PERSPECTIVE

Focusing on caveolin-1 in CNS autoimmune disease: multiple sclerosis

Multiple sclerosis (MS) is the leading autoimmune disorder in the central nervous system (CNS) that affects over 2.5 million people globally. Clinically, the disease is characterized with severe neurological defects and motor disabilities such as paresis and paralysis. Experimental autoimmune encephalomyelitis (EAE) is a well-defined laboratory animal model of MS that mimics key features of disease including aberrant auto-reactive immune activations in the periphery, CNS oriented pathogenic immune infiltrations, the pathological formation of demyelination in the CNS lesions and symptomatic consequences such as motor disabilities (Ontaneda et al., 2012). Although the etiopathogenesis of MS remained largely obscured, it is well recognized that the trafficking of encephalitogenic leukocytes, from the circulating blood across the blood-brain barrier (BBB) and infiltrated into the parenchyma of CNS tissues, is a hallmark process that greatly contributes to disease development. In fact, the efficient trafficking and extravasations of these highly pathogenic immune cells into the CNS tissues are prerequisites for triggering neuroinflammation, the formations of pathological lesions in the CNS and sebsequently the development of the clinical symptoms of MS and EAE (Goverman, 2009).

Among various pathogenic immune cells, antigen specific CD^{4+} T cells specifically TH1 and TH17 cells have been considered as crucial drivers in EAE provoked neuroinflammation (Huppert et al., 2010). For instance, antigen specific TH17 cells could infiltrate into the CNS parenchyma *via* CCR-6 dependent recruitment (Reboldi et al., 2009) where they re-activate local resident cells by secreting interleukin (IL)-17. IL-17 activated wide range of cells including different immune cells, endothelial cells, fibroblast, myeloid cells and enhanced the positive feedback for the productions of pro-inflammatory mediators including CXCL1, CXCL-12, CXCL6, IL-1β, IL-6, TNF-α, GM-CSF and CCL2. These actions lead to the attraction other pathogenic leukocytes including pro-inflammatory macrophages, cytotoxic T cells, B cells and dendritic cells in the CNS tissues and the perpetuated neuroinflammation *in situ* (Bettelli et al., 2007). Thus the suppression of encephalitogenic TH1 and TH17 cell populations and their trafficking frequencies into the CNS tissues by either genetic modification or molecular/pharmacological modulation could directly lead to the alleviation of disease outcomes.

The trans-endothelial extravasation of pathogenic lymphocytes is a multi-step process each of which is strictly regulated by the active interactions of activated lymphocytes and primed endothelial cells. For instance, cell adhesion molecules and chemokine receptors presented on the luminal surface of microvascular endothelial cells of the CNS bind to their ligands on the surface of polarized lymphocytes and initiate the process of trans-migration. Among those adhesion molecules presented on the endothelial surface, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two distinct representatives for regulating leukocytes diapedesis into the CNS parenchyma, directly contributing to the development of MS and EAE. The strategies targeting these adhesion molecules by either pharmacological agents or genetic modifications exert promising results for treating MS. For instance, VLA-4 is ligand for VCAM-1 that presented on the majority of immune cells. Functional blockage of VLA-4 significantly compromised the trans-migration of leukocytes and showed potent efficacy in the treatment of MS (Gandhi et al., 2016). However, the underlying mechanisms regarding how the process of adhesion molecules helping the trafficking of immune cells into the CNS is regulated remains largely unknown.

Caveolins are 22 kDa integral membrane proteins in caveolae, the plasma membrane invaginations (50-100 nanometers). There are three subtypes of caveolins including caveolin-1 (Cav-1), caveolin-2 (Cav-2) and caveolin-3 (Cav-3). Cav-1 and Cav-2 are widely expressed in fibroblasts, adipocytes, neuronal cells and endothelial/epithelial cells whereas cav-3 is muscle specific. Physically, cav-1 interacts with numbers of molecules by amino-terminal membrane-attachment region named cav-1 scaffolding domain (CSD). Molecules bind to CSD *via* binding domain, namely cav-1 binding motif (CBM) with the hydrophobic sequences of "φXφXXXXφ" or "φXXφXXXXφ", where φ is aromatic residue such as tyrosine, tryptophan or phenylalanine. Proteins with these character domains include cav-2, Src tyrosine kinases, TGFβ receptor, endothelial NOS (eNOS), amyloid precursor protein (APP), epidermal growth factor receptor (EGFR) and so on (Parat, 2009). By interacting with multiple cellular signaling molecules, cav-1 participates in diverse cellular events such as transcytosis, cholesterol trafficking, signal transductions and directional cell migration. The diverse regulatory interactions of cav-1 with proteins and receptors suggest the divergent functions of cav-1 in different cellular events and diseases.

Cav-1 appears to play a role in the pathological process of EAE, a laboratory animal model of MS. Shin et al. (2005) previously reported that the expression of cav-1 was increased in the spinal cord of EAE lesions, yet the functions of cav-1 in the pathogenesis of EAE or MS remained unknown. Thus, we studied the pathogenic involvement of cav-1 in the development of EAE. We found that the serum secretion of cav-1 and its expressions in the spinal cord were increased after active immunization and the increase was highly coincident with the progression and severity of EAE (Wu et al., 2016). Furthermore, cav-1 deficient mice were highly refractory to EAE with declined disease incidence, delayed symptoms presentations and improved neurological deficient sufferings. In the peripheral spleen and draining lymph nodes of cav-1 deficient mice, we observed comparable activation/priming of auto-reactive T cells, indicating that the loss of cav-1 did not compromise the auto-reactive immune priming in periphery. In fact, loss of cav-1 could still sustain the immune activation in peripheral lymphoid organs but significantly alleviated the trafficking of encephalitogenic lymphocytes into the CNS parenchyma (Wu et al., 2016). To the best of our knowledge, this is the first time to demonstrate the crucial involvement of cav-1 in EAE pathogenesis.

A critical hallmark in the pathogenesis of EAE and MS is that the trafficking of encephalitogenic leukocytes from the circulating blood into the parenchyma of CNS tissues. The efficient trafficking of these highly encephalitogenic leukocytes into the CNS parenchyma is a key prerequisite in MS and EAE for the development of pathological leisions such as demyelination and subsequent motor disabilities such as paresis or paralysis. During the process of trans-migration, inflamed endothelial cells are crucial participants. Cellular mediators for endothelial activations may actively contribute to the trans-endothelial diapedesis. Cav-1 is abundantly presented in vascular endothelial cells. Cav-1 regulates vascular properties and endothelial functions including vascular permeability, clathrin independent endocytosis, macromolecular transport as well as inflammatory induced cytoskeleton transformation under diverse conditions (Sowa, 2012). For example, cav-1 positively modulates the activation of Src and Rho GTPases, thereby controlling the polarization of inflamed endothelial cells and its directional mobility. At site of inflammation, adhesion molecules presented on endothelial cells cluster near the transcelluar pores where caveolae and caveolins are enriched

(Millan et al., 2006). Attenuation of cav-1 in endothelial cells by pharmacological blockage or siRNA partially reduced the pathological leukocytes diapedesis while restoration of cav-1 attenuated such effects (Zhong et al., 2008; Xu et al., 2013). Subsequently, we hypothesized that cav-1 could be responsible for facilitating the trans-endothelial extravasations of pathogenic lymphocytes into the CNS. We found that cav-1 deficiency alleviated the efficient trafficking of pathogenic helper T cells, specially TH1 and TH17 cells, into the CNS parenchyma. In consistent, down-regulation of cav-1 in endothelial cells by using siRNA inhibited the trans-endothelial diapedesis of pathogenic TH1 and TH17 cells *in vitro* (Wu et al., 2016). These results highlighted the critical requirement of cav-1 in endothelial cells for directing lymphocytes trafficking during inflammation.

We next addressed the question whether adhesion molecules are the molecular targets of cav-1 in promoting trans-endothelial migration of encephalitogenic TH1 and TH17 cells during EAE. After inflammatory stimulation, adhesion molecules, such as ICAM-1 and VCAM-1, were increased in the inflamed endothelial surface companied with the ICAM-1 translocation into cav-1 enriched lipid raft domains (Millan et al., 2006). With active EAE induction, cav-1 was highly co-localized with adhesion molecule ICAM-1 and VCAM-1 within the CNS lesions where inflammatory infiltrations existed. Moreover, the *in vitro* knockdown of cav-1 partially compromised the increase of ICAM-1, VCAM-1 and attenuated the lymphocytes trans-endothelial diapedesis (Wu et al., 2016). These results, when taken together, suggest the critical roles of cav-1 in CNS oriented encephalitogenic lymphocyte trafficking by targeting ICAM-1 and VCAM-1.

Interestingly, as a cellular trafficking protein, cav-1 could dissociate from the membrane caveolae structure and release into the circulating system, which might account for its appearance in serum. As we have showed the increased serum cav-1 secretion after EAE induction, further explorations should be conducted to evaluate the diagnostic value of serum cav-1 secretion for indicating the occurrence of MS or disease severity. To this end, we should further investigate the potential correlations of serum cav-1 levels in MS patients at different phases of disease development.

Of note, the roles of cav-1 in neurological diseases are not limited to the regulatory role in lymphocytes trans-endothelial migration. Our previous studies indicate cav-1 diverse functions in different neurological diseases. For instance, in cerebral ischemic-reperfusion injury, cav-1 could help to sustain BBB integrity and prevent tight junction degradations (Gu et al., 2012). On the other hand, cav-1 regulates post stroke neurogenesis negatively (Li et al., 2011). Down-regulation of cav-1 could benefit neuronal differentiation and improve symptomatic relief in cerebral ischemic stroke to some extent. The complexity of the bioactivities of cav-1 and its dual effects in particular physiological or pathological conditions suggested us that consideration must be taken with great prudence we aim to modulate cav-1. In our case, the attenuation of cav-1 clearly benefits from EAE sufferings with compromised CNS trafficking (Wu et al., 2016). The heterogeneity of cav-1 may mark the complicated network that links the beneficial effects and side effects when modulating cav-1 in a certain pathological conditions. Thus for further investigations, we should carefully evaluate the dual sides of the value of cav-1 when we aim to serve cav-1 as a promising molecular target to attenuate.

Taken together, current knowledge has demonstrated the crucial contributions of cav-1 in the pathogenesis of EAE (Wu et al., 2016). Loss of cav-1 in vivo significantly protected from EAE with alleviated clinical symptoms and neuroinflammation. We have elucidated the regulatory functions of cav-1 in modulating the trans-endothelial diapedesis of lymphocytes. The study suggested a comprehensive understanding of the roles of cav-1 in CNS oriented lymphocytes diapedesis during EAE and marked the first step of the journey to serve cav-1 as a potential molecular target, which would lead to the exploration of new treatment strategy for MS and other neuroinflammatory diseases.

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