

● PERSPECTIVE

Tenascin-C in aneurysmal subarachnoid hemorrhage: deleterious or protective?

Subarachnoid hemorrhage (SAH) caused by the rupture of a cerebral aneurysm is a well-known devastating cerebrovascular disease. Post-SAH brain is vulnerable, associated with early brain injury (EBI; Suzuki, 2015). The first step for intensive care of aneurysmal SAH patients is aneurysmal obliteration to prevent rebleeding as well as further aggravation of EBI (Suzuki, 2015). The subsequent treatment requires intensive medical care to manage the associated problems including hydrocephalus, cerebral vasospasm and delayed cerebral ischemia (DCI). Despite improvements in the clinical management of SAH, DCI remains one of the most important causes of morbidity and mortality in SAH patients who survive the initial bleeding. Recently, EBI as well as cerebral vasospasm is considered to be a cause of DCI (Suzuki, 2015). However, the pathogenesis of EBI, cerebral vasospasm and DCI remains unclear, precluding the development of new therapies against them.

Many molecules may be involved acting simultaneously or at different stages during post-SAH brain injury *via* multiple independent or interconnected signaling pathways. Tenascin-C (TNC) is a representative of matricellular proteins that are a class of inducible, non-structural, secreted and multifunctional extracellular matrix glycoproteins (Midwood and Orend, 2009). TNC is composed of 14 epidermal growth factor-like repeats and a series of fibronectin type III repeats, and forms a typical disulfide-linked hexamer, in which six flexible arms emanate from a central globular particle. TNC levels are low in steady-state condition in adult tissues, but are readily and transiently upregulated in pathological conditions such as myocarditis, arteriosclerosis and cancer, or by inflammatory reaction (Midwood and Orend, 2009). TNC directly binds to many cell surface receptors and other matrix proteins, modulating their functions (Midwood and Orend, 2009). However, the biological functions of TNC are highly variable, and often seemingly contradictory, depending on the biological scenario surrounding its induction.

In a clinical setting, TNC levels in the cerebrospinal fluid (CSF) were below the diagnostic threshold level in patients with an unruptured cerebral aneurysm, and markedly increased after the rupture of an aneurysm (Suzuki et al., 2008). Significantly higher CSF TNC levels were observed in patients with worse admission clinical grade, more severe SAH on admission computed tomography, acute obstructive hydrocephalus, subsequent angiographic vasospasm, DCI, chronic shunt-dependent hydrocephalus, and a worse outcome (Suzuki et al., 2011). Clinical findings suggest that more severe SAH or EBI may induce more TNC, which may cause angiographic vasospasm and DCI separately or simultaneously; that is, DCI may occur by severe angiographic vasospasm with more TNC induction, and/or by vasospasm-unrelated causes with TNC induction that are supposed to be EBI (Suzuki et al., 2011; Suzuki, 2015).

Experimental studies also demonstrated that TNC is induced in both cerebral arterial wall (Shiba et al., 2012) and brain parenchyma (Shiba et al., 2014) after experimental SAH by endovascular puncture in rats. TNC induction in the cerebral artery caused cerebral vasospasm, potentially leading to DCI (Shiba et al., 2012). On the other hand, TNC was increased in neuropil mainly in the brain surface of the cerebral cortex irrespective of cerebral vasospasm development at both 24 and 72 hours

after SAH (Shiba et al., 2014). It was considered that astrocytes, neurons and capillary endothelial cells produced TNC. A previous study used imatinib mesylate (a selective inhibitor of the tyrosine kinases of platelet-derived growth factor [PDGF] receptors [PDGFRs]) to block endogenous TNC induction and to examine the effects on brain injury after SAH, because PDGF is a well-known potent inducer of TNC (Shiba et al., 2014). Imatinib mesylate prevented brain PDGFR activation, TNC upregulation and mitogen-activated protein kinase (MAPK) activation, and suppressed neuronal apoptosis and neurological impairments after SAH in rats. In addition, a cisternal injection of recombinant TNC (murine myeloma cell line, NS0-derived, Gly23-Pro625, with a C-terminal 6-His tag) caused cerebral vasospasm, neuronal apoptosis and neurological impairments in imatinib mesylate-treated filament puncture SAH rats (Shiba et al., 2012, 2014). Exogenous TNC activated MAPKs in the cerebral artery, causing cerebral arterial contraction in both healthy rats and imatinib mesylate-treated SAH rats (Suzuki et al., 2016). However, effects of exogenous TNC on brain may be different between healthy rats and SAH rats, because SAH may induce matrix metalloproteinases (MMPs) and serine proteases, which can cleave TNC (Midwood and Orend, 2009; Fujimoto et al., 2015). Cleavage of TNC may release cryptic sites that create adhesive sites for cell surface receptors, activating different signaling and exerting diverse cell responses *via* the receptors (Midwood and Orend, 2009). It is most likely that hexameric, monomeric and protease-cleaved TNC exhibit distinct functions, although the full extent of these functions is not currently clear. In fact, exogenous TNC had no effects on brain in healthy rats (Shiba et al., 2014). However, TNC may have the positive feedback mechanisms on upregulation of TNC itself in an acute phase of SAH, leading to more activation of the signaling transduction and the development or aggravation of cerebral vasospasm or brain injury after SAH. PDGF may induce endogenous TNC, and an exogenous TNC injection also induced endogenous TNC in addition to PDGFR- β upregulation and PDGFR activation in the cerebral artery and brain after SAH (Shiba et al., 2012; Shiba et al., 2014). That is, PDGF-induced TNC may positively feedback on PDGFR activation *via* PDGFR upregulation and crosstalk signaling between receptors as well as upregulation of TNC, leading to more MAPK activation and therefore internally augmenting cerebral vasospasm, neuronal apoptosis and neurological impairments in SAH rats.

On the other hand, TNC has fibrosis-promoting effects, and exerted potent aneurysm repair effects in an aneurysm model in rats, possibly by recruiting macrophages, which secrete cytokines to induce migration and proliferation of smooth muscle cells (Hamada et al., 2014). In this context, TNC is protective, because both arterial rebleeding-related brain injuries and an increase in subarachnoid and intraventricular blood volume, which is closely correlated with the severity of secondary brain injuries (Suzuki, 2015), are precluded. However, the fibrosis-promoting effects of TNC may impair circulation and reabsorption of CSF, causing chronic hydrocephalus that also results in secondary brain injuries after SAH (Suzuki et al., 2008). Taken together, TNC induction may be protective if it is induced in the ruptured cerebral aneurysm wall, while TNC induction may be deleterious if it is induced in post-SAH brain, cerebral artery, subarachnoid space or CSF. To address the issue directly, it is useful to investigate the significance of TNC induction after SAH using TNC-knockout mice.

Recently, we investigated the role of TNC induction in post-SAH brain in terms of blood-brain barrier (BBB) disruption and brain edema formation using TNC-knockout mice (Fujimoto et al., 2015). TNC-knockout mice do not show an

apparent phenotype after gene disruption, however those mice exhibit various phenotypes in response to various insults. Expression of TNC in the brain was weakly detected in wild-type sham mice and was upregulated in wild-type SAH mice. TNC knockout suppressed neurological impairments, decreased brain edema and prevented BBB disruption associated with an inhibition of MMP-9 induction and the consequent preservation of the tight junction protein zona occludens-1. The reaction was also associated with inactivation of MAPKs in brain capillary endothelial cells in SAH brain. In TNC-knockout sham mice, furthermore, exogenous TNC treatment had no effects on neuroscore, brain edema formation and BBB disruption. In contrast, in TNC-knockout SAH mice, exogenous TNC treatment aggravated these findings compared with the vehicle treatment. Our recent preliminary study also showed that TNC-knockout mice had less severe cerebral vasospasm (unpublished data). Notably, TNC knockout did not increase the severity of SAH or subarachnoid blood volume. Thus, the studies demonstrated that TNC is deleterious and plays an important role in the development of DCI in terms of cerebral vasospasm and BBB disruption, which is a key pathological manifestation of EBI after SAH.

The experimental findings as to the role of TNC after SAH have been obtained using the endovascular perforation model in rats or mice as described above. The endovascular perforation model mimics clinical mechanism of artery rupture and shows a high mortality and acute metabolic changes similar to clinical findings, and thus being the most attractive model for studies of EBI after SAH (Suzuki, 2015). However, it is one of limitations that the model has no cerebral aneurysm, which is a major cause of SAH. As far as we know, no studies are reported regarding the role of TNC in the genesis, growth, rupture or subsequent hemostasis of a cerebral aneurysm. In addition, no one knows if the existence of a ruptured aneurysm affects the role of TNC after SAH. Thus, it would be worthwhile studying the role of TNC in intracranial aneurysm animal models. In addition, it is well known that TNC modulates cellular signaling through induction of pro-inflammatory cytokines, which may cause post-SAH brain injury (Midwood and Orend, 2009; Liu et al., 2015). However, the role of inflammatory responses in TNC signalings has been hardly studied in the context of SAH, needing further studies.

It becomes clear that matricellular proteins are key mediators of various pathological conditions. Although the information is still limited, our recent studies strongly support that TNC is deleterious for brain after SAH and involved in post-SAH brain injury at several different levels. It is also interesting to investigate a possibility of the interaction between TNC and other matricellular proteins or other molecules in the extracellular space or inside the cells. For example, the function of osteopontin (OPN), another matricellular protein, seems to be conflicting with that of TNC in the setting of SAH (Suzuki et al., 2016). As to cerebral vasospasm after SAH, OPN activated the protective pathways including MAPK phosphatase-1, an endogenous MAPK inhibitor, *via* binding to L-arginyl-glycyl-L-aspartate-dependent integrins. Although the mechanisms of how OPN antagonizes TNC's effect remain unclear in cerebral vasospasm after SAH, another possibility includes that OPN may inhibit TNC's binding to its receptor competitively, because they share some receptors. It is unknown if the signaling of TNC and OPN in the setting of EBI or BBB disruption is identical with or different from that observed in cerebral vasospasm after

SAH, but it may include MMP-9 as a key player. In addition, PDGF, vascular endothelial growth factor and cytokines may be upstream of TNC or interrelated with TNC in SAH (Shiba et al., 2012, 2014; Liu et al., 2015). Further studies will provide more valuable information that TNC potentially play a key role in the pathophysiology including EBI, cerebral vasospasm and DCI, and can be future therapeutic targets after SAH, leading to the development of new protective and restorative therapies. As post-SAH brain injury has both hemorrhagic and ischemic elements and includes arterial injuries, the information could be applicable to other types of brain injuries and vascular diseases. Matricellular proteins including TNC would be good therapeutic targets in many brain injuries having different causes.

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