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***In Vivo* Administration of Cerebrospinal Fluid of Patients with Multiple Sclerosis; A New Model to Study Adult Neurogenesis in Different Stages of Disease**

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Abstract

Multiple sclerosis (MS) is an autoimmune and demyelinating disease, affecting the central nervous system causing a wide spectrum of signs and symptoms. In adult mammalian brain, neural progenitor cells (NPCs) are placed in the subventricular zone of ventricles, subgranular zone of the dentate gyrus and give rise to new cells. In addition, alterations in adult neurogenesis are implicated in psychiatric disease in humans. Currently, there is no satisfactory evidence to describe how neurogenesis changes during pathogenesis of MS disease. It can be because of the lack of suitable animal model that represents pathogenesis of different stages of MS disease. Given that, cerebrospinal fluid (CSF) as a mirror can reflect major part of pathological conditions of disease *in vivo* administration of CSF derived from patients in to the brain ventricles of mice can lead to an experimental animal model for studying neurogenesis and other pathophysiology of multiple sclerosis. This strategy will help researchers to unravel the mechanisms in which neurogenesis changes in different stages of MS disease.

Keywords: Multiple sclerosis; Cerebrospinal fluid; Adult neurogenesis**Abbreviations:** MS: Multiple Sclerosis; CNS: Central Nervous System; R-R MS-Relapsing: Remitting Multiple Sclerosis; PMS: Progressive Multiple Sclerosis; SVZ: Subventricular Zone; SGZ: Subgranular Zone; DG: Dentate Gyrus; NPC: Neural Progenitor Cells; GCL: Granular Cell Layer; TNF α : Tumor Necrosis Factor Alpha; IFN γ : Interferon Gamma; BrdU: Bromodeoxyuridine; GFAP: Glial Fibrillary Acidic Protein; NGF: Nerve Growth Factor

periods of remission and exacerbation, referred to as RRMS [1,2].

While patients typically return to near normal neurologic function at the end of each episode, over time, failure of the CNS to regenerate MS lesions can lead to an invariable progression of clinical disability (PMS) [3]. In addition to motor and sensory deficits, cognitive reduction is found in up to 65% of patients with MS [4]. Apart from impaired information processing and working memory performance, deficiency in spatial memory is generally reported [5]. The hippocampus is of crucial importance to spatial memory formation, so part of the observed memory deficit in MS [6] is likely to be caused by damage to this tissue. It has been shown that the inflammation-induced deficits in cognitive performance may be related to the reduction of neurogenesis, especially when the inflammatory reaction continues firmly for extended periods of time [7]. In the adult mammalian brain, neurogenesis under physiological conditions occurs in the subventricular zone (SVZ) of lateral ventricles and in the subgranular zone (SGZ) of dentate gyrus (DG) of hippocampus [8]. Neural progenitor cells (NPCs) located in the SGZ of the DG give rise to thousands of new cells every day. The majority of these new cells migrates up into the granule cell layer (GCL) and differentiates into granule cells. Over time, these new granule cells are inserted into the functional hippocampal circuitry through the formation of specific granule-cell afferent and efferent synaptic contacts and interfere in spatial memory establishment [9]. There is an intrinsic neurogenesis throughout life in the human hippocampus, and only a modest reduction in neurogenesis occurs in aging. Moreover, there is also a preferential loss of adult-born neurons and a larger ratio of hippocampal neurons are replaced with newborn neurons in humans relative to the mice [10,11]. The vast majority of hippocampal neurons are included to exchange in humans in compare to mice. Furthermore, adult hippocampal neurogenesis displays a much less reduction with aging in humans relative to mice [12]. These interesting studies emphasize that the crucial role of adult neurogenesis in physiology of human brain relative to other rodents. Thus, understanding the molecular mechanisms that trigger neurogenesis modifications in human neurological disorders have piqued the curiosity of many.

Background

Multiple sclerosis (MS) is an inflammatory, autoimmune disease of the central nervous system (CNS), which is commonly diagnosed in the prime of life. In most cases results in chronic disability. MS mostly begins by a series of alternative

The complete molecular mechanisms that control NPC proliferation and differentiation under neuroinflammation are largely unclear. Under pathological conditions of the CNS associated with neuroinflammation, inflammatory cytokines and chemokines can also affect the proliferative potential of NPCs and modify neurogenesis [13]. The impact of inflammation associated with MS in hippocampal neurogenesis is still unknown [14]. There is now a body of evidence that indicate an important role for CSF in brain physiology and development. CSF contains cytokines and growth factors secreted by the choroid plexuses and the sub-commissural organ [15]. From the lateral ventricles CSF moves on into the third ventricle and then the cerebral aqueduct (of Sylvius) to the fourth ventricle the fourth ventricle. It departs the ventricular system and enters the subarachnoid spaces [16]. It has been shown that components carried in the CSF not only circulate quickly through the CSF pathways but also have quick availability to most regions of the brain [17]. CSF acts as a signaling pathway for physiological control systems because it has been shown to contain molecules such as corticotrophin releasing factor, adrenocorticotropin, leptin, interleukins and growth factors. Moreover, concentrations of these components change with the physiological activity of the animal, pathogenesis of neurological disorders and also during different stages of the embryonic development [18].

It has been represented that spatial gradients of CSF proteins play an active role to guide the distinct cell populations, including the migration of neuroblasts from the subventricular zone (SVZ) to the olfactory bulb in the adult brain. Therefore, CSF distributed factors have essential effects in many aspect of normal adult brain activity [19]. In addition, CSF alone can promote the development and growth of neural stem cells and cortical explants [20]. Regarding to the point that CSF components have fast access to all of brain regions and a large number of different CSF components are changed during pathogenesis and different stages of disease, neurogenesis modifications can caused by summation of all effects of these components, such as growth factors, inflammatory cytokines and metabolic mediators. Different kinds of proinflammatory cytokines are upregulated in MS and it is very interesting that different proinflammatory cytokines may have different roles in neurogenesis. For instance, TNF can inhibit proliferation of NPCs [21] and IFN gamma and NGF are important in self renewal of precursor cells in neurogenic zone [22,23].

Hypothesis presentation and testing

Given that, a vast majority of CSF components in which change during pathogenesis of multiple sclerosis can affect directly or indirectly on adult neurogenesis, CSF can be considered as a mirror that reflects major parts of pathological condition of disease. Moreover, tissue specimens from patients with MS are not generally accessible, and post-mortem pathology weakly displays the biological events connected to ongoing pathogenesis. By contrast, CSF is acquirable and can be investigated all over the course of the disease. Considering that, no single existing animal model can translate every aspect of MS contributes to our incomplete

understanding of pathological mechanisms that lead to impairment of adult neurogenesis. We hypothesized that intracerebroventricular injection of spinal fluid derived from patients with MS patients into mice can lead to modifications of neurogenesis in adult mice. In this experimental model of MS, human CSF acts as a pathology initiating factor. Knowing that, CSF components in different phases of MS disease are changed, thus the effects of R-R MS CSF in neurogenesis of adult mice would be different relative to Progressive MS CSF. Moreover, we can also investigate whether patient treatment might effect on regulation of neurogenesis. To this aim, CSF from treatment responders (for instance, with intrathecal methotrexate or natalizumab) can be applied instead untreated ones. These data suggest whether the factors involved in neurogenesis can be favorably altered with treatment chosen. Obtained CSF should be injected to the ventricles of mice with mini-pompe for one month. The injection protocol of the cell cycle marker bromodeoxyuridine (BrdU) and the immunohistochemical techniques can reveal the number of proliferating cells in neurogenic niches. In addition, double-label fluorescence staining for BrdU/glia fibrillary acidic protein (GFAP), BrdU/double cortin, BrdU/NeuN and BrdU/Cleaved Caspase 3 on the one hand can show the level of gliogenesis, neurogenesis and cell death [24,25] of precursor cells and on the other hand it can display the destination of newborn neurons. Regarding that in inflammatory disorders, such as MS, neurogenesis and gliogenesis considered as part of self-repair process and endogenous NPCs participate to repair the damaged CNS, they may become the target of the disease itself [26]. By means of viral vector, green fluorescent protein (GFP) can be selectively deposited into newborn cells. Therefore, it is possible to track GFP positive cells and perform electrophysiological tests on them to observe their progress into fully mature neurons.

Recently it has been demonstrated that spatial memory impairs in patients with MS [27,28]. Adult neurogenesis is essential for formation of spatial memory [29]. Morris water maze as a test of spatial memory in rodents can be used to show the connection between neurogenesis alteration and spatial memory impairments. Moreover, a bunch of evidence has demonstrated that depression is common in patients with multiple sclerosis [30-32]. Snyder JS and his colleagues have shown that adult hippocampal neurogenesis buffers depressive behavior [33]. Elongated immobility in the forced swim test (FST), long latency to eat in the novelty-suppressed feeding (NSF) test, and reduction of sucrose preference in the sucrose preference test (SPT) are the most commonly used rodent behavioral tests of depressive behavior [33]. Application of these behavioral tests can indicate the relation between neurogenesis modifications and depression in different stages of MS disease.

Implications of the hypothesis

Neurogenesis is critical for maintaining the normal physiology of brain. Impairing or altered neurogenesis has been associated with various neuropsychiatric disorders in mice models [34].

In same manner, it has been demonstrated that age-associated cognitive decline is in line with decrement of newborn granule cells numbers in the DG [35]. It is supposed that remarkable alteration in the integration pattern of newborn cells in the DG may lead to the development of cognitive impairment observed in patients suffering from MS.

Therefore, *in vivo* administration of CSF acquired from R-R and progressive MS patients can give us new insights into the mechanisms that regulate neurogenesis in different phases of MS disease and eventually to target neural stem cells or their progeny to design new effective treatment strategies to improve of cognitive deficits in patients with MS.

Moreover, using the CSF as a therapeutic vehicle to promote CNS homeostasis has many potential benefits. Interestingly, many proteins such as neurotrophic factors have been demonstrated to be delivered to the CSF via intranasal administration [36]. Thus, it seems there is a noninvasive method with therapeutic point of view that provides the possibility of simultaneously administering a cocktail of neurogenic and neuroprotective factors to the CNS for alteration of adult neurogenesis. Taking together, this hypothesis can be considered as one of the most reliable ways to address neurogenesis modifications in different phases of MS.

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