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Role of TGF-β1 in the Behavior Disorders

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Abstract. Transforming growth factor 1 (TGF-β1) is an anti-inflammatory cytokine that is expressed in different regions of the mammalian brain, and at all developmental ages. This cytokine can modulate neuron differentiation and survival, and also participate in the tissular response to injury. Based on clinical evidence, different approaches have been used to study the role of TGF-β1 on modulating brain function and behavior. Here, we review evidence showing a role of TGF-β1 in circadian rhythms, locomotion, sociability and depression-related behaviors. For these behaviors, suprachiamatic, hippocampal and cerebellar expression of TGF-β1 have been manipulated. Further studies are required to extend these results to other brain regions and different behaviors, but so far evidence points to a role of TGF-β1 on behavior disorders such as schizophrenia, depression and autism.

Keywords: Neurogenesis, sociability, depression-related behavior, locomotion, autism

TRANSFORMING GROWTH FACTOR BETA 1 (TGF-β1)

Transforming growth factor betas (TGF-βs) are a large family of proteins that regulate the growth of many organs [1]. Although they were originally discovered as a factor (TGF-β1) capable of inducing transformation in rat kidney and fibroblast cell lines [2, 3], we now know that they guide embryonic stem cells through differentiation, and that TGF-β signaling also controls the expression of a variety of homeostatic genes, which in turn regulate cell proliferation, extracellular matrix production, cell-cell attachment, immune function and tissue repair.

Three isoforms of TGF-β have been identified in mammalian tissue [4, 5]. TGF-β regulation of gene transcription involves two types of receptors (types I and II) that phosphorylate Smad2 and Smad3 transcription factors, which then associate with Smad4 and translocate to the nucleus. Smads bind to specific sequences in the DNA, activating the transcription of target genes [6]. TGF-β1 and TGF-β2 bind to the same receptor, TGF-βRII, and share their biological activities [7].

TGF-βs are expressed in diverse tissues both during development and in adulthood, e.g. mesenchyme, connective tissue, bone, endothelium, platelets, immune cells (reviewed in [1, 8]).

TGF-β1 IN THE CENTRAL NERVOUS SYSTEM

The TGF-β family not only plays an important role in the development and function of the immune system, but also affects central nervous system development (reviewed in [9]). TGF-β2 and 3 are expressed as early as E15 in the embryonic brain [10], mainly in regions where neuronal differentiation is occurring, and they are excluded from regions of active proliferation such as the ventricular zone [10]. Moreover, these molecules are expressed in the midbrain and they are required for survival of dopaminergic neurons [11–13] and evidence from TGF-β1 knockout mice also shows that this molecule is involved in neuronal survival [14]. However, other reports showed that, during brain development, TGF-β2 and 3 inhibit neuronal survival,

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whereas TGF-β1 does not have this effect [10], and that TGF-β inhibits the proliferation of cultured cerebellar neurons [15]. So, the effect of TGF-β expression on brain development can be diverse, depending on the specific developmental age and the neuronal type.

TGF-β2 and 3 are widely expressed also in the adult rodent brain, in particular they were detected in cerebral cortex, hippocampus, striatum, cerebellum and brainstem [15, 16]. Both astrocytes and neurons are immunoreactive for TGF-β2 and 3 in these regions [17]. Conversely, different studies show that, as it was observed during development, TGF-β1 is not expressed by neurons or glial cells in the adult brain, but circumscribed to meninges and choroid plexus [16]. However, more recent reports found expression of TGF-β1 in the adult brain, in cortical pyramidal neurons [18] and in proliferative regions such as the dentate gyrus of the hippocampus [19].

TGF-β receptors are also expressed in different regions of the brain. Types I and II receptors were detected in neurons and astrocytes in cerebral cortex, midbrain, hippocampus, brain stem and cerebellum [1, 18, 20]. So, both the expression of TGF-βs and their receptors strongly points to a role of this cytokine in brain function.

TGF-β1 IN BRAIN FUNCTION

The initial attempts to elucidate the effects of TGF-β1 expression in the brain focused on the response to degeneration and tissular damage, or to the effect of overexpressing this molecule in the central nervous system. In the injured brain, TGF-β1 is actively secreted by astrocytes and it contributes to scar formation [21]. This goes in line with the role of this cytokine in scarring in peripheral tissues. However, overexpression of TGF-β1 in astrocytes results in brain inflammation and neurodegeneration in transgenic mice [22–24], thus suggesting that the effect of TGF-β1 on astrocytic function depends on the developmental age and/or levels of expression.

In addition, the parenchyma response to an inflammatory stimulus (i.e. IL-1β) involves TGF-β1 in the striatum and the substantia nigra, but it is expressed in basal levels in IL-1β-injected hippocampus and cortex [25], thus suggesting that the effect of TGF-β1 expression is also dependent on the brain structure considered.

However, the basal expression of TGF-β1 in the adult rodent brain puts forward the hypothesis that this cytokine should modulate neuronal and/or glial physiology, and not only act upon and injury or external stimulus.

ROLE OF TGF-β1 IN BEHAVIOR

Despite the wide evidence of TGF-β1 expression in the developing and adult brain, the role of TGF-β in the modulation of behavior has only started to be evaluated. Some clinical studies proposed a contribution of TGF-β1 on behavioral symptoms, which were then tested in animal models. For example, the hippocampal TGF-β1 signaling pathway was reported as altered in psychiatric disorders characterized by profound alteration of social behavior, such as autism, schizophrenia and bipolar disorder [26–28]. Moreover, manipulation of TGF-β signaling has been proposed as a possible treatment for anxiety and depression [29–32]. Others and we generated animal models to test the role of TGF-β1 in modulating these behaviors.

For example, the expression of TGF-β in the suprachiasmatic nucleus of the mouse follows a circadian pattern [33] and they regulate the expression of clock genes [34]. Thus, the increase of this cytokine in the cerebrospinal fluid of Alzheimer's disease patients have been suggested as a possible cause for alterations in sleep [34].

Hippocampal TGF-β1 modulates the response to spatial novelty and pre-pulse inhibition of the startle reflex, PPI [35]. Forebrain-specific conditional Smad4 knockout mice showed increased locomotion in a novel environment (open field), although they showed no differences in the exploration of a novel object or the avoidance of the center of the open field. This increased response to novelty was further confirmed in a new home cage. Finally, these mice showed impaired PPI. As Smad4 KO mice show abnormal GABAergic transmission in the CA1, TGF-β1 was proposed to alter response to novelty and PPI by altering the excitatory-inhibitory balance in the hippocampus. It was proposed that this could be a potential mechanism for behavioral symptoms observed in schizophrenic patients.

In rats, we showed that prenatal maternal inflammatory stimuli have long-term consequences on the TGF-β1 expression levels in the hippocampus [19]. Interestingly, this decrease in hippocampal TGF-β1 correlates with reduced neurogenesis and reduced response to novelty. Using an adenoviral vector, we then proved that reestablishing TGF-β1 expression in the dentate gyrus of these rats could revert both neurogenesis and novel object recognition. Indeed, TGF-β1 is pro-neurogenic in adult neuronal stem cells via its canonic pathway, Smad2/3 [36]. Whether the effect of TGF-β1 on novel object recognition was through modulation of neurogensis or directly affecting neuronal function, remains to be investigated. Indeed, previous

reports showed that overexpressing TGF-β1 in astrocytes results in reduced neurogenesis in the dentate gyrus in aged mice, showing an effect of TGF-β1 on neurogenesis opposed to what we had observed [37].

To further test the effect on behavior of TGF-β1 expression in the adult brain, we used an adenoviral vector expressing this molecule (AdTGF-β1) and we injected it in different regions of the mouse brain. We evaluated behavior 14 days after injection. When injected in the adult hippocampus, TGF-β1 overexpression resulted in increased sociability, reduced self-grooming and reduced depression-related behaviors [38]. Interestingly, when we inoculated AdTGF-β1 in the postnatal hippocampus -at postnatal day 14- and we evaluated behavior in adulthood, we observed the opposite effect: reduced social interaction, increased repetitive behaviors and increased depression related behaviors. These results prompted us to conclude that the effect of TGF-β1 expression in the hippocampus depends on the developmental age when it occurs. Different mechanisms can be proposed for these effects. For example, inhibition of TGF-β receptors inhibits serotonergic differentiation of ES cells [39] suggesting a developmental mechanism leading to behavioral abnormalities later in life. In addition, we observed less Reelin-positive cells in the dentate gyrus and reduced expression of the synaptic protein neuroligin-3. These results support a role of TGF-β1 in autism, as was previously suggested by clinical studies [27, 28, 32].

To evaluate whether TGF-β1 can have a role in modulating other behaviors, we injected AdTGF-β1 in the adult cerebellum. Behavior was evaluated as previously described [38]. We did not find differences in open field behavior (Fig. 1A-B) or T-maze alternation (unpublished data), suggesting that locomotion is not altered and that these mice have normal anxiety-related behaviors. Although both experimental groups spent more time in the social compartment when a strange mouse was present, AdTGF-β1 mice spent a significantly larger amount of time in that compartment than Adβgal mice (Fig. 1C). This suggests that cerebellar

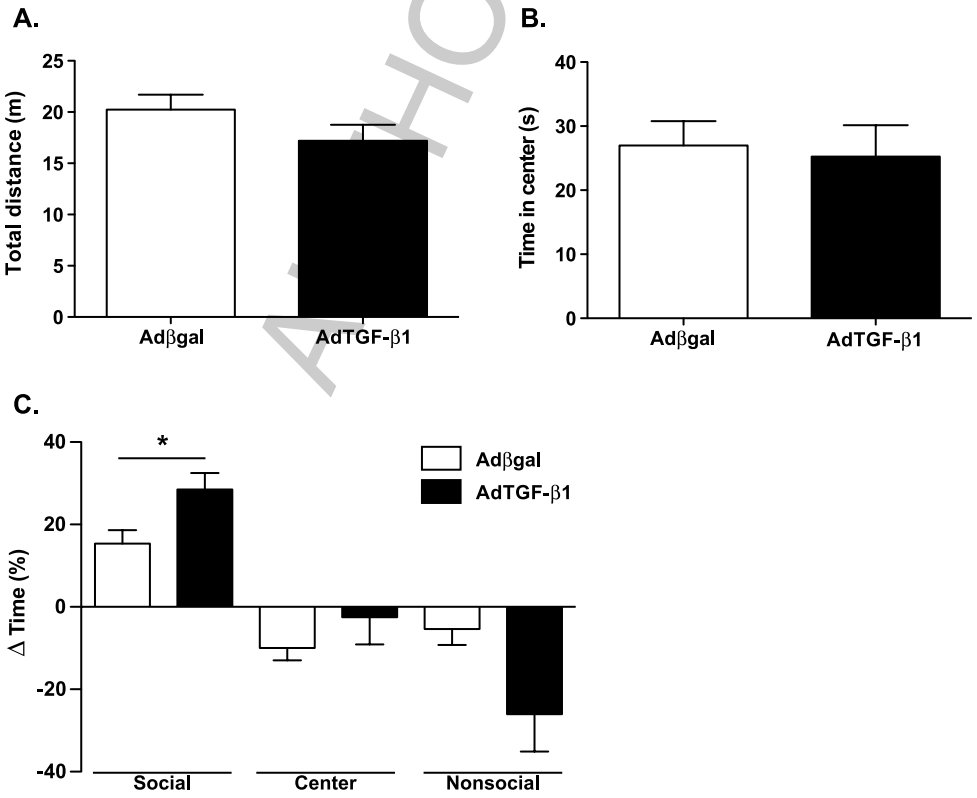

Fig. 1. Overexpression of AdTGF-β1 in the lobule 7 of the adult cerebellum results in increased sociability in mice. Mice injected with AdTGF-β1 showed similar levels of locomotion (A) and avoidance of the center of an open field (B) than control mice injected with Adβgal. AdTGF-β1 mice spent a higher percentage of time in the social compartment than Adβgal mice, in the social interaction test (C). Mice were injected at 8 weeks of age with adenoviral vectors and tested two (open field) or three (social interaction) weeks after surgery. $^{*}p < 0.05$, Student's t test. $N = 8$-9 per group.

TGF-β1 can modulate social behavior is a similar manner as hippocampal TGF-β1.

CONCLUSIONS

Only recently a role of TGF-β1 in modulating behavior has been described. Considering the spread expression of this molecule and other TGF-βs in the developing brain, TGF-βs could have a relevant role in normal and pathological behavior. Clinical evidence also supports a role of TGF-β1 in psychiatric and neurological disorders. The elucidation of the role and effects of modulating TGF-β1 levels in the brain at specific developmental ages could help advancing efficient therapies for these disorders.

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CONFLICT OF INTEREST

There is no conflict of interest.

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