TGF-β1 factor in the cerebrovascular diseases of Alzheimer’s disease

X. ZHANG, W.-J. HUANG, W.-W. CHEN

The Third Department of Neurology, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing, China

Abstract. - Transforming growth factor betas (TGF-βs) belong to three isoforms (TGF-β1, TGF-β2 and TGF-β3) members of a large pleiotropic superfamily of around 100 distinct proteins participating in the regulation of key events of development and disease, and tissue repair. In the central nervous system (CNS), all the three isoforms are produced by both glial and neuronal cells and are involved in essential tissue functions such as cell-cycle control, regulation of early development and differentiation, neuronal survival and astrocytes differentiation. Recent findings have shown abnormally increase of the levels of TGF-β1 in the brain of patients suffering Alzheimer’s disease (AD), an elderly pathology reaching individuals over 65-years-old which present well-known hallmarks, including cerebrovascular deficiency, abnormal deposition of amyloid beta (Aβ), cholinergic denervation, neuroinflammation, neurofibrillary tangles and progressive loss of memory. However, related to the pathological features of AD, the brain overexpression of TGF-β1 was associated with neuroinflammation, accumulation of extracellular matrix compounds and cerebrovascular stiffness, neuronal apoptosis along with the development of vascular hypertrophy. Consistent with these observations, transgenic mice model (TGF mice) overexpressing constitutively TGF-β1 fully mimicked AD-like cerebrovascular pathology. Taken altogether, these data suggest the involvement of TGF-β1 in the pathogenesis of AD, particularly in the cerebrovascular pathology which is of interest in the present review that will discuss the contribution of TGF-β1 in the cerebrovascular physiopathology of AD.

Key Words: TGF-β1, Cerebrovascular deficits, Neuroinflammation, Alzheimer’s disease.

Introduction

Discovered in the early 1980s for its transforming properties1-2, transforming growth factor betas (TGF-βs) soon appeared as pleiotropic cytokines orchestrating many critical physiological processes including embryogenesis, immune response, extracellular matrix metabolism as well as cell circle. Indeed, TGF-βs belong to a subfamily of a large family of more than 40 structurally related regulatory protein expressed in mammals among which are included bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), Mullerian inhibitory factor (MIF), activins and inhibins3,4 (Figure 1). TGF-βs subfamily express by human are three genetically distinct isoforms (TGF-β1, β2 and β3) with high homology, located on chromosomes 19q13, 1q41 and 14q24, respectively5. TGF-βs play pleiotropic roles in the growth organs and systems6 including, cell-cycle control, differentiation, regulation of early development, extracellular matrix formation, angiogenesis, hematopoiesis and immune functions7.

Brain TGF-β1 may have neuroprotective functions, but under some conditions8,9, thereby preventing neuronal apoptosis through the inhibition of caspase-310 mitochondrial membrane potential and increases the expression of the antiapoptotic protein, Bcl-2 and Bcl-xL11. As well, TGF-β1 protects neurons against damage induced by excitotoxins, hypoxia/ischemia, deprivation of trophic factors, and aggregates of amyloid beta (Aβ)12-14, a distinct feature of Alzheimer’s disease (AD) which is a neurodegenerative disease affecting mostly individual over 65 year old, characterized by cerebrovascular deficiency, abnormally deposition and aggregation of amyloid beta (Aβ), cholinergic denervation, neuroinflammation, neurofibrillary tangles and the progressive loss of memory15.

In line with the hallmarks of AD, recent findings have shown the increased of the levels of TGF-β1 inside of the brain and cerebral vessels tissues of patients suffering Alzheimer disease (AD)16,17. These increases of TGF-β1 were likely associated with the development of neuroinflammation, vascular hypertrophy, fibrosis, and the accumulation of extracellular matrix components18-22 (Figure 2).
Accordingly, transgenic mice overexpressing a constitutive form of TGF-β1 (TGF mice) in astrocytes exhibited AD-like neuroinflammation and cerebrovascular dysfunction. Consequently, more to suggest the involvement of TGF-β1 in AD pathogenesis, these findings revealed a fundamental novel role and distinct mechanisms through which cerebral blood vessels and brain perfusion can be endangered. In this review, we will focus our attention on the involvement of TGF-β1 in the cerebrovascular disease physiopathology of AD.

**Presence and Role of Transforming Growth Factor Beta (TGF-β) in the Mammalian Brain**

In the central nervous system, (CNS) of animals and humans, from early embryonic stages, all three isoforms are expressed (mRNA and protein) by both glial and neuronal cells. However, TGF-β2 and -β3, but not TGF-β1, and their respective receptors are expressed in early embryonic structures where they play essential role in the induction of midbrain dopaminergic neurons survival, acting in concert with other molecules such as Fibroblast Growth Factor (FGF2, FGF8) and glial cell line-derived neurotrophic factor (GDNF)42. TGF-β2 and -β3 are also localized in radial glial cells, neuronal cell bodies in the telencephalic cortex and cerebellum, suggesting a role in the regulation of neuronal migration and differentiation as well as glial cell proliferation and differentiation. In addition, the expression of TGF-β2 and -β3 persists in the entire adult CNS areas including cortex, hippocampus, striatum, brainstem and cerebellum.

However, immunoreactive mRNA of TGF-β1 was observed in white matter astrocytes as well as neurons within the hippocampal pyramidal neuron, dentate gyrus granule cells, large cortical neurons within layer II, III and V, and a sub-population of cerebellar Purkinje cells. Although the distribution of TGF-β1 in the brain is controversial and restricted to meninges and choroid plexus in the intact brain and upon lesion, it is induced in neurons, astrocytes and microglia. In vivo role of TGF-β1 in the unlesioned brain is supported by in vitro studies in neurons and astrocytes in culture. Indeed, TGF-β1 has been widely considered as an injury-related cytokine which is a crucial regulator of nervous system physiology and its vasculature. It has been reported a reduction of cell-cell-contacts and increase focal contacts, thus inducing astrocytes motility. However, TGF1-β1 production and release increase significantly in response to CNS lesions, with astrocytes and microglia as the major sources of TGF-β1 in the injured brain. Indeed, an increase of TGF-β activation can be observed in response to injury and subsequent extracellular matrix perturbation. The TGF-β activity is primarily regulated through the conversion of latent TGF-β to an active form by a variations.
iety of molecules, from protons to proteases, and once released from its latent secreted complex, TGF-β isoforms elicits their cell type-specific responses through the ligand-induced formation of heteromeric receptor complex between the serine/threonine kinase receptors. The subsequent functional complex of TGF-β family receptors at the cell surface consists of the activin-like kinase 5 (ALK5)/TGF-β type I (TβRI) receptor and the TGF-β type II (TβRII) subunits with the latter having serine/threonine kinase domain. The consequently activated type I receptor phosphorylates selected Smads, and these receptor-activated Smads (R-Smads) then form a complex with Smad 4. Activated Smad complexes translocate into the nucleus where they regulate the expression of various target genes involving cell proliferation (Figure 3). However, the TGF-β/Smad signaling cascade can be regulated at many levels. For example, inhibitory-Smads, namely Smad 6 and Smad 7, counteract the signaling of R-Smads through diverse mechanisms. Particularly, Smad 7 binds to activated type I receptors, thus inhibiting the phosphorylation and the following nuclear translocation of R-Smads. The same way, Smad 7 can also target TGF-β receptors to the ubiquitin degradation pathway with the following inhibition of TGF-β/Smad signaling cascade. Accordingly, the increase of Smad 7 protein could oppose the cell-cycle control operate by TGF-β or other protein or pathways. In addition to Smad-mediated gene transcription, TGF-β can activate Smad-independent pathways including the extracellular-regulated kinase (ERK) pathway, the nuclear factor κB (NF-κB) pathways, and the PI-3-K-serine-threonine protein kinase (AKT) pathway. TGF-β/Smad-independent pathways have a key role in mediating various biological effects of TGF-β, such as cell-cycle inhibition, epithelial-to-mesenchymal transdifferentiation, immune suppression, and neuroprotective effects.

However, in AD, impairment of TGF-β-activated Smad signaling has been demonstrated, particularly, an ectopic localization of phosphorylated Smad2/3 has been detected in the cytoplasm.
of hippocampal neurons, within Aβ plaques\textsuperscript{48}. One of the specific features arriving in the earlier course of AD, but not in other neurodegenerative conditions, is the reduction of neuronal expression of TβRII\textsuperscript{49}. This suggests that a dysfunction of TGF-β signaling is probably a causal factor in AD progression. Accordingly, neuronal expression of the kinase deficient TβRII in AD transgenic mice promotes both Aβ deposition and dendritic loss\textsuperscript{49}. In turn, Aβ might decrease the expression of TβRII through the induction of the micro RNA-106β\textsuperscript{50}. In vitro studies have demonstrated that TGF-β1 prevents Aβ-challenged cortical neurons from entering the S-phase of the cell cycle\textsuperscript{13}. The cell-cycle inhibition is one of the mechanisms by which TGF-β1exerts its neuroprotective effects against Aβ toxicity\textsuperscript{13} or preventing Aβ-inducing τ hyperphosphorylation\textsuperscript{51} through a mechanism involving PI-3K- promoted inhibition of the τ-phosphorylating enzyme GSK-3β\textsuperscript{13}. Another important mechanism is the capacity of TGF-β1 to synergize with neurotrophins which required for full neuroprotective activity of brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF)\textsuperscript{52-53}. In AD, TGF-β1 reduces the chemotactic migration of microglial cells toward Aβ aggregates through Smad-dependent pathways to prevent microglia-mediated neuroinflammation\textsuperscript{54,55}. The anti-inflammatory effects are seemingly mediated by the inhibition of the NADPH oxidase (PHOX) subunit P47phox from cytosol to the membrane in microglia\textsuperscript{9}. However, in animal models of AD, TGF-β1 has been found to block the TGF-β/Smad 2/3 signaling in peripheral macrophages and to reduce cerebrovascular Aβ deposits in Tg2576 mice, while mice overexpressing TGF-β1 develop AD-like vascular alterations, and the overexpression of TGF-β1 in AD transgenic mice seems to accelerate the deposition of Aβ in cerebral blood vessels also termed cerebral amyloid angiopathy (CAA)\textsuperscript{56}. These data suggest that vessel-derived TGF-β1 might contribute to inflammatory processes in AD brain\textsuperscript{16,17}.

**Involvement of TGF-β1 in the Cerebrovascular Pathology of Alzheimer’s disease**

The deregulation of TGF-β1 signaling pathways are involved in several pathological conditions\textsuperscript{53,58}, including Alzheimer’s disease (AD)\textsuperscript{54}. Indeed, in AD brain, cytokines, chemokines, and free radicals emanating from activated glial cells recruited at the site of plaques may promote an inflammatory, oxidative state and chronic overproduction of TGF-β1may promote microvascular degener-
The overexpressed TGF-β1 inside of the brain and cerebral vessels tissues of Alzheimer disease (AD) patients is associated with the release of pro-inflammatory cytokines IL-1β and TNF-α that induce inflammatory cycle in astroglial cells and neuronal apoptosis along with the development of vascular hypertrophy, fibrosis, and the accumulation of extracellular matrix components. Likewise in the injured central nervous system (CNS), astrocytes which appear as a key component of reactive gliosis characterized the response of the nervous system to many kinds of lesions such as ischemia, excitotoxic lesion and several neurodegenerative diseases and particularly AD. Similar to ischemia, excitotoxic lesion and several neurodegenerative diseases such as AD and PD are characterized by an increase in the expression of transforming growth factor beta (TGF-β1), a key mediator of fibrogenesis in a variety of tissues and has been shown to induce expression of components of the extracellular matrix (ECM) and other genes that act to regulate the composition of the ECM. The extensive deposition of collagen and other ECM components that are characteristic of fibrosis is thought to be the result of deregulation of fibrogenesis, a normal part of the wound healing response that occurs in almost all tissues after exposure to a destructive stimulus. TGF-β1 stimulates also the production of laminin and fibronectin and their incorporation into the ECM of primary culture of cerebellar astrocytes. The result of such fibrosis in the brain is an inability of the cerebral blood vessel to maintain autoregulation of cerebral blood flow and therefore predisposes to ischemia leading to localized ischemic areas of necrosis. Studies of fibrosis in a variety of tissue have established that collagen-secreting cells may also derive from resident epithelial cells by way of a mechanism termed epithelial-mesenchymal transition which is involved in inducing the formation of fibroblasts after injury. However, under the influence of combinations of cytokines, including TGF-β1, epithelial cells begin to secrete matrix metalloproteinase that degrades the extracellular matrix.

Conclusions

Data suggest well-established dysfunction of TGF-β1 signaling in AD. However, cerebrovascular pathology appears as the earlier marker of AD and likely a most important contributor to cerebrovascular and cognitive deterioration, even if TGF mice displayed unaffected memory. Indeed, TGF-β1 is associated with the release of pro-inflammatory cytokines IL-1β and TNF-α, and induces initiation and propagation of destructive inflammatory cycle in astroglial cells and neuronal apoptosis along with the development of vascular hypertrophy, fibrosis, and the accumulation of extracellular matrix components. Thus, targeting TGF-β1 to prevent cerebrovascular pathology or neuroinflammation may constitute an interesting avenue for AD therapeutic.

Conflict of interest

The authors declare no conflicts of interest.

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