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REVIEW ARTICLE

Recent advances with liposomes as drug carriers for treatment of neurodegenerative diseases

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Abstract
A major challenge in treating neurogenerative diseases is delivering drugs across the blood–brain barrier (BBB). In this review, we summarized the development of liposome-based drug delivery system with enhanced BBB penetration for efficient brain drug delivery. We focused on the liposome-based therapeutics targeting Alzheimer's disease and Parkinson's disease because they are most common types of adult chronic neurodegenerative disorders. A variety of liposome with surface modification of BBB-targeting ligands have been created to cross the BBB via transcytosis to the therapeutic efficacy of Alzheimer's disease and Parkinson's disease drugs. Recent advances in liposome are providing alternatives to overcome BBB for more efficient therapeutic strategy. To improve the BBB penetration of liposomes, we need to completely understand the pathophysiological changes at the BBB.

Keywords Blood–brain barrier · Liposome · Alzheimer's disease · Parkinson's disease

1 Introduction

Neurodegenerative diseases are debilitating conditions that result in progressive degeneration of nerve cells [1]. It is accompanied by accumulation of protein misfolding, increase in reactive oxygen species and mitochondrial dysfunction, resulting in neuronal cell death and damage in function of synapses [2]. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most prevalent neurodegenerative diseases [3, 4]. As life expectancies continue to increase worldwide, the prevalence of AD and PD rise dramatically increased in many countries, which has greatly affected society and the economy [5]. Studies in molecular and cellular mechanisms of AD and PD have led to development of new therapeutic strategies, however a major challenge in the development of treatment is delivering drugs across the blood–brain barrier (BBB).

The BBB is a physiological barrier that controls transport of molecules from blood to the brain and vice versa. The central player in maintaining the BBB is the cerebral capillary endothelium, which limits passive transport from the blood by forming a monolayer with tight junctions and by actively pumping unwanted molecules back into the blood [6]. The endothelium may be the central player in maintaining the BBB, but it has become increasingly clear that it relies heavily on its direct cellular and acellular microenvironment to maintain differentiation and functionality [6]. Key factors in the cerebral endothelial microenvironment are cerebral pericytes, astrocytes, neurons and the extracellular matrix. Together, these cells and biomolecules form the neurovascular unit, which is a key organ subunit that is known to be important in neurological function and disease. The brain microvascular endothelium differs from that found in peripheral capillaries based on its complex tight junctions, which restricts para-cellular transit and instead, require that molecules use transcytosis to pass from the blood through the endothelial cell into the central nervous system [7]. The brain endothelium also expresses multiple broad-spectrum efflux pumps on their luminal surface that inhibit uptake of lipophilic molecules [8, 9].

It is estimated that more than 98% of molecules with small sized chemical drugs and 100% with big sized biomaterial cannot cross BBB and reach the brain [10]. Drugs for neurodegenerative diseases are no exception to this limited transmission. As therapeutic strategies for AD and PD have been developed from symptom-reducing small molecules to

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disease-modifying biologics based on understanding of disease mechanism, delivering big sized biologics is becoming big challenges [11]. In this review, the current advances in the development of nanotechnology for delivering therapeutics for AD and PD will be discussed.

2 Liposomes for crossing over BBB

Nanoparticles (NPs) are therapeutic carriers on a scale between 1 to 1000 nm and represent promising approaches for brain drug delivery due to their physiochemical features and easy surface functionalization. Liposomes are highly flexible and biocompatible NPs considered to be the most successful NPs in the clinic [12]. They have capacity to incorporate hydrophilic drugs in the aqueous core or hydrophobic compounds within the lipid layers [13] and easily functionalized to interact with specific molecular target [12].

With the remarkable advancements in nanotechnology, a variety of liposome with surface modification of BBB-targeting ligands have been created to cross the BBB via transcytosis (Fig. 1). It facilitates the delivery of cargos from apical to basolateral plasma membrane of BBB through "Trojan horse strategy". The trojan horse strategy includes carrier-mediated transcytosis (CMT), receptor-mediated transcytosis (RMT), and adsorptive-mediated transcytosis (AMT). CMT relies on molecular carriers present at membranes of the BBB including large neutral amino acid transporters (LAT1) and glucose transporter (GLUT1). For example, binding of glucose to GLUT1 triggers a conformational change in the transporter allowing glucose transported following a high-to-low concentration gradient. While CMT usually transports small molecules, RMT offers selective transport of relatively large molecules through vesicular transport across the cells upon the binding of ligands including lactoferrin (Lf), transferrin (Tf), and insulin to the specific receptors expressed on BBB [14, 15]. Thus, RMT has been considered to be a state-of-art strategy for transporting liposomes across the BBB [16]. Liposomes canalso utilize AMT based on electrostatic interactions between the positively charged surface and the negatively charged plasma membrane, however it is not specific to BBB.

3 Liposome-based therapy for AD

AD is represented by concurrence of accumulation of amyloid plaque and neurofibrillary tangles in brain tissue. According to the traditional amyloid hypothesis, the accumulation of aggregates of the amyloid-beta protein in the brain results in complex pathological cascade that accelerate tau phosphorylation [13, 17]. The current AD therapeutic strategies include amyloid-beta clearance, tau protein deposits, neuroprotection, as well as non-mechanism-based approaches such as symptomatic cognitive stimulation [13, 17].

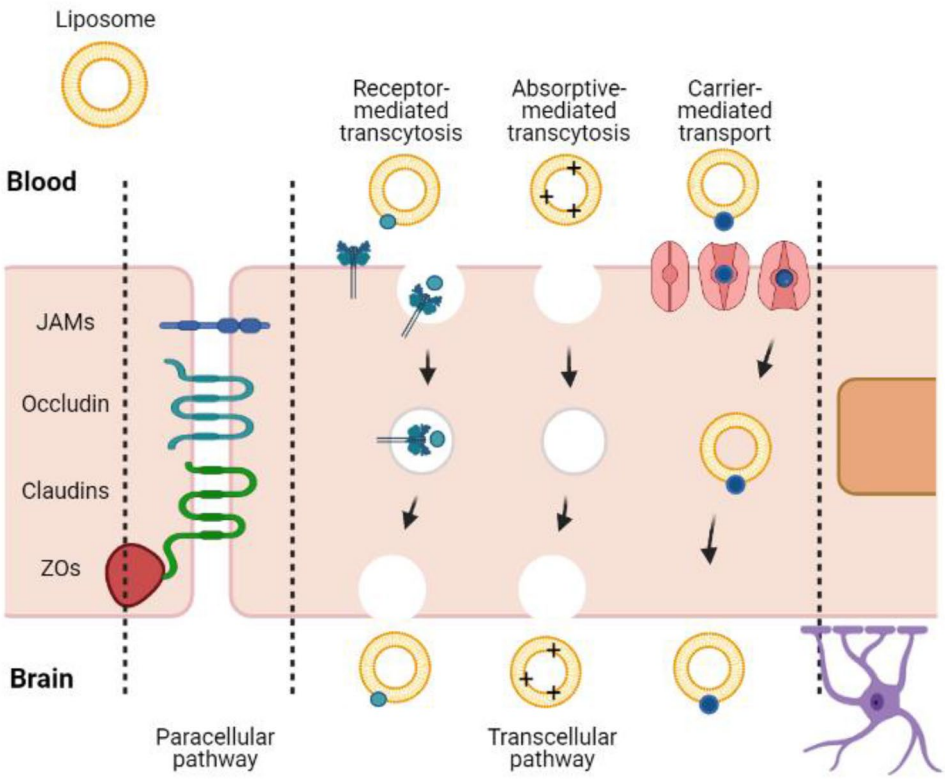

Fig. 1 Drug transport mechanism of liposomes in BBB

To deliver therapeutics using liposomes, many studies have focused on modifying the surfaces of liposomes by conjugation with ligands such as Tf and Lf to cross over BBB via RMT. The Tf receptor is originally responsible for transporting iron into the brain to maintain iron homeostasis. This transport mechanism of the BBB was hijacked to deliver Osthole (Ost) to stimulate the neural stem cells promoting the synaptic growth [18]. For this, the polyethylene glycol (PEG) and Tf were incorporated on liposomes and Ost, a natural coumarin derivative (7-methoxy-8-isopen-tenoxycoumarin), was entrapped inside the liposome. Increased BBB penetration of Tf-Ost-liposomes was demonstrated using in vitro brain endothelial cells (hCMEC/D3) and APP/PS-1 AD mouse model. The rate of Ost across the BBB was approximately twice higher in Tf-Ost-liposome compared to Ost-liposome both in vitro and in vivo. In addition, encapsulation of Ost with liposome resulted in enhanced pharmacodynamic and pharmacokinetics, showing significantly decreased Amyloid beta$_{1\text{-}42}$ in vivo. Similarly, Kuo et al. [19] reported liposome NPs conjugated with Lf to deliver neuronal growth factors (NGF) across the BBB. Because Lf receptor is over-expressed in the capillaries and neurons in AD and PD, Lf is a promising biological ligand for drug delivery for treatment of those diseases [20]. The Lf-NGF-liposomes showed higher permeability on BBB in vitro and protective effect against Aβ-induced neurotoxicity in vitro. The targeting efficiency of Lf was also demonstrated in different study [21]. The Lf-modified liposomes were grafted with RMP-7 (bradykinin B2 receptor agonist) and used to encapsulate quercetin (QU) for recovery of degenerated neurons [21]. Due to the Lf, RMP-7-Lf-QU-liposomes successfully permeated BBB and significantly interrupted the expression of the p38 and p-S202 proteins which are highly induced by amyloid beta in vitro.

Absorptive transcytosis-based brain-drug delivery can be designed by modifying liposomes with cationic proteins as targeting moieties. Yang et al. [22] developed cell penetration peptides (CPP)-modified liposomes to improve the drug distribution in brain (Fig. 2). The CPP, short peptides having a positive charge facilitated the penetration of cargoes into plasma membrane by AMT. The CPP-modified liposomes incorporated with rivastigmine, a cholinesterase inhibitor, showed the enhanced therapeutic effect with improved uptake by BBB in vivo. Distinct from liposome strategies aiming for enhanced BBB penetration, some research groups suggested the opposite.

In 2015, Ordóñez-Gutiérrez et al. [23] reported that unilamellar vesicles containing phosphatidic acid or cardiolipin which has high affinity with amyloid beta significantly reduced the level of amyloid beta in the plasma, while achieving a minor reduction in brain amyloid beta of APP/PS1 transgenic mice. In fact, the unilamellar vesicles were not able to penetrate BBB, however the reduction of amyloid beta in plasma led to shift an equilibrium to the side of the reaction towards the enhanced efflux of amyloid beta through BBB which is referred as “peripheral sink” hypothesis [24]. Based on this finding, the same group constructed multivalent immunoliposomes decorated with monoclonal IgG antibodies targeting circulating amyloid beta (STAB-Maβ) [25]. The treatment of APP/PS1 transgenic mice with immunoliposomes reduced the level of amyloid beta in the plasma as well as in the brain due the promotion of amyloid beta efflux from the brain into the systemic circulation, which demonstrates that amyloid beta level can be also modulated without delivering drugs into the brain [25]. While STAB-Maβ has shown therapeutic activity for "aged" (16 month-old) mice, it has not shown any beneficial effect on "adult" (10 month-old) mice. It is assumed that STAB-MAβ has differential reactivity with circulating forms of amyloid beta in “adult” and “aged” mice. The peripheral sink hypothesis is very promising in the sense that the site of therapeutic action of liposome is a plasma not a brain, however, it needs further studies to fully understand its molecular mechanism.

4 Liposome-based therapy for PD

PD is a progressive neurodegenerative disease affecting around 1–3% of the population over the age of 65 [26]. The main feature of PD is the abnormal accumulation and aggregation of α-synuclein in the form of Lewy bodies leading dysfunctions of somatomotor system, which is by progressive degeneration of nigrostriatal dopaminergic pathway with substantial loss of dopamine neurons in the substantia nigra [26]. Thus, delivering dopamine or dopamine derivatives in the brain has been a mainstream of PD therapeutic strategy.

Qu et al. developed liposomes conjugated with a 29 amino-acid peptide (RVG29) as aligand and incorporated with dopamine derivative N-3,4-bis (pivaloyloxy)-dopamine [27]. The RVG29 is derived from the rabies virus glycoprotein which targets nicotinic acetylcholine receptors expressed on BBB and neuron [27]. The liposomes showed high efficiency of absorption and RMT-based penetration across in vitro BBB. And these carriers showed selective distribution to the brain, striatum, and substantia nigra in a mouse model of PD (6-OHDA) after intravenous administration, demonstrating the RVG29 as an efficient target ligand for liposome delivery into the brain [27] (Fig. 3).

Targeting the receptors of amyloid precursor protein in the BBB can also facilitate the RMT-mediated BBB penetration. Kahana et al., reported the liposomes linked to a peptide of five amino acids (RERMS) selected from APP and dopamine loaded inside [28]. Intra-peritoneal injection of the APP-targeted liposomes resulted in a significant increase in striatal dopamine within 5 min (6.9-fold, $p < 0.05$) in PD

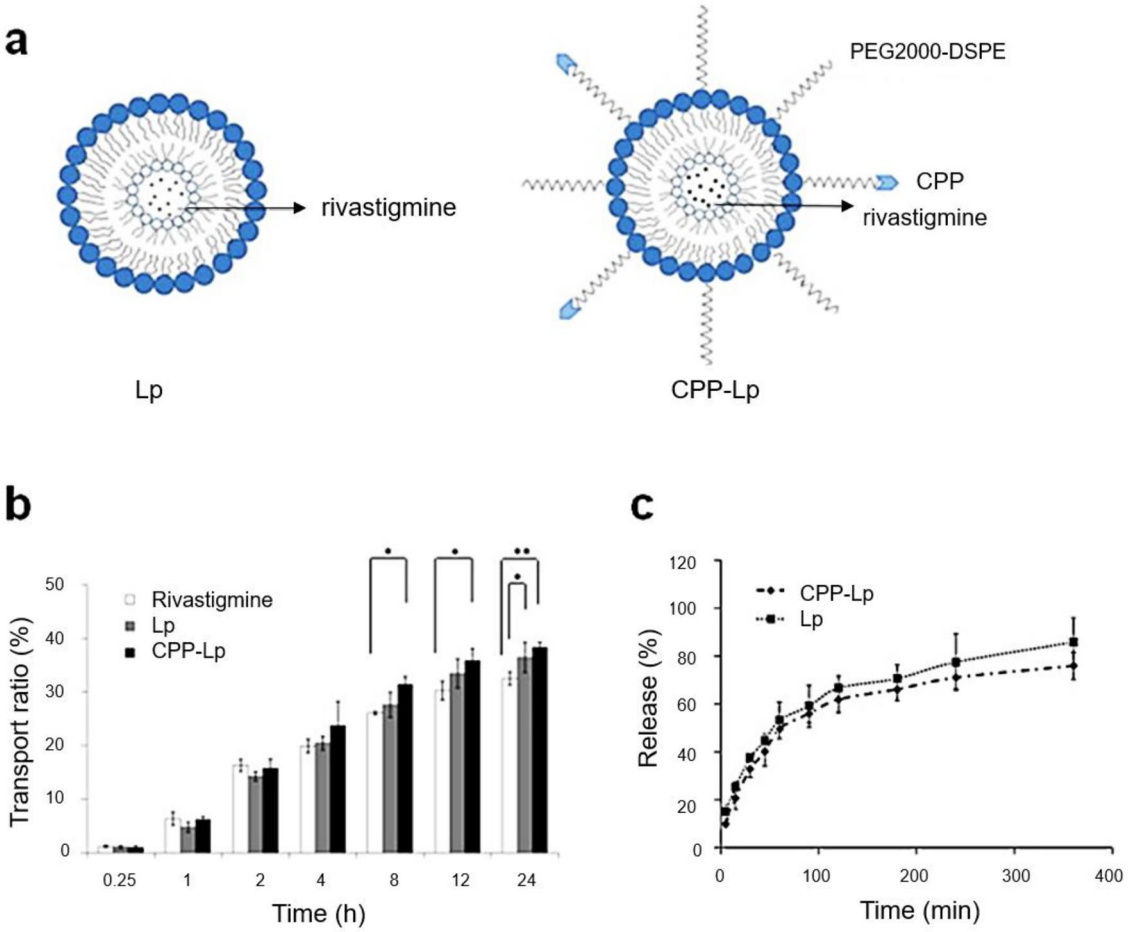

Fig. 2 Enhanced brain distribution and pharmacodynamics of rivastigmine by liposomes following intranasal administration [22]. **a** Schematic diagram of rivastigmine liposomes (Lp) and CPP modified rivastigmine liposomes (CPP-Lp). **b** The transport ratio (%) across the BBB model in vitro of CPP-modified rivastigmine liposomes (CPP-Lp), rivastigmine liposomes (Lp) and rivastigmine solution. The results represent as mean ± SD (n = 3). $*P < 0.05$, $**P < 0.01$ vs rivastigmine. **c** Rivastigmine release from Lp and CPP-Lp. The results represent as mean ± SD (n = 3). Copyright (2013) Elsevier

mouse model, rats and mini-pigs. They also showed the improved behavioral impairments by functionalizing the stratal circuit [28].

Recently, a magnetic liposomal delivery system has been explored to improve the brain drug delivery efficiency [29]. The magnetic liposome (Fe_3O_4- nimodipine liposome (NMD)-liposome) was obtained by modifying NMD with PEG-coated Fe_3O_4 by mixing the liposomal suspension and Fe_3O_4 solution. In a PD rat model, NMD retention in the brain was 2.5-fold higher in Fe_3O_4-NMD-liposomes treatment group compared to NMD treatment group under the action of external magnetic field. By attenuating the PD neurotoxin through nimodipine incorporated in liposomes, they also showed enhanced protection of dopaminergic neuron in vivo. The therapeutic effect of Fe_3O_4-NMD-liposomes was evidently larger than that of free NMD group as Fe_3O_4-NMD-liposomes were efficiently attracted toward the brain. In a follow-up study, the Fe_3O_4-NMD-liposomes incorporated with a Resveratrol, a natural product and neuroprotective drug also showed a promising therapeutic activity for PD rat model [30]. These studies indicate that the use of a magnetic field for focused delivery of liposomes into the brain can be a promising strategy for treatment of PD. However, to move from the lab environment to clinical setting, some technical challenges need to be addressed including rapid reduction of field strength in deep tissue and the need of driving a large number of magnetic objects to get a therapeutic result.

5 Summary and perspectives

Recent advances in liposome are providing alternatives to overcome BBB for more efficient therapeutic strategy. Especially, future developments of liposomes aiming for RMT-mediated BBB penetration will offer new opportunities

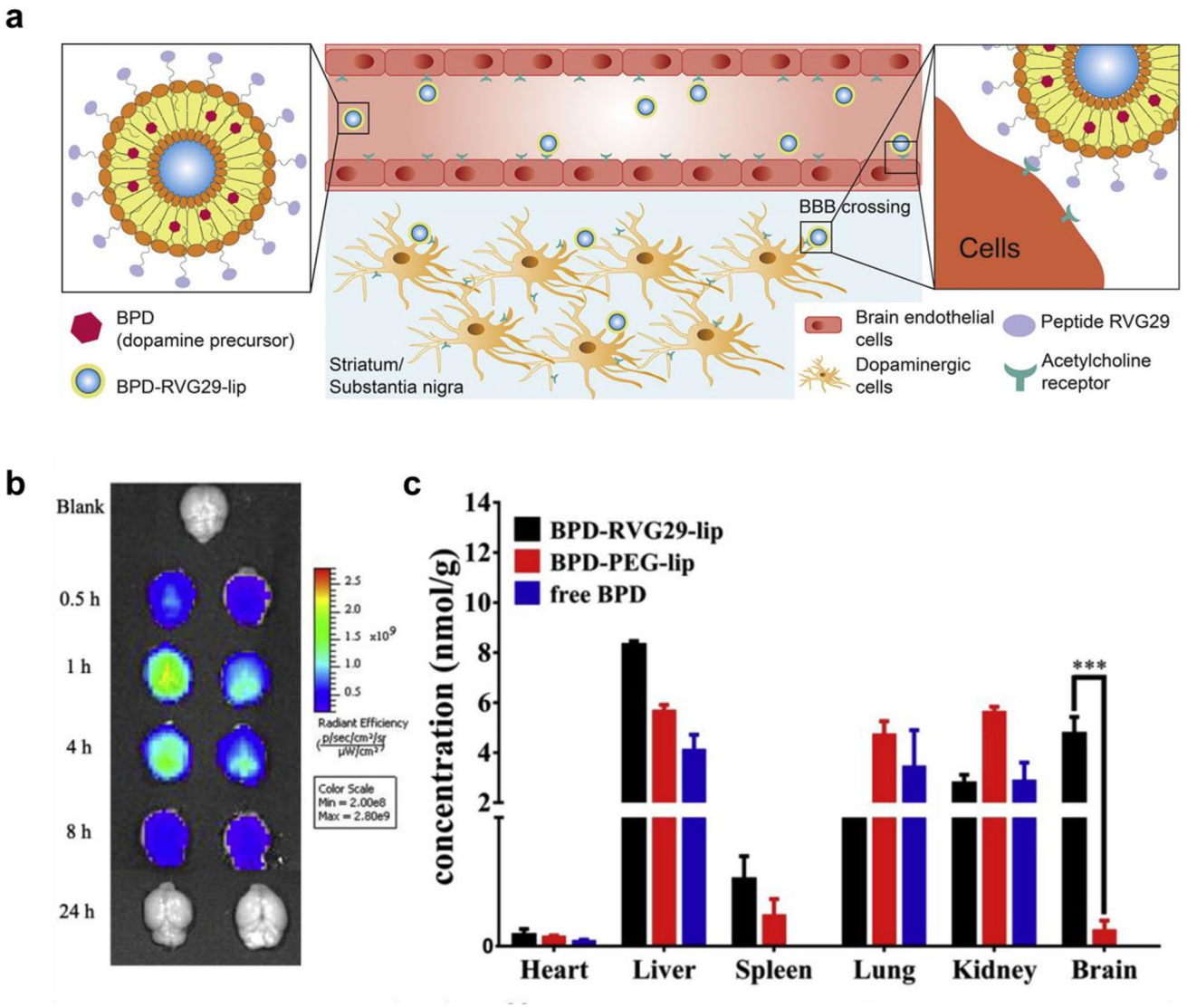

Fig. 3 A brain targeting functionalized liposomes of the dopamine derivative for treatment of Parkinson's disease [27]. **a** schematic illustration of liposome linked with RVG29 peptide incorporating dopamine derivative N-3,4-bis(pivaloyloxy)-dopamine (BPD). **b** Ex vivo brain images of nude mice after systemic administration of DiD-labeled liposome at different time point (left: BPD-RVG29-lip, right: BPD-PEG-lip). **c** BPD distribution in mice organ at 1 h after intravenous injection. Adapted with permission from reference [27]. Copyright (2018) Elsevier

to treat AD and PD. To enhance the delivery efficiency of liposomes, pathophysiological changes in BBB in AD and PD must be completely understood. Dysfunction of the BBB is not only involved in disease progression but also may affect the RMT mechanism of liposomes because of altered expression of receptors, efflux pump and endocytic dynamics. Most studies still use the animal model or brain endothelial cell line to test the drug delivery across the BBB, however, they are not relevant to the physiological function of human BBB in AD and PD. To understand the pathological BBB for better drug delivery strategy, it is necessary to consider advanced cell models including BBB-on-a-Chip [31] and BBB organoids [32].

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Ethical Declarations

Conflict of interest Park TE and Seo MW declares that s/he has no conflict of interest in relation to the work in this article.

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