral ischemia [60]. The molecular mechanisms by which HGF prevents the disruption of the BBB are as follows: 1) prevention of the apoptotic endothelial cell death through attenuating the decrease in the level of Bcl-2 [60]; and 2) a decrease in the expression of the tight-junction proteins, occludin and zonula occludens (ZO)-1 in cerebrovascular endothelial cells [61]. Therefore, HGF-mediated prevention of endothelial cell injury and maintenance of tight junctional proteins in endothelial cells may be a possible mechanism for the protective effect of HGF against the disruption of the BBB after ischemia.

6.1.3. Prevention of Ischemic Cerebral Edema

Treatment with rhHGF suppressed the microsphere embolism (ME)-induced increase in tissue water. Although an ME-induced increase in water content was associated with increases in tissue Na⁺ and Ca²⁺ content and decreases in tissue K⁺ and Mg²⁺ content, HGF suppressed the increases in Na⁺ and Ca²⁺ content, but not the decreases in K⁺ and Mg²⁺ content [62].

6.1.4. Prevention of Dysfunction of Learning and Memory

Administration of rhHGF into the ventricle prevented learning and memory dysfunction in the water maze task by protecting against injury to the endothelial cells [58, 63]. rhHGF and gene transfer of HGF using HVJ envelope vector significantly improved learning and memory after cerebral ischemia [64]. Therefore, HGF promotes the recovery of cognitive function in the chronic stage of ischemia through reconstitution of the neuronal network.

6.2. Neurodegenerative Diseases

Neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral sclerosis are characterized by neuronal degeneration, resulting in severe nervous system dysfunction. Although several hypotheses have been proposed, the actual pathogenic mechanisms that lead to selective neuronal degeneration are largely unknown, and no effective treatment is currently available to arrest or reverse neuronal cell death and to improve the functional recovery at each stage of neurodegenerative diseases. Molecules with neurotrophic activity have long been thought of as beneficial agents for the treatment of neurodegenerative diseases, not only because of their survival-promoting activity, but also because of their neurite-promoting activity, which may assist in the reorganization of neural networks [62]. The role of HGF in neurodegenerative diseases is summarized in Table 4.

6.2.1. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is increasingly considered to be a disorder of multiple etiologies, which have in common the progressive degeneration of both upper and lower motor neurons and their axons, accompanied by intense gliosis in lesioned areas of the brainstem, motor cortex, and spinal cord. These ultimately give rise to a relentless loss of muscle function, such as spasticity, hyper-reflexia, generalized weakness of the limbs, and muscle atrophy and paralysis [65]. However, the actual pathogenic mechanisms that lead to the selective motor neuronal degeneration are largely unknown. Riluzole is a currently available drug that prolongs survival by several months. Riluzole protects against motor neuronal injury by inhibiting glutamate release, inactivating voltage-dependent sodium channels and interfering with intracellular events that follow transmitter binding at excitatory amino acid receptors [66]. However, no other effective treatment against ALS is currently available [67], and a large worldwide effort and challenges are in progress to find new therapeutic intervention(s).

The beneficial effects of HGF on motor neurons in vitro and in vivo have been reported. For example, HGF promotes survival of embryonic spinal motor neurons in vitro and in vivo [68-70]. Recombinant HGF application and adenoviral gene transfer of HGF prevent the death of injured adult motoneurons after peripheral nerve avulsion, such as hypoglos sal, facial and spinal nerves in vivo [71-73]. Consistent with these findings, beneficial effects of HGF application have been shown in animal models of ALS. For example, overexpression of HGF in the nervous system has attenuated
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spinal and brainstem (facial and hypoglossal) motor neuronal cell death and prolonged the lifespan in a mouse model of ALS in which mutated Cu/Zn superoxide dismutase 1 was overexpressed [21, 25]. Continuous intrathecal administration of rhHGF attenuates motor neuron degeneration, improves motor performance and has prolonged survival even with treatment beginning around the onset of paralysis [74]. The molecular mechanisms by which HGF attenuates neuronal cell death are as follows: 1) inhibition of apoptosis by preventing the induction of pro-apoptotic proteins, including caspase-1, -3 and -9; and 2) enhancement of the induction of inhibitors of apoptosis family proteins, including X chromosome-linked inhibition of apoptosis protein (XIAP), in the motor neurons of ALS model mice [21, 25, 74]. In addition, HGF retained the levels of the glial-specific glutamate transporter (excitatory amino acid transporter 2/glutamate transporter 1) in reactive astrocytes, thereby reducing the glutamate neurotoxicity of motor neurons [25, 74], and reduced both microgliosis (accumulation of activated microglia) and astrocytosis in ALS model mice [21, 25]. Gliosis in the vicinity of degenerating motor neurons may contribute to ALS disease progression, raising the possibility that gliosis might be a good target for curative efforts. The regulation of the HGF-c-Met system in the spinal cords of sporadic and familial patients with ALS is very similar to that in the transgenic mouse model of ALS, which overexpresses mutant SOD1(G93A) [26]. Thus, HGF is an endogenous neurotrophic/ neuroregenerative factor that is regulated in ALS, and supplementation of HGF exerts neuroprotective effects by acting on motor neurons and glial cells, which might be valuable for ALS therapy for both familial and sporadic patients. The direct role of HGF in microglia/macrophage in the disease progression of ALS is the next issue to be studied, since c-Met is expressed in the subpopulation of microglia/macrophage in ALS model mice and in primary culture (Ohya-Shimada and Funakoshi, unpublished results). Previous in vitro studies demonstrated that phosphorylation status of the serine residue at position 985 of c-Met is associated with decreased HGF-induced phosphorylation of the tyrosine residue and its phosphorylation state is regulated in vitro through protein phosphatase 2A (PP2A), a serine/threonine protein phosphatase, and protein kinase C (PKC), a serine/threonine protein kinase [75, 76]. Furthermore, reciprocal phosphorylation of serine and tyrosine residue(s) of c-Met is shown in motor neurons of a transgenic mouse model of amyotrophic lateral sclerosis (ALS), suggesting the potential contribution of reciprocal phosphorylation in vivo [77]. Such reciprocal regulation of the phosphorylation states of serine and tyrosine residues of c-Met supports the notion that HGF functions more efficiently when animals are affected by mutant SOD1(G93A) (under ALS-stress) and this mechanism might be beneficial in avoiding the non-essential activation of c-Met in non-ALS mice in the presence of a certain amount of HGF, suggesting that HGF could be a safe and effective therapeutic agent for the treatment of ALS and related disorders [77].

6.2.2. Alzheimer’s Disease

Alzheimer’s disease (AD) is a progressive, age-related dementia of unknown etiology characterized by neuronal degeneration, synaptic loss and cholinergic deficits, leading to cognitive, behavioral and psychological impairment. Histologically, AD neurodegeneration is distinguished by neuropathological changes and deposits of misfolded proteins, mainly consisting of amyloid β (Aβ) and hyperphosphorylated Tau in neurofibrillary tangles in the form of senile plaques and deposits in the neocortex, hippocampus and cerebral blood vessels [78]. Current drugs improve symptoms, but have no profound disease-modifying effects [79].

Beneficial effects of HGF on AD therapy have been reported. Takeuchi et al. have reported that overexpression of human HGF plasmid DNA injected into the cerebral ventricle improved Aβ-induced memory impairment in Aβ(1-40)-injected mice by increasing HGF protein in the injection site and around the cerebral ventricles [80]. This effect is associated with significant recovery of vessel density in the dentate gyrus of the hippocampus, recovery of blood flow, upregulation of brain-derived neurotrophic factor (BDNF) (which enhances long-term potentiation (LTP) in the hippocampus [81]), a significant decrease in oxidative stress, and synaptic enhancement in mice treated with Aβ [80]. In vitro studies have shown that HGF increased the expression of synaptic proteins including the NR2B subunit of the NMDA receptor, calcium/calcmodulin-dependent protein kinase II, and the GluR1 subunit of the AMPA receptor [82], rapidly enhanced induction of synaptic transmission and synaptic LTP in the CA1 region of the hippocampus, and augmented NMDA receptor-mediated currents [83]. Furthermore, HGF attenuated the decreased synaptic localization of NR2B and PSD-95 [33]. HGF increased the number of puncta of PSD-95 in young hippocampal neurons [32]. Since functional localization of the NMDA receptor at synapses depends on PSD-95 [84], HGF might modulate the synaptic expression of the NMDA receptor by regulating the localization of PSD-95, leading to the modification of cognitive impairment in AD.

6.2.3. Parkinson’s Disease

Parkinson’s disease (PD) is characterized by dopaminergic striatal insufficiency, secondary to a progressive loss of dopaminergic neurons in the substantia nigra and intracellular inclusions called Lewy bodies. At present, available therapies, such as treatment of levodopa combined with carbidopa, aim to replace dopamine in the brain to restore motor function [85]. These drugs provide dramatic relief from the symptoms, but do not seem to stop or reverse the progression of PD.

Neurotrophic effects of HGF in primary midbrain dopaminergic neurons and beneficial effects of HGF on PD therapy have been reported [31, 86]. Koike et al. reported that gene transfer of human HGF plasmid DNA by direct injection into the rat striatum inhibited dopaminergic neuronal cell death in 6-hydroxydopamine (OHDA)-induced PD model rats by increasing HGF protein in the injection site [86]. 6-OHDA is a neurotoxin used to selectively degenerate dopaminergic neurons and induce Parkinsonism in laboratory animals [87]. Furthermore, HGF significantly inhibited amphetamine-induced abnormal rotation in rats [86]. These results demonstrate that HGF inhibits the loss of dopaminergic neurons in the substantia nigra in a rat PD model, providing a potential novel therapy for PD.
6.3. Injuries of the Nervous Systems

6.3.1. Spinal Cord Injury

Spinal cord injuries (SCI) are classified as partial or complete, depending on how much of the cord width is damaged. In general, injuries that are higher in the spinal cord produce more paralysis. For example, a spinal cord injury at the neck level may cause paralysis in both arms and legs and make it impossible to breathe without a respirator, while a lower injury may affect only your legs and lower parts of the body. Currently, there are no effective treatments, and the mechanisms underlying these neuropathological changes are not completely understood. Methylprednisolone is the presently available drug, and it appears to reduce the damage to neuronal cells if it is given within the first 8 hours after injury. SCI is followed by secondary degeneration, which is characterized by progressive tissue necrosis. Several experimental approaches have been employed to minimize tissue damage and to enhance axonal growth and regeneration throughout the lesion epicenter after SCI. Although several neurotrophic factors that augment axonal pathfinding and neuronal survival improve the neurological deficit after spinal cord injury, angiogenesis after SCI is also a critical factor in the endogenous regenerative response to trauma [88]. Since HGF is widely known as a potent neurotrophic and angiogenic factor, it might be more effective than other neurotrophic factors for treatment of spinal cord injury. Kitamura et al. demonstrated that the application of exogenous HGF genes into the injured spinal cord, using a replication-incompetent herpes simplex virus-1 vector, prevented motor neuronal loss by attenuating caspase-3 activation after SCI, thereby promoting the survival of motor neurons and reducing the size of the damaged area [27]. HGF promoted the survival of oligodendrocytes, as well as neurons, by reducing caspase-3 activation after SCI, resulting in a significant reduction in the area of demyelination after SCI [27]. Taken together, the antiapoptotic and neurotrophic effects of HGF on the neurons and oligodendrocytes contributed to a significant reduction in the area of parenchymal damage after SCI. Furthermore, the introduction of HGF into the injured spinal cord increased the total area of endothelial cells and the number of vessels with abnormally large lumina after SCI [27]. HGF appears to exert significant neuroprotective and antiapoptotic effects that promote the survival of neurons and oligodendrocytes, and also enhances angiogenesis around the lesion epicenter after SCI. These effects significantly reduced the area of damage and provided a better scaffold for axonal regeneration.

6.3.2. Peripheral Nerve Injury and Neuropathy

Peripheral nerve avulsion is an injury caused when the nerve root is severed or cut from the spinal cord, causing marked motor neuronal degeneration. The therapeutic effects of HGF on motor neurons in vitro and in vivo have been reported, as described in the “Amyotrophic Lateral Sclerosis” section. In animal models of peripheral nerve avulsion (adult motor neuron injury), Okura et al. have demonstrated that application of rat recombinant HGF protein prevents the rapid decrease of choline acetyltransferase (ChAT) mRNA and protein after axotomy [71]. In addition, Hayashi et al. have reported that the treatment of an adenoviral vector encoding HGF after facial nerve and spinal root avulsion significantly improved the survival of injured facial and spinal motor neurons and ameliorated ChAT immunoreactivity in these neurons [72]. These lines of evidence suggest that HGF may be a potential neuroprotective agent against motor neuron injury in adult humans.

6.4. Neuroimmune Disease

Immune-mediated diseases of the CNS, for example multiple sclerosis (MS), is a devastating autoimmune disease that affects more than 1 million people worldwide and severely compromises motor and sensory function through demyelination and axonal loss. MS and its animal model, experimental autoimmune encephalomyelitis (EAE), are characterized by the activation of antigen-presenting cells and the infiltration of autoreactive lymphocytes within the CNS, leading to demyelination, axonal damage, and neurological deficits. Because HGF shows immunomodulatory effects and neuroprotective effects, the effects of HGF on animals of EAE were examined, using transgenic mice over-expressing rat HGF in a neuron-specific manner. The protein produced was secreted into extracellular space in the nervous system and functioned in an autocrine, as well as a paracrine, fashion (HGF-Tg mice [25]) [35]. EAE induced either by immunization with myelin oligodendrocyte glycoprotein peptide or by adoptive transfer of T cells was inhibited in HGF-Tg mice. Notably, the level of inflammatory cells infiltrating the CNS decreased, except for CD25(+)Foxp3(+) regulatory T (T(reg)) cells, which increased. A strong T-helper cell type 2 cytokine bias was observed: IFN-gamma and IL-12p70 decreased in the spinal cord of HGF-Tg mice, whereas IL-4 and IL-10 increased. Antigen-specific response assays showed that HGF was a potent immunomodulatory factor that inhibited dendritic cell (DC) function and the differentiation of IL-10-producing T(reg) cells, decreased in IL-17-producing T cells, and down-regulated surface markers of T-cell activation. These effects were fully reversed when DC were pretreated with anti-cMet (HGF receptor) antibodies. These results suggest that, by combining both potentially neuroprotective and immunomodulatory effects, HGF is a promising candidate for the development of new treatments for immune-mediated demyelinating diseases associated with neurodegeneration such as multiple sclerosis.

Given that c-Met is expressed and phosphorylated in both OPCs and oligodendrocytes [12], and that HGF promotes proliferation of OPC [12] and promotes its chemotaxis [89], and that HGF attenuates activation of caspase-3 in oligodendrocytes in the animal model of spinal cord injury [27], it would be intriguing to examine whether HGF suppresses the degeneration of oligodendrocytes in neuroimmune disease model(s), and whether HGF promotes the proliferation of oligodendrocyte precursor cells (OPCs) to increase the numbers of reserve cells for oligodendrocytes, chemotaxis of OPCs and subsequent remyelination [34]. Considering that HGF attenuated gliosis, including astrocytosis and microgliosis in the animal model of ALS [21, 25] and that c-Met is expressed in reactive astrocytes [21, 25] and in the subpopulation of microglia in primary culture and in the animal model of ALS, the role of the HGF-c-Met system in other types of cells including astrocytes, microglia and macrophages is another interesting issue for future study.
6.5. Other Diseases

In addition to these animal models, therapeutic roles of HGF have been shown in other neuronal and neuroimmune diseases, such as hydrocephalus, seizures, retinal and hearing impairment, and neuropathic pain (Table 4) [90-94].

7. CONCLUSIONS

Many studies have demonstrated that HGF is a powerful neurotrophic factor with a great deal of demonstrative activity that is beneficial in various animal disease models in the nervous system (Table 4). These findings suggest the possibility of therapeutic application of HGF in neurological, neuroimmunological, and psychiatric diseases. Clinical trials of recombinant HGF protein in subjects with ALS and spinal cord injury have been designed and planned as collaborative projects among Osaka University, Asahikawa Medical University, Tohoku University and Keio University with the support of the Japanese Ministry of Health Labor and Welfare.

ACKNOWLEDGEMENTS

We are grateful to Ms. Kanai for the help with figure preparation and Ms. Ikushima for secretarial assistance. This work was supported by Research Grants from the Japanese Ministry of Health Labor and Welfare to H.F. and by a grant-in-aid from the Global Centers of Excellence (COE) program of the Ministry of Education, Science, and Culture of Japan to Osaka University.

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Hepatocyte Growth Factor (HGF): Neurotrophic Functions


