Revitalise hippocampal neurogenesis via increasing BDNF and IGF-1 in aged murine

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Abstract

Adult neurogenesis continuously produces new neurons for integration into the neural circuits throughout adulthood, which has been implicated in cognitive functions. Neurogenesis in the hippocampus is highly regulated by a number of regulators, including BDNF and IGF-1, and their decrease with age is responsible for the decline in neurogenesis. Accumulating studies suggest that age-related decrease in hippocampal neurogenesis could be restored through multiple interventions, covering small molecular drugs, natural compounds and physical exercise. In this review, I present a discussion of current findings to revitalise hippocampal neurogenesis via enhancing BDNF and IGF-1 effects in the brain.

Introduction

Neurogenesis is an endogenous process that involves neural stem cell (NSC) proliferation and differentiation, immature neurons survival and maturation as well as dendrite development [1]. In most mammals, adult neurogenesis occurs in the subventricular zone of the lateral ventricle and in the subgranular zone of the dentate gyrus in the hippocampus [1-2]. Although it has been verified that neurogenesis occurs throughout life [1, 2, 3], its progressive decline with age still causes problems of cognition impairment. In healthy ageing, many studies have confirmed that age-related deficits in learning and memory performance are associated with the reduction of hippocampal neurogenesis [3]. Hippocampus is vulnerable to detrimental effect of ageing and is always the first regions of brain to suffer injury. The decline in hippocampal neurogenesis is notably shown in reduced number of newborn neurons, decreased synaptic plasticity and poor dendrite morphology [2, 3], which together impair brain functioning in old age. In this regard, hippocampus has become one of the most important targets of attempts to alleviate aged-related brain damages.

The idea to rejuvenate old bodies became popular since the anti-aging effect was reported in heterochronic parabiosis, which is joining two animals of different ages. Aged mice sharing circulatory systems with young mice gained improvements in multiple tissue regeneration, including neurogenesis [4]. This suggested that neurogenesis could be promoted by endogenous factors exist or have higher concentration in the young organisms, compared to aged ones. Adult hippocampal neurogenesis is highly regulated by diverse neurotrophic growth factors, among which brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1) are investigated most within the past two years [2,5]. The factors upregulate adult neurogenesis, stimulate neural stem cell differentiation, and subserve newborn neurons survival and maturation [5, 6]. Reductions in the expression levels of BDNF and IGF-1 have been observed during ageing and could account for the age-related hippocampal neurogenesis attenuation [5, 3]. Accordingly, BDNF and IGF-1 are potential targets in therapeutic applications to revitalise hippocampal neurogenesis in old age. Recent researches proposed multiple strategies to reverse age-associated cognition decline via increasing the effects of BDNF and IGF-1.

Improve neurogenesis via increasing BDNF

BDNF promotes hippocampal neurogenesis through specific binding and activation of its receptor, tropomyosin-related kinase receptor B (TrkB) [7]. It has been reported that increasing BDNF level improves dendritic complexity during hippocampal neurogenesis [8] and subserves neural circuit formation and brain computation [9]. However, the limited delivery, short half-life [10] and poor permeability trough brain-blood barrier (BBB) [11] of BDNF impede its therapeutic potential, resulting in disappointed performance of direct use of BDNF in clinical trials. Hence, people are looking for strategies to induce BDNF production*in vivo*and for the past few decades, antidepressant treatment has been studied widely [12]. In recent years, more researches focus on alternatives to accelerate BDNF-induced neurogenesis.

In 2010, 7, 8-dihydroxyflavone (DHF) was first identified as a TrkB agonist that has potent neurotropic activities [10,11]. DHF imitates the behaviour of BDNF and activates downstream signaling pathways upon DHF-TrkB binding, which improves neurogenesis and dendrite development in adult mice after brain injury [10, 11, 13]. Compared to administration of BDNF, DHF has higher affinity for TrkB and is more permeable through BBB, making it a widely used TrkB agonist since its discovery. Lately, Wang *et al.*demonstrated the beneficial effects of DHF on promoting newborn neuron dendritic development in aged mice [11]. Administration of 5mg/kg DHF for 2 weeks significantly increased the average dendritic length per branch and also dramatically reduced the percentage of newborn neurons with short total dendritic length [11].Alternatively, Guilloux *et al.*discovered that S38093, a H3 receptor antagonist, also improved dendritic morphology in aged mice hippocampus, possibly through releasing growth factors including BDNF [14]. Administration of 3mg/kg S38093 not only increased the dendritic length but also contributed to a significant increase in the number of newborn neurons with tertiary dendrites [14]. In addition, S38093 dramatically increased cell proliferation, survival and maturation in the hippocampus, which eventually improved context discrimination performance in aged mice. Guilloux *et al* also observed an improvement in BDNF transcripts level in aged mice treated with S38093 [14]. Previous studies suggested that blockage of H3receptor with S38093 facilitated glutamate release [15] and glutamate signaling triggered BDNF expression in the hippocampus [16]. Therefore Guilloux *et al.*speculated that S38093 revitalised hippocampal neurogenesis in aged mice via releasing growth factors like BDNF. S38093 also showed similar effect on mouse module of Alzheimer's' disease [14].

In addition to small molecules, other studies are using natural compounds to restore hippocampal neurogenesis in healthy ageing. Tomato ethanolic extracts (TEE) has been previously reported as a natural neuroprotective agent that reverses neurogenesis impairments in Parkinson's disease [17-19]. Recent work by Bae and colleagues further confirmed its cognitive-enhancing effect in healthy ageing. TEE treated aged-mice had shown reversal of memory impairment, increase in newborn neurons and synaptic plasticity. This restoration of hippocampal neurogenesis was attributed to enhancement of BDNF and reduction of corticosterone after oral TEE supplement to aged mice [19]. Corticosterone has been shown to down-regulate BDNF expression [20], suppress synaptic plasticity [21] and induce adult neurogenesis decline as well as cognitive impairment in rodents [22], which indicated that inhibition of corticosterone levels by TEE also involved in BDNF promotion and cognition restoration.

BDNF binds with TrkB and accomplishes neurogenesis improvement via activation of downstream signaling pathways, mainly involves withτηε πηοσπηολιπασε ̃γ (ΠΛ̃γ),phosphatidyl inositol-3 kinase (PI3K) and extracellular signal- regulated kinase (ERK) cascades [11, 12]. These pathways all eventually induce activation and phosphorylation of cAMP response element-binding protein (CREB), the transcription factor mediating expression of genes essential for survival and development of neurons during hippocampal neurogenesis [12]. For example, higher levels of ERK and CREB phosphorylation has been detected in aged mice treated with TEE feeding [19], which demonstrated the important role of ERK/CREB pathway in promoting neurogenesis and cognition.

Improve neurogenesis via increasing IGF-1

Similar to BDNF, IGF-1 is a restorative factor that facilitates hippocampal neurogenesis and has been used as a promising therapeutic molecule to revitalise cognitive abilities in aged animals [5]. Researches in the past focused on using IGF-1 peptides to overcome cognitive deficits in aged brain. Administration of IGF-1 peptides via intracerebroventricular (ICV) infusion [23] or osmotic minipumps implants [24] has successfully increased hippocampal neurogenesis and ameliorated age-related cognitive decline. By contrast, current studies are now paying more attention to the application of genes.

Pardo *et al.*exemplified the potential of gene therapy via introducing adenovectors expressing IGF-1 into aged mice through delivery of ependymalroute. This treatment distinctly raised IGF-1 levels in the cerebrospinal fluid (CSF), which was paralleled by the pronounced increase of hippocampal neurogenesis as well as improvement of spatial memory accuracy [25]. Importantly, for the first time, this study reported the positive effect of *in vivo*administration of IGF-1 on restoring astrocyte branching in aged rats [25], which could also contribute to memory improvement. Furthermore, specific introduction of IGF-1 gene to NSCs of dentate gyrus was sufficient to mitigate cognitive decline in aged mice [26]. These studies demonstrate that overexpression of IGF-1 *in vivo*has beneficial effect and could be considered as a therapeutic strategies to deal with ageing. In addition to gene therapy, administration of antiepileptic drugs, such as levetiracetam[27] and aripiprazole [28], has been found as promising drugs to rescue cognitive deficits. Previous researches reported that rufinamide(RUF), an antiepileptic drug, had therapeutic potential recovering functional and behavioral damage caused by diabetic neuropathy [29]. Chen *et al.*first investigated the beneficial effect of RUF regarding hippocampal neurogenesis in their latest research [30]. Treatment with 3mg/kg RUF for 4 weeks in aged gerbils led to considerable increase in neurogenesis, newborn cell proliferation and maturation, with memory and learning abilities simultaneously improved. Finally, elevated levels of IGF-1, its receptor IGF-1R and CREB phosphorylation after RUF treatment together accounted for the mechanism of RUF. It is concluded that RUF improved neurogenesis in the hippocampus through stimulating IGF-1 signaling pathways [30].

Nevertheless, the dualistic nature of IGF-1 is a big challenge of applying IGF-1 treatment to clinical trials [31]. Recent studies proposed that in humans, optimal cognitive functioning required an optimum level of IGF-1 and both low and high levels of IGF-1 may impair cognition [32, 33]. Chigogora*et al.* discovered that both low and high levels of IGF-1 might be associated with higher risk of depression in humans [33]. Moreover, a recent report proposed pro-epileptic effect of IGF-1 and showed that long-term IGF-1 exacerbated epileptogenesis *in vitro*after brain injury [31]. Further researches are needed to determine the optimum dose of IGF-1 required in human.

The effect of IGF-1 on hippocampal neurogenesis is known to rely on the activation of diverse intracellular regulatory cascades, including ERK and PI3K, following IGF-1 binding to IGF-1R [34]. Besides, overexpression of IGF-1 in aged mice also significantly increased the phosphorylation level ofCa2+- calmodulin-dependent kinase II (CaMKII) [26], a downstream protein of PLCγ pathway [12]. Taken together these studies indicate that IGF-1 exerts regulatory control over hippocampal neurogenesis sharing similar signals (PLCγ, PI3K and ERK) with BDNF pathways (Figure 1).

Exercise increases neurogenesis via combined effects of BDNF and IGF-1

Physical exercise has always been one of the most promising methods aimed at brain wellbeing improvement, due to its distinct neuroprotective benefits. It has been illustrated that running rescues brain functions by boosting the expression levels of neurotrophic factors, including BDNF and IGF-1 [35,36]. Significantly, depletion of BDNF or IGF-1 through their specific antagonist eliminated exercise-induced improvements in cognition and hippocampus [36-38], showing that BDNF and IGF-1 are essential for exercise to promote neurogenesis in the hippocampus. Recently, treadmill running has been reveled to enhance neurogenesis, notably cell proliferation and neuroblast differentiation, in aged gerbil hippocampus following ischemic shock [39]. Later its effect on healthy ageing was confirmed in old Wister rats [40]. The study demonstrated that restoration of memory by treadmill exercise was correlated with enhanced expression of BDNF and

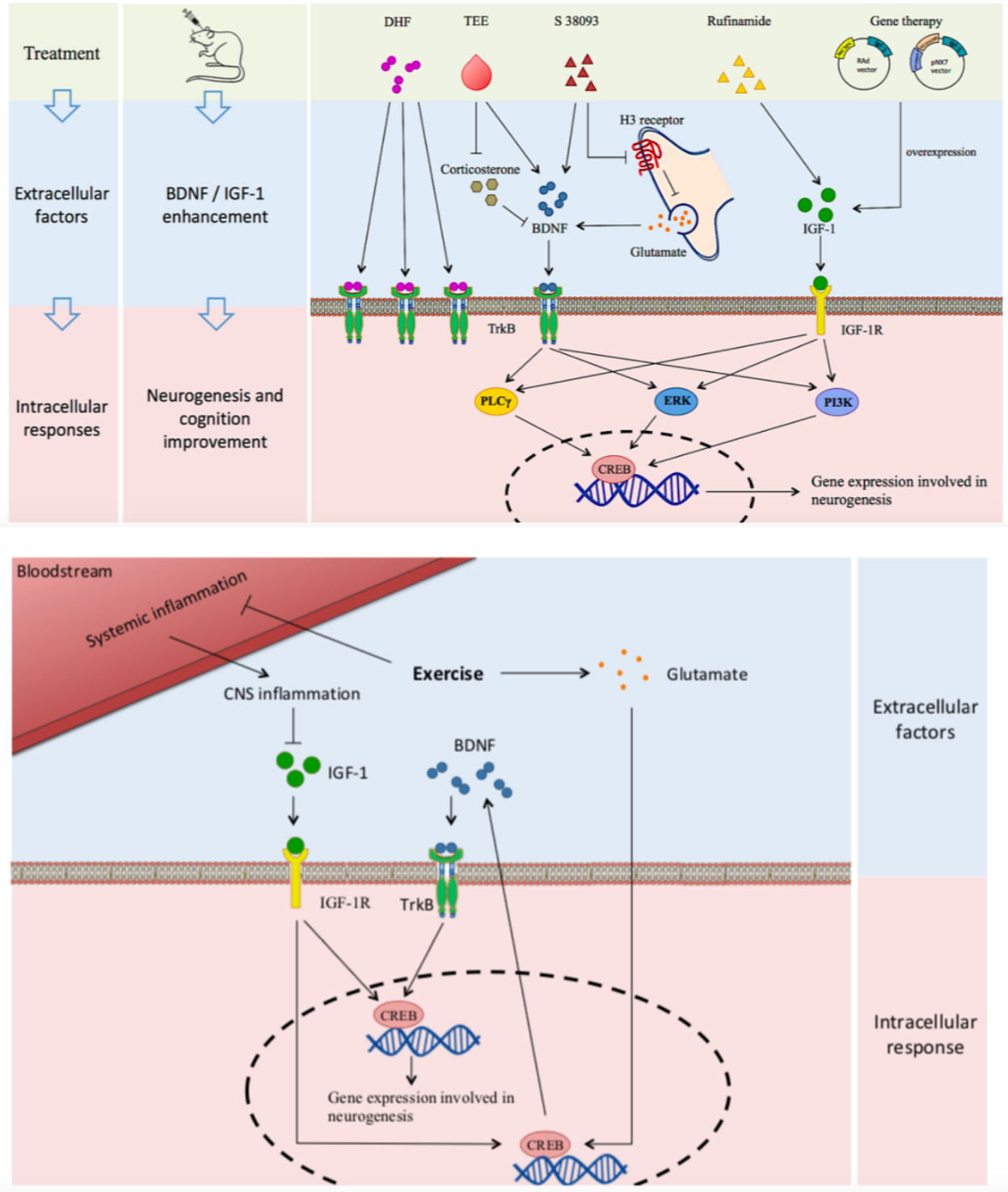
IGF-1 [40]. Recent studies suggested that IGF-1 interfaces with BDNF to regulate exercise-dependent cognitive improvement [36]. IGF-1-induced protein synthesis is inhibited by central nervous system (CNS) inflammation [41], which is known to increase with age [42]. Chronic aerobic exercise, such as jogging, has been reported to reduce systematic inflammation that contributes to local inflammation in the brain [37]. Accordingly, chronic aerobic exercise recovers age-inhibited IGF-1 through reducing inflammation, which conduces to higher IGF-1 levels in the bloodstream and therefore increases neuronal IGF-1 uptake [37]. Neurons uptaking IGF-1 increase their CREB-mediated BDNF expression [38] and together contribute to neurogenesis enhancement. The effect of IGF-1 on BDNF expression during exercise was confirmed when intra-carotid injection of IGF-1 resulted in similar enhancement in BDNF expression to exercise [38]. Taken together these studies, Stimpson *et al.*proposed a model for the mechanism of exercise-induced cognitive improvement, in which IGF-1 increases BDNF expression, mediated by CREB [37]. Moreover, glutamate also involves in the upregulation of BDNF in response to exercise. It is well acknowledged that glutamate signaling triggers BDNF expression [16] and many studies indicate that exercise increase glutamate release [43]. In brief, physical activity exerts its beneficial effects on hippocampal neurogenesis and brain functioning through increasing IGF-1 and BDNF (Figure 2).

Conclusion

Neurogenesis is ongoing in adult hippocampus but dramatically decrease with age. As a result, with age the brain is more susceptible to neurodegenerative diseases such as Alzheimer's disease. In recent years, extensive studies have reported potential approaches that can improve hippocampal neurogenesis and alleviate age-related cognitive decline, through increasing BDNF and IGF-1 (Table 1). However, since most of findings are discovered in rodent models, their effects on humans remain unclear due to the inabilities to access live samples. Recent progress in organoid, such as a 3D brain structure developed from human pluripotent cells [44], provides promising methods to test drugs in a human brain microenvironment [45]. By contrast, exercise is more easily applicable in humans and along with the recent advances in non-invasive imaging technology like MRI and PET that measure neurogenesis in human [2], transferring exercise-related researches into human trials would be possible. Furthermore, as interaction between BDNF and IGF-1 has been identified in the mechanism of exercise-induced cognitive amelioration, it is reasonable to speculate that interaction effects of these two factors also exist in other mechanisms affecting neurogenesis. Further studies are needed to explore the comprehensive relationship between BDNF and IGF-1. In conclusion, advances in technology development allow further studies to understand the age-associated neurogenesis in human hippocampus and confirm potential strategies to restore the decline. And, BDNF and IGF-1 are important targets to discover novel alternatives that intervene brain-ageing process.

Figure 1. Hippocampal neurogenesis in aged mice can be improved through direct or indirect stimulation of BDNF and IGF-1 pathways *in vivo*. BDNF and IGF-1 binds to the extracellular domains of their receptors, TrkB and IGF-1R respectively, which activate three major intracellular cascades of signaling pathways: PLCγ, ERK and PI3K. These pathways ultimately lead to the activation of CREB, a transcription factor mediating gene expression essential for neurogenesis. Other kinases involved in these pathways are not listed in the figure.

Figure 2. Physical activities enhance hippocampal neurogenesis through interactions between IGF-1 and BDNF. Systematic and CNS inflammation is inhibited following chronic aerobic exercise, reversing the inhibition of IGF-1 signaling and subsequently increases neural IGF-1 uptake. Besides, glutamate release also increases in response to exercise. Under the regulation of CREB, BDNF expression in hippocampal



neurons uptaking IGF-1 or glutamate increases. Exercise-induced alternations in hippocampus eventually lead to neurogenesis improvement and are shown in cognitive benefits. Other kinases involved in the pathways are not listed in the figure.

Table 1

Recently studied methods to improve neurogenesis in aged murine

Administration	Dose	Species age	Factors		proliferation		regeneration	Dendritic morphology
			BDNF	IGF-1	$Ki67^+$	$BrdU^+$	DCX^+	
S 38093 oral treatment	1/3mg/kg – 28 days	15-month–old mice	↑	-	↑	↑	↑	↑
DHF injection	5mg/kg– 14 days	12-month-old mice	↑	/	/	/	-	↑
TEE oral feeding	400mg/kg – 42 days	12-month-old mice	↑	/	/	/	↑	↑
RUF injection	3mg/kg – 28 days	24-month-old gerbils	-	↑	↑	↑	↑	↑
Treadmill exercise	28 days	22-24-month-old mice	↑	↑	/	↑	↑	↑
	Vector / promoter							
IGF-1 cDNA introduction	Adenovector / mCMV	28-month-old SD rats	/	↑	/	/	↑	↑
	pcDNA3.1 / nestin gene intron	18-month-old mice	/	↑	/	↑	/	/

Table 1. The table summarizes some recent studies on restoring neurogenesis in aged murine, via regulation of BDNF and/or IGF-1. Ki67 and BrdU are proliferative markers of adult neurogenesis. DCX is an immature neuron marker that can be used to identify newborn neurons or neuroblast. Dendritic morphology was indicated by dendritic complexity, length and branching in the above studies. '—'indicates a significant increase in the corresponding index and '-'indicates no significant differences are observed. '/' refers to non-tested index in that particular research.

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