The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Review

J Atheroscler Thromb, 2023; 30: 1085-1094. http://doi.org/10.5551/jat.RV22007

Pericyte-Mediated Molecular Mechanisms Underlying Tissue Repair and Functional Recovery after Ischemic Stroke

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There are still many patients suffering from ischemic stroke and related disabilities worldwide. To develop a treatment that promotes functional recovery after acute ischemic stroke, we need to elucidate endogenous tissue repair mechanisms. The concept of a neurovascular unit (NVU) indicates the importance of a complex orchestration of cell–cell interactions and their microenvironment in the physiology and pathophysiology of various central nervous system diseases, particularly ischemic stroke. In this concept, microvascular pericytes play a crucial role in regulating the blood–brain barrier integrity, cerebral blood flow (CBF), and vascular stability. Recent evidence suggests that pericytes are also involved in the tissue repair leading to functional recovery following acute ischemic stroke through the interaction with other cell types constituting the NVU; pericytes may organize CBF recovery, macrophage-mediated clearance of myelin debris, intrainfarct fibrosis, and periinfarct astrogliosis and remyelination. In this review, we will discuss the physiological and pathophysiological functions of pericytes, their involvement in the molecular mechanisms underlying tissue repair and functional recovery after ischemic stroke, and a therapeutic strategy to promote endogenous regeneration.

Key words: **Acute ischemic stroke, Pericytes, Blood–brain barrier, Extracellular matrix, Functional recovery**

1. Introduction

Ischemic stroke is a leading cause of mortality and disability worldwide. It is characterized by the obstruction of blood flow to the brain, which leads to brain damage and loss of neurological function. Recanalization therapy, such as intravenous thrombolysis and endovascular thrombectomy, in the acute phase of ischemic stroke has made remarkable progress. However, the number of patients who can benefit from it is limited, and there have been no established treatments except rehabilitation that bring functional recovery. Therefore, our urgent task is to elucidate the endogenous mechanisms promoting functional recovery and develop novel treatments to enhance recovery during the subacute phase.

Over the past decades, many neuroprotective agents, such as NMDA antagonists[1], AMPA receptor antagonists[2], and antiICAM1 antibodies[3], have been tried in clinical trials but have failed to show their efficacy. One of the causes of the failure may be that only neuroprotective effects targeting the inhibition of excitotoxicity and apoptosis have been investigated. Then, the concept of a neurovascular unit (NVU) gradually permeated, and the progress of research focused on this has increasingly suggested the importance of a complex orchestration of cell–cell interactions and their microenvironment to repair the damaged tissue and promote functional recovery[4, 5]. One of the cell types that has gained attention in the NVU is pericytes. Pericytes are perivascular mural cells that wrap around the endothelial cells of small vessels. They play a crucial role in regulating the blood–brain barrier (BBB) integrity, cerebral blood flow (CBF), and vascular stability. In addition, pericytes have emerged as key players not only in the pathophysiology of various central nervous system (CNS) diseases[6] but also in the repair process after ischemic stroke through the interaction among cell types in the NVU. In this review, we will discuss the

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Received: May 19, 2023 Accepted for publication: May 19, 2023

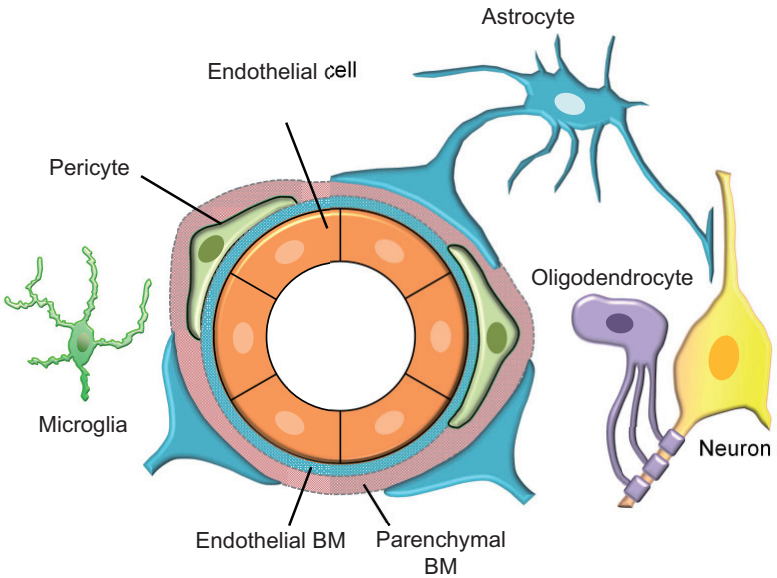

Fig. 1. Schematic diagram showing the NVU

The NVU is a concept in which neurons, cerebrovascular endothelial cells, pericytes, astrocytes, oligodendrocytes, microglia, and ECM are considered one structural unit. BM surrounds blood vessels with two layers of endothelial BM and parenchymal BM and regulates barrier function and interactions between cells.

physiological and pathophysiological functions of pericytes, their involvement in the molecular mechanisms underlying tissue repair and functional recovery after ischemic stroke, and a potential therapeutic strategy to promote endogenous regeneration.

2. Physiological and Pathophysiological Functions of Pericytes

Pericytes are located at the abluminal side of endothelial cells of precapillary arterioles, capillaries, and postcapillary venules and are embedded in the basement membrane (BM) **(Fig. 1)**. One of the important roles of brain pericytes is to maintain BBB integrity through direct interaction with endothelial cells[7] and the regulation of CBF. The BBB is formed by tight junctions between adjacent endothelial cells, functioning as a highly selective barrier that regulates the exchange of substances between the blood and the brain parenchyma to maintain CNS homeostasis. Although pericytes exist throughout the body at different covering ratios against endothelial tubes, they are most abundantly present in the brain and retina, where a high level of blood–tissue barrier is formed[8]. An important signaling pathway for pericyte recruitment around endothelial tubes is PDGF-BB–PDGFRβ. Pericyte recruitment is essential for the maturation of newly formed vessels harboring the BBB during development; the lack of pericyte recruitment results in embryonic lethality due to increased cerebral vascular permeability and rupture of microaneurysms leading to cerebral hemorrhage in *Pdgfb*- or *Pdgfrb*-deficient mice[9, 10].

Pericytes regulate CBF by contracting and relaxing in response to neuronal activity (neurovascular coupling) through vasoactive substances, such as nitric oxide and prostaglandins[11-13], whereas there is also a report that smooth muscle cells, not the capillary pericytes, primarily regulate CBF[14]. Compared with endothelial cells, pericytes are more easily injured or degenerated under stressed conditions, such as aging, diabetes mellitus, and ischemia. Pericyte loss, microaneurysms, and acellular capillaries, which are induced in *Pdgfb*-deficient mice, are characteristics of diabetic retinopathy[15, 16]. Age-dependent chronic pericyte loss results in capillary rarefaction, leading to CBF reductions and BBB breakdown. It also disturbs the clearance of metabolic wastes that can cause vascular permeability increase and neurotoxicity in the brain, such as soluble Aβ, thereby leading to the development of Alzheimer disease-like pathology[17, 18]. The "no-reflow" phenomenon, that is, impaired blood flow restoration even after successful recanalization of the proximal occluded artery, may be caused by inappropriate contraction of distal pericytes in the brain following ischemia–reperfusion[19]. Thus, pericytes are in the limelight in various pathological conditions, such as lifestyle-related diseases, cerebrovascular diseases, cerebral white matter lesions,

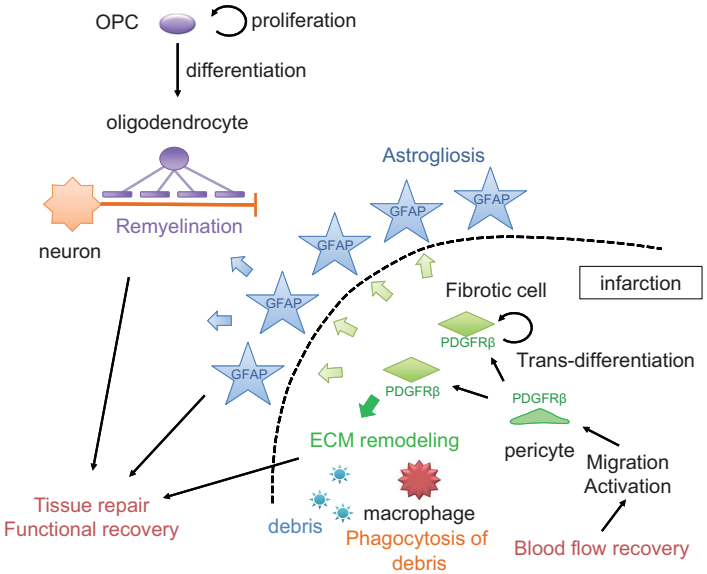

Fig. 2. Interaction of NVU components in tissue repair and functional recovery after ischemic stroke (modified from Shibahara *et al.* [31])

After cerebral ischemia, pericytes accumulate around the infarct lesions, differentiate into fibroblast-like cells, and produce ECM proteins such as fibronectin (fibrotic scar formation). Pericytes promote macrophage-mediated clearance of myelin debris within the infarct, periinfarct astrogliosis, and remyelination and thereby contribute to functional recovery by interacting with astrocytes and OPCs.

and cognitive dysfunction.

3. Origin of Pericytes and Endogenous Stem Cells

Mesenchymal cells and neural crest cells are recognized as the main sources of pericytes; however, there have been some recent reports showing that macrophages or tissue-localized myeloid progenitors can be the origin of pericytes[20]. Kokovay *et al.* described that pericytes are recruited from peripheral bone marrow-derived cells and are involved in blood vessel stabilization during angiogenesis following ischemia[21]. It has also been elucidated that mature macrophages undergo transdifferentiation after attaching to microvessels during neurogenesis and contribute to vascular maturation as pericytes[22]. Therefore, pericytes may be a heterogeneous population, considering that the molecular markers of pericytes vary and change over time depending on the tissue and pathology. Pericyte-derived cells may behave like mesenchymal stem cells that produce neurotrophic and immunomodulatory factors[23]. Following brain infarction, pericytes in the pia mater can transdifferentiate into multipotent stem cells (ischemic-induced neural stem/progenitor cells) that have the potential to differentiate into both neural and vascular cells[24, 25].

It is known that neural stem cells, located in the subventricular zone even in human adults, migrate toward infarct areas. They differentiate primarily into reactive astrocytes in periinfarct areas and may contribute to functional recovery while harboring the potential to differentiate into neurons by forced expression of *Ascl1* [26]. Circulating endothelial progenitor cells adhere to ischemic vessels and participate in new vessel formation by supplying endothelial cells[27], and their potential therapeutic effects in ischemic stroke have been gradually identified[28].

4. Pericyte-Mediated Poststroke Tissue Repair and Functional Recovery

(1) CBF Recovery

Restoration of blood flow within infarct areas is the most important process for poststroke functional recovery through clearance of debris and tissue repair (Fig. 2). According to the RESCUE-Japan registry, functional outcome at 3 months after stroke onset was better in patients with reperfusion by endovascular treatment, even in cases of similar neurological severity at 24 hours after the onset[29]. Basic research using mouse middle cerebral artery occlusion (MCAO) stroke models supports this clinical data; even though brain infarction is similarly produced in the perfusion areas on day 1, infarct volume became significantly smaller with enhanced intrainfarct fibrotic responses

and better functional recovery on day 7 in mice with efficient reperfusion than without reperfusion[30]. The extent of tissue repair and functional recovery was better in mice with earlier reperfusion. An important pathological difference between transient MCAO (tMCAO) and permanent MCAO (pMCAO) was the extent of pericyte survival within infarct areas, which maintains blood flow within infarct areas, thereby promoting subsequent tissue repair even after the development of brain infarction. Unless the occluded artery is recanalized, remodeling of the pial collateral circulation and recruitment of pericytes around surviving microvascular endothelial cells are required to promote efficient reperfusion and subsequent repair within infarct areas. Because these processes following pMCAO were significantly suppressed in pericyte-deficient *Pdgfrb* heterozygous knockout (*Pdgfrb*$^{+-}$) mice, pericytes play important roles in poststroke intrainfarct reperfusion, leading to tissue repair and functional recovery even in cases without recanalization[31].

(2) Macrophage-Mediated Clearance of Debris Within Infarct Areas

A major purpose of blood flow restoration within infarct areas may be the recruitment of phagocytes, particularly macrophages, into infarct areas to remove cellular and myelin debris that encloses cytotoxic and proinflammatory molecules. It has been thought that infiltration of inflammatory cells is a detrimental factor to be targeted because it enhances vascular edema and cell death in periinfarct areas during the acute phase; however, recent evidence demonstrates that functional recovery may be worse without debris clearance or fibrotic repair during the subacute phase. Thus, proper macrophage-mediated debris clearance may be a prerequisite for promoting poststroke functional recovery (the clean-up hypothesis)[32-35]. Maintenance or immediate remodeling of the microcirculation by pericytes is important for macrophages to infiltrate into infarct areas through the blood. In addition to damage-associated molecular patterns (DAMPs), such as high mobility group box-1 and peroxiredoxin, which recruit macrophages and trigger inflammation[36], PDGFRβ-positive pericytes around endothelial cells may recruit macrophages into infarct areas: 1) pericytes produced a chemokine CCL2 and a trophic factor CSF1 within infarct areas; 2) macrophages infiltrated around PDGFRβ-positive microvessels within infarct areas[37]; and 3) macrophage infiltration and debris clearance were significantly suppressed in *Pdgfrb* knockout mice. Resident microglia are also activated immediately after brain ischemia. However, since microglia can neither survive within infarct areas nor infiltrate freely into infarct areas, blood-derived macrophages rather than microglia may be the predominant phagocytes within infarct areas. Macrophages removing intrainfarct debris can function as reparative ones (M2 type)[38]; they can produce various trophic factors supporting intrainfarct fibrosis and periinfarct astrogliosis and oligodendrogenesis, leading to functional recovery, in contrast to proinflammatory ones (M1 type)[36, 39]. Resident microglia may function as an eliminator of DAMPs and neuron-repairing cells primarily in periinfarct areas[38].

Poststroke excessive inflammation and immune responses may have a downside that deteriorates periinfarct edema and expands infarct lesions. Macrophages produced IL-23, which induced IL-17 production from $\gamma\delta$T cells around infarct areas and enhanced inflammation during the subacute phase[40]. Conversely, recent lines of evidence demonstrate that microglia- and macrophage-mediated immediate resolution of inflammation is a key to obtaining better functional recovery. Elimination of DAMPs via the upregulation of AIM or MSR1, efferocytosis, or accumulation of FOXP3-positive regulatory T cells may be involved in the mechanism[33, 35, 38, 41, 42].

(3) Intrainfarct Fibrosis and Periinfarct Astrogliosis

Following macrophage-mediated phagocytosis of dead cells and debris, PDGFRβ-positive fibroblast-like cells gradually occupy and produce extracellular matrix (ECM) proteins within infarct areas with the aid of infiltrating macrophages. Göritz *et al.* demonstrated that type A pericytes leave the blood vessel and proliferate and differentiate into fibroblast-like cells after spinal cord or brain injury, completing wound healing[43, 44]. Infiltrating macrophages phagocytosing debris effectively produce PDGF-B and bFGF, an upregulator of PDGFRβ, and prompt PDGFRβ-positive fibroblast-like cells to produce ECM proteins, such as fibronectin and collagen type I[45-47]. It is an important fact that fibrotic responses occur even in the brain, similar to other major organs such as the heart, lungs, and liver. Different from chronic fibrosis leading ultimately to organ failure, poststroke fibrosis may work beneficially for functional recovery following acute ischemic stroke because the suppression of pericyte-mediated fibrosis results in impaired functional recovery, as evidenced by a stroke model using *Pdgfrb* knockout mice[31].

Periinfarct accumulation of GFAP-positive reactive astrocytes, that is, astrogliosis or glial scar, clearly demarcates infarct areas where pericyte-derived PDGFRβ-positive fibroblast-like cells and macrophages would occupy over subacute phases[46].

Table 1. Remodeling of extracellular matrix proteins after acute ischemic stroke

ECM proteins	Remodeling after ischemic stroke	Main cell source	Possible function in ischemic stroke
Collagen IV	vascular BM	endothelial cells	BBB maintenance [60]
Perlecan	vascular BM	endothelial cells	BBB maintenance pericyte recruitment [63]
Fibronectin	diffuse within infarction	PDGFRβ-positive fibroblast-like cells liver	fibrosis [46] macrophage phagocytosis [62]
Collagen I	diffuse within infarction	PDGFRβ-positive fibroblast-like cells	fibrosis [46]
Vitronectin	diffuse within infarction	PDGFRβ-positive fibroblast-like cells astrocytes liver	BBB maintenance [66]
Laminin α2	boundary of infarction	reactive astrocytes	glial scar OPC differentiation [62] limiting inflammatory cells

ECM: extracellular matrix, BM: basement membrane, BBB: blood-brain barrier, OPC: oligodendrocyte precursor cell.

Because *Pdgfrb*-deficient mice had poor astrocyte accumulation[31, 48], PDGFRβ-positive cells may participate positively in astrocyte proliferation and migration in periinfarct areas. Indeed, PDGFRβ-positive cells can produce various trophic factors, such as NT-3, IL-6, and TGFβ, that can activate astrocytes[49]. Glial scar has traditionally been recognized to impede post-damage axonal regeneration in the CNS because astrocytes can produce chondroitin sulfate proteoglycans, potential inhibitors of axonal regeneration[50, 51]. However, there are increasing lines of evidence that inhibition of astrogliosis attenuates axonal regrowth in CNS injury [52]. Thus, glial scar, positively regulated by intrainfarct fibrosis, does not necessarily inhibit functional recovery but may promote regeneration depending on the interaction with trophic factors and disease states.

(4) Periinfarct Oligodendrogenesis and Functional Recovery

Because oligodendrocytes are high-energy-demanding cells and are most vulnerable to energy deprivation among neural cells, demyelination easily occurs under stressed conditions, including ischemia. Oligodendrocyte precursor cells (OPCs) are widely distributed in the brain, mainly in the white matter, and function as a reservoir of oligodendrocytes to prepare for demyelination. Periinfarct OPC-mediated remyelination is a key factor promoting poststroke functional recovery[53]. OPCs can rapidly proliferate in periinfarct areas; however, clearance of myelin debris and an appropriate blood supply are absolutely required for their differentiation into mature oligodendrocytes accompanied by remyelination. Intrainfarct pericyte-derived cells and macrophages phagocytosing myelin debris can produce trophic factors, such as IGF-1 and BDNF, inducing periinfarct oligodendrogenesis[31]. Because pericyte-deficient mice showed white matter injury with fibrin(ogen) accumulation and blood flow reduction, pericytes alone may have the capacity to induce oligodendrogenesis[54]. It has been shown that AKAP12, a scaffolding protein organizing intracellular signal transduction expressed in pericytes, may play important roles in promoting OPC differentiation through the production of trophic factors, such as BDNF, LIF, GRO-α, and HGF[55, 56].

(5) Maintenance and Remodeling of ECM Proteins

ECMs are roughly divided into those existing in the BM or in the interstitial matrix[57] and regulate many cellular functions, such as cell survival, proliferation, differentiation, and migration, as the structural and functional core of the microenvironment around cells. ECM composition shows diversity across tissues under physiological conditions[58]. The vascular BM in the brain is composed of two layers, the endothelial BM and the parenchymal BM, separating the systemic circulation from the CNS to support the barrier function of the BBB[58-60]. Following brain ischemia, proteases such as matrix metalloproteinases (MMPs) and cathepsins degrade the ECMs of the BM, leading to the disruption of BBB integrity[58, 61]. Then, surviving cells remodel ECMs dynamically within infarct areas (Table 1)[61, 62]. While the expression of perlecan and collagen IV was enhanced mainly in the vascular BM in intrainfarct areas, fibronectin and collagen I, produced primarily by PDGFRβ-positive cells, accumulated diffusely within infarct areas[46, 62, 63]. Although the BBB appeared to be intact under healthy conditions in mice lacking perlecan, it was severely disrupted after brain ischemia[63, 64]. Macrophages adhere to fibronectin via integrins and enhance their phagocytic ability, thereby effectively removing debris within infarct areas[62]. Astrocyte-associated fibronectin

also supports axonal regeneration in the CNS[65]. Vitronectin, a plasma glycoprotein similar to fibronectin, can also be produced by pericytes and may strengthen the interaction with endothelial cells via the integrin $\alpha 5$ receptor[62, 66]. Laminin $\alpha 2$ is a major BM protein that limits the infiltration of inflammatory cells in the intact brain. It was degraded by MMPs within infarct areas and was redistributed to the infarct border[62]. Laminin $\alpha 2$ induced the differentiation of OPCs for remyelination, while it may limit the infiltration of inflammatory cells into the intact brain. Laminin $\gamma 1$, derived from astrocytes, regulates pericyte differentiation and BBB integrity, and thus astrocytic laminin $\gamma 1$-deficiency leads to severe BBB disruption and age-dependent intracerebral hemorrhage[67]. However, mural cell-derived laminin $\alpha 5$-deficient mice showed less severe vascular damage and attenuated ischemic injury in a tMCAO model, indicating a detrimental role of mural cell-derived laminin $\alpha 5$ in ischemic stroke[68].

5. Strategy for Pericyte Protection

As we mentioned above, based on basic research, pericytes may be a therapeutic target to promote poststroke functional recovery. Clinical studies support this concept because poststroke functional outcome at 3 months is significantly poorer in patients with aging, poor-controlled diabetes mellitus[69-71], proteinuria[72], and current smoking[73], that are situations where pericyte dysfunction can be predicted[74]. Therefore, strategies to protect pericytes from these risk factors may improve functional outcomes after ischemic stroke. Furthermore, as a means of protecting and activating pericytes, it may be useful to focus on the intracellular metabolism, the acquisition of ischemic tolerance, and the functional activation of pericytes.

The expression of sodium glucose cotransporter 2 (SGLT2) was induced in periinfarct pericytes in a mouse stroke model. Preadministration of a low-dose SGLT2 inhibitor reduced pericyte/BBB injury during the development of brain infarction without affecting blood glucose levels, possibly by enhancing the ischemic tolerance of pericytes by increasing mitochondrial activity[75]. Although the clinical benefits of SGLT2 inhibitors are still unclear for the prevention of ischemic stroke, it is interesting that preconditioning of pericytes by a common clinical medication could result in a better outcome following ischemic stroke.

Administration of nanoparticle-mediated PDGF-BB, a growth factor promoting proliferation and migration of pericytes, may potentially reduce infarct volume following tMCAO through pericyte-mediated blood flow maintenance and neuroprotection[76]. However, it should be noted that administration of PDGF-CC, similar to VEGF[77, 78], may increase vascular permeability in mice treated with rt-PA following tMCAO.

Some of the ECM protein fragments have recently raised attention due to their various physiological activities, and the development of biomaterials targeting cell culture scaffolds and angiogenesis is progressing. Poststroke administration of perlecan domain V (endorepellin) increases the accumulation of pericytes around endothelial tubes and alleviates BBB breakdown within infarct areas through the cooperative functioning of PDGFRβ and integrin $\alpha 5\beta 1$ [63]. Moreover, perlecan domain V has been reported to have neuroprotective and proangiogenic effects after ischemic stroke through VEGF secretion by brain endothelial cells via integrin $\alpha 5\beta 1$ and ERK-dependent signaling pathways[79, 80].

6. Future Directions and Conclusions

In summary, brain pericytes not only play a wide variety of functions in microvessels, such as regulation of microcirculation and maintenance of the BBB, but also play a crucial role in blood flow recovery and tissue repair after brain ischemia through their interaction with various cell types in the NVU. In particular, pericytes take command and systematically perform these tissue repair processes by regulating macrophage-mediated clearance of debris and the production of ECM proteins, providing the optimal microenvironment required for functional recovery **(Fig. 2)**. Based on this, it may be useful to focus on the intracellular metabolism and functionality of pericytes. However, there are still many unclear points about how pericytes accumulate around infarct areas and become transformed to play a wide variety of roles beyond mural cells. Further studies are expected to develop a new treatment targeting pericytes for functional recovery after ischemic stroke.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research (B 16H05439 and B 20H03791 to T.K. and T.A.; C 20K09373 to T.A.; C 19K09511 and C 22K09236 to K.N.) from the Japan Society for the Promotion of Science (JSPS); a grant from Mochida Memorial Foundation for Medical and Pharmaceutical Research (K.N.); a grant from SENSHIN Medical Research Foundation, Japan (K.N., and T.A.); a grant from the Smoking Research

Foundation (T.A.); and research grants from Astellas, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Eisai, MSD, Sanofi, Taisho and Takeda (T.K. and T.A.).

Competing Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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