Genetic knockout of myosin light chain kinase (MLCK210) prevents cerebral microhemorrhages and attenuates neuroinflammation in a mouse model of vascular cognitive impairment and dementia

David J. Braun · Adam D. Bachstetter · Tiffany L. Sudduth · Donna M. Wilcock · D. Martin Watterson · Linda J. Van Eldik

Received: 11 February 2019 / Accepted: 25 April 2019 / Published online: 19 May 2019
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Abstract The blood-brain barrier (BBB) is critical in maintenance of brain homeostasis, and loss of its functional integrity is a key feature across a broad range of neurological insults. This includes both acute injuries such as traumatic brain injury and stroke, as well as more chronic pathologies associated with aging, such as vascular cognitive impairment and dementia (VCID). A specific form of myosin light chain kinase (MLCK210) is a major regulator of barrier integrity in general, including the BBB. Studies have demonstrated the potential of MLCK210 as a therapeutic target for peripheral disorders involving tissue barrier dysfunction, but less is known about its potential as a target for chronic neurologic disorders. We report here that genetic knockout (KO) of MLCK210 protects against cerebral microhemorrhages and neuroinflammation induced by chronic dietary hyperhomocysteinemia. Overall, the results are consistent with an accumulating body of evidence supporting MLCK210 as a potential therapeutic target for tissue barrier dysfunction and specifically implicate it in BBB dysfunction and neuroinflammation in a model of VCID.

Keywords Myosin light chain kinase · Cerebrovascular · Microhemorrhage · Neuroinflammation · Vascular cognitive impairment and dementia · Knockout

Introduction

Loss of blood-brain barrier (BBB) functional integrity is a major pathological component of age and disease-associated dementia (Zlokovic 2008; Daneman 2012), and the severity of barrier dysfunction is often associated with worsened cognitive function (Montagne et al. 2015; Janelidze et al. 2017). Attenuation or rescue of BBB dysfunction is therefore a promising target for treatment of a variety of neurodegenerative diseases disproportionately affecting older adults. The BBB is a specialized system of endothelial cell junctions and glial associations that are dynamically regulated by a
signaling network composed of discrete regulatory points (for review see Daneman and Prat 2015). For example, myosin light chain kinase (MLCK) is a signaling protein involved in regulation of barrier integrity that has been identified as a potential therapeutic target in disease-associated tissue barrier dysfunction. There are multiple MLCK proteins, but the focus of the present study is a 210-kDa MLCK protein encoded by the mylk1 genetic locus, referred to as MLCK210 (for review see Khapchaev and Shirinsky 2016). Prior studies on the role of MLCK210 in tissue barrier dysfunction and the potential of selective inhibitors have largely focused on non-CNS disorders (for recent reviews see Cunningham and Turner 2012; Rigor et al. 2013; Khapchaev and Shirinsky 2016; Xiong et al. 2017), including acute lung injury models (Wainwright et al. 2003; Rossi et al. 2007; Mirzapoiazova et al. 2011; Usatyuk et al. 2012; Fazal et al. 2013; Wang et al. 2014; Wang et al. 2016; Zhou et al. 2015), burn injury (Reynoso et al. 2007; Guo et al. 2012; Zahs et al. 2012), acute diarrhea (Clayburgh et al. 2005; Clayburgh et al. 2006), endotoxic shock (Rayl Ranaivo et al. 2007; Gaceb et al. 2016), cardiovascular shear stress (Ollmann et al. 2005), atherosclerosis (Sun et al. 2011), hypoxia (Arnaud et al. 2018), and intestinal injury models (Al-Sadi et al. 2012; Gilbert et al. 2012; Wu et al. 2014; Lorentz et al. 2017; Nighot et al. 2017; Al-Sadi et al. 2019). Additionally, there exists a smaller literature exploring the benefit of inhibition of MLCK in the context of BBB dysfunction. This includes in vivo models of traumatic brain injury (Luh et al. 2010; Rossi et al. 2013), cerebral ischemia (Zhang et al. 2015), subarachnoid hemorrhage (Luh et al. 2018), and in vitro experiments modeling cerebral hypoxia (Kuhlmann et al. 2007; Hicks et al. 2010) and cytokine elevation (Huppert et al. 2010; Beard et al. 2014). The in vivo data show MLCK suppression can ameliorate acute cerebrovascular injury, while the in vitro data suggest a link to chronic stressors commonly underlying cerebrovascular dysfunction. To extend these findings, we performed comparative studies of the MLCK210 KO mouse response to a diet-induced hyperhomocysteinemia (HHcy) model of chronic VCID. This model uses a B vitamin-deficient diet to induce elevated levels of plasma homocysteine, which leads to progressive BBB dysfunction and reproducible and quantitative cerebrovascular changes that mimic many of those found in clinical VCID (for review see Price et al. 2018). Thus, subjecting the MLCK210 KO model to the diet-induced HHcy model of chronic VCID allows a direct test of the hypothesis that MLCK210 is a viable target for progressive CNS diseases such as VCID. We report here that MLCK210 KO mice are protected from HHcy-induced microhemorrhage formation and pro-inflammatory biomarker changes, justifying further exploration of MLCK210 inhibition as a therapeutic strategy for chronic neurological diseases involving a BBB dysfunction mechanism.

Methods

Animals and experimental diet

The experiment was carried out in a 2 × 2 diet by geno-type design. All animals received 6 weeks of the HHcy diet (Envigo, #TD.97345)—deficient in vitamins B6, B9, and B12 with excess methionine—or nutritionally matched control diet with normal methionine and vitamin levels (Envigo, #TD.01636) (Sudduth et al. 2013; Sudduth et al. 2014; Sudduth et al. 2017). C57BL/6J mice (The Jackson Laboratory strain #664) were used as wild-type (WT) controls for the MLCK210 KO mice that were generated as previously reported (Wainwright et al. 2003). Eight MLCK210 KO mice (4 male/4 female) received control diet, and 8 (4M/4F) received HHcy diet. Eight WT mice (3M/5F) received control diet, and 10 (3M/7F) received HHcy diet. Animals were housed 1–4 per cage (503.22 usable cm²) in a room at 23 °C ± 2 °C, under a 14/10-h light/dark cycle beginning at 6:00 AM. All mice were administered experimental diet between 2 and 3 months of age and were sacrificed at 3.5–4.5 months of age after 6 weeks on diet. Mice had ad libitum access to water and chow.

Tissue collection

Mice were deeply anesthetized with 5% isoflurane and arterial blood collected from the left ventricle for measurement of homocysteine levels by the University of Kentucky Hospital clinical laboratory. Mice subsequently underwent transcardial perfusion with 50 ml ice-cold phosphate-buffered saline (PBS) at a flow rate of 10 ml/min before decapitation and brain removal and dissection. The left hemisphere was post-fixed in 4% paraformaldehyde for 24 h at 4 °C and cryo-protected in 30% sucrose for 48 h at 4 °C before sectioning. A portion of frontal cortex from the right hemisphere was...
dissected, flash frozen in liquid nitrogen, and stored at −80 °C until processing for biochemistry.

Immunohistochemistry for Prussian blue

The left hemisphere was cut coronally into 30 μm sections, with every 24th section collected for staining. A total of 5–7 sections per hemibrain were mounted and stained for hemosiderin using Prussian blue as described previously (Wilcock et al. 2004). Slides were incubated in a 2% potassium ferrocyanide in 2% hydrochloric acid solution for 15 min, followed by a counterstain in a 1% neutral red solution for 10 min. The number of Prussian blue positive profiles were counted across each section, and an average per-section value was generated for analysis.

Quantitative reverse-transcriptase polymerase chain reaction

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) was performed as previously reported (Sudduth et al. 2013), with all reagents acquired from Thermo Scientific (Rockford, IL, USA). Briefly, RNA was extracted from the right frontal cortex using the Trizol plus RNA purification system according to the manufacturer’s instructions. Total RNA was quantified with a nanodrop spectrophotometer, and cDNA made using the cDNA High Capacity kit according to instructions. Real-time PCR was performed using the TaqMan Gene Expression assay kit, and genes normalized to 18s rRNA. TaqMan probes were used to measure transcript levels of Arg1 (Mm00475988_m1), IL-1β (Mm00434228_m1), IL-10 (Mm00439616_m1), IL-12A (Mm00439506_m1), MMP2 (Mm00439506_m1), MMP3 (Mm00440295_m1), MMP9 (Mm00600163_m1), MMP14 (Mm00485054_m1), TIMP1 (Mm00441818_m1), TIMP2 (Mm00441825_m1), TNF-α (Mm00443258_m1), and YM1 (Mm00657889_mH). Fold change values were determined for mice receiving experimental diet relative to mice receiving control diet within the same genotype. For comparisons between WT and MLCK210 KO mice on control diet, fold change values were determined for the KO mice relative to WT. All fold change values were calculated using the $2^{(-ΔΔC_t)}$ method, and log2 normalized.

Statistical analyses

Statistical analyses and figure generation were performed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). Two-way analysis of variance (ANOVA) with Sidak’s post hoc testing was performed for most comparisons, with a statistical significance level set to $\alpha = 0.05$. For comparison of gene expression between WT and MLCK210 KO mice on control diet, $t$ tests were performed for each gene, followed by the two-stage step-up method (Benjamini et al. 2006) to control false discovery rate, with $Q = 10\%$. All graphs show means with error bars representing the standard error of the mean (SEM). Where reported in the text, data are described with $M = \text{mean}, SD = \text{standard deviation},$ and $CI = 95\% \text{ confidence interval.}$

Results

Experimental diet induces HHcy in both MLCK210 KO and WT mice, but MLCK210 KO mice are protected from HHcy-induced microhemorrhages

Plasma homocysteine concentration was measured in a subset of 3 mice per group, and levels on control diet were not significantly different between WT ($M = 5.89 \, \mu M, SD = 0.67, CI = 4.23–7.55$) and MLCK210 KO mice ($M = 6.34 \, \mu M, SD = 0.74, CI = 4.50–8.18$) (Fig. 1a). The HHcy model has been reported to induce cerebrovascular pathology such as cerebral microhemorrhages (Sudduth et al. 2013). Therefore, we measured microhemorrhages by Prussian blue staining. The average number of Prussian blue positive profiles per section was significantly elevated in the WT mice on HHcy diet ($M = 3.124, SD = 0.760, CI = 2.580–3.667$) versus control diet ($M = 0.421, SD = 0.297, CI = 0.173–0.669$) (Fig. 1b). In contrast, the MLCK210 KO mice were completely protected from HHcy-induced microhemorrhages, with no difference found between those mice on HHcy diet.
A gene expression panel related to neuroinflammation and BBB disruption, previously shown to change in response to HHcy, was screened for differences between WT and MLCK210 KO mice. The panel contained the inflammation-associated genes Arg1, IL-1β, IL-10, IL-12A, IL-1Ra, IL-6, TNF-α, and YM1, and the hemorrhage-implicated matrix remodeling genes MMP2, MMP3, MMP9, MMP14, TIMP1, and TIMP2 (Sudduth et al. 2013; Weekman et al. 2017). We first compared gene expression levels between the two genotypes at baseline, with values of the MLCK210 KO mice on control diet normalized to WT mice on control diet (Fig. 2). Unexpectedly, 6 genes were differentially expressed between the strains on control diet at baseline: MMP14, MMP3, MMP9, MMP14, TIMP1, and TIMP2. The basal mRNA levels of these 6 genes were significantly lower in MLCK210 KO compared to WT mice. Because baseline levels were different between strains, we normalized expression levels from mice on HHcy diet to within-genotype controls for characterization of diet-effects. Compared to WT control diet, plasma homocysteine was significantly elevated in both strains relative to control diet. There were no differences between WT and MLCK210 KO mice at baseline. Average Prussian blue positive microhemorrhages per section were elevated in WT mice in response to HHcy diet, but unchanged in MLCK210 KO mice. ***p < .001, ****p < .0001 vs. within-strain control, two-way ANOVA with Sidak’s post hoc tests.
associated changes. Interestingly, levels of MMP14, MMP2, MMP3, and MMP9 were all significantly up-regulated in the MLCK210 KO mice on the HHcy diet, in contrast to the WT mice on HHcy diet that showed no changes in MMP levels (Fig. 3a). No HHcy-induced changes were detected in either the TIMP1 or TIMP2 mRNA levels (Fig. 3b).

MLCK210 KO reduces HHcy-induced pro-inflammatory changes

Increased levels of pro-inflammatory cytokines have been implicated as both a driver and consequence of BBB hyperpermeability, and MLCK210 specifically plays a role in BBB dysfunction induced by IL-1β (Beard et al. 2014) and pro-inflammatory processes mediated via NFκB signaling (Recoquillon et al. 2015). To determine whether the rescue of BBB integrity in this model corresponds with an overall shift from pro- to anti-neuroinflammatory responses, we measured mRNA levels of pro-inflammatory cytokines IL-1β, TNF-α, IL-6, and IL-12A, and the generally anti-inflammatory immune modulators Arg1, IL-10, IL-1Ra, and YM1. In line with previous studies (Sudduth et al. 2013; Sudduth et al. 2017), mRNA levels of IL-1β, TNF-α, and IL-6 were significantly upregulated in response to HHcy diet in the WT mice (Fig. 4a). This effect was reduced in the MLCK210 KO mice, in which

![Graphs showing MMP and TIMP levels](image)
no significant HHcy-induced changes in these cytokines were detected. Interestingly, we found IL-12A mRNA significantly downregulated in the WT mice after HHcy, an effect reversed in the MLCK210 KO mice (Fig. 4a). In line with the attenuated pro-inflammatory response to HHcy diet in the MLCK210 KO mice, there was a concomitant increase in the anti-inflammatory factors IL-10, IL-1Ra, and YM1, an effect not observed in WT mice (Fig. 4b). Arg1 levels were unchanged in either strain in response to HHcy diet (Fig. 4b).

**Discussion**

Blood-brain barrier dysfunction is a critical factor in neurological dysfunction and neurodegeneration, contributing to multiple causes of cognitive impairment and dementia primarily affecting older populations. MLCK210 is an established regulator of tissue barrier permeability, the suppression of which is related to its potential as a therapeutic target in diverse preclinical models. The present study was designed to address whether such an approach might also be useful in some of the chronic stressors contributing to cerebrovascular damage. For this purpose, we subjected the MLCK210 KO mice to the dietary HHcy model of VCID that recapitulates many core pathophysiological features of cerebrovascular damage, including neuroinflammation and BBB disruption. We found that knockout of MLCK210 abrogated HHcy-induced microhemorrhage formation. In addition, MLCK210 KO mice showed a reduced neuroinflammatory profile after HHcy, as reflected in decreased transcript levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and...
increased levels of anti-inflammatory modulators (IL-10, IL-1Ra, YM1). Analysis of baseline expression levels of these genes between WT and MLCK210 KO mice indicated that these results were not likely due to a generally immunosuppressed phenotype in the KO animals. Overall, our findings using the MLCK210 KO mice extend the preponderance of data across diverse tissue barrier injury models that there is a potential for therapeutic benefit by targeting MLCK210 in future drug discovery efforts.

There were two interesting observations during the course of this study that remain to be addressed in future investigations. First, the finding that MMP levels were unchanged in the WT mice on HHcy diet despite significant microhemorrhage pathology and, conversely, MMP levels were elevated in the MLCK210 KO mice on HHcy diet without significant microhemorrhages was unexpected. Caution needs to be taken in the interpretation, however, as mRNA levels of the MMPs do not necessarily reflect the levels of the protein or protein activity (for review see Rempe et al. 2016). Therefore, it is not known if MMP enzyme activity differs between WT and MLCK210 KO mice on HHcy diet. To address whether MLCK210 might play a role in the homeostatic maintenance of brain extracellular matrix as mediated via the MMP system, future studies should directly measure MMP enzyme activity at baseline and in response to HHcy in MLCK210 KO mice. Second, IL-12A was an exception to the trend where most of the immunomodulatory gene expressions were altered. We previously found an increase in IL-12A transcript levels in hippocampus after 6 weeks on HHcy diet (Sudduth et al. 2017) compared to the decrease in cortical levels observed here (Fig. 3d). The main difference might simply reflect the anatomical region of measurement. This raises the potential of regional variability in brain neuroinflammatory responses to HHcy. IL-12A is also interesting in that it encodes a protein that can function as part of two different heterodimer cytokines: the pro-inflammatory IL-12 and the anti-inflammatory IL-35 (for review see Vignali and Kuchroo 2012). While not widely studied in the brain, IL-35 has recently been shown to have neuroprotective effects in a cerebral ischemia mouse model (Xu et al. 2018). Therefore, whether this HHcy-induced cortical decrease in IL-12A in the WT mice is reflective of pro- or anti-inflammatory processes remains to be determined.

In summary, the present study demonstrates that suppression of MLCK210 can provide protection in a model of chronic cerebrovascular dysfunction, and supports the hypothesis that MLCK210 is a viable target for progressive CNS diseases such as VCID. Future studies should explore MLCK210 inhibition as a therapeutic strategy for chronic neurological diseases involving cerebrovascular pathology and BBB dysfunction mechanisms.

Funding information This work was supported in part by a NIH postdoctoral fellowship F32 AG058456 (DJB).

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References

Al-Sadi R, Guo S, Dokladny K, Smith MA, Ye D, Kaza A, Watterson DM, Ma TY (2012) Mechanism of interleukin-1β induced-increase in mouse intestinal permeability in vivo. J Interf Cytokine Res 32:474–484. https://doi.org/10.1089/jir.2012.0031

Al-Sadi R, Youssef M, Rawat M, Guo S, Dokladny K, Haque M, Watterson DM, Ma TY (2019) MMP9-induced increase in intestinal epithelial tight permeability is mediated by p38 kinase signaling pathway activation of MLCK gene. Am J Physiol Gastrointest Liver Physiol 316:G278–G290. https://doi.org/10.1152/ajpgi.00126.2018

Arnaud C, Bouyon S, Recoquillon S, Brasseur S, Lemari E, Briancon-Marjollet A, Gonthier B, Toral M, Faury G, Martinez C, Andriantsitohaina R, Pepin JL (2018) Nonmuscle myosin light chain kinase: a key player in intermittent hypoxia-induced vascular alterations. J Am Heart Assoc 7:e007893. https://doi.org/10.1161/JAHA.117.007893

Beard RS, Haines RJ, Wu KY, Reynolds JJ, Davis SM, Elliott JE, Malinin NL, Chatterjee V, Cha BI, Wu MH, Yuan SY (2014) Non-muscle MLCK is required for β-catenin-and FoxO1-dependent downregulation of Cldn5 in IL-1β-mediated barrier dysfunction in brain endothelial cells. J Cell Sci 127:1840–1853. https://doi.org/10.1242/jcs.144550

Benjamini Y, Krieger AM, Yekutieli D (2006) Adaptive linear step-up procedures that control the false discovery rate. Biometrika 93:491–507. https://doi.org/10.1093/biomet/93.3.491

Clayburgh DR, Barrett TA, Tang Y, Meldings JB, Van Eldik LJ, Watterson DM, Clarke LL, Mrsny RJ, Turner JR (2005) Epithelial myosin light chain kinase–dependent barrier dysfunction mediates T cell activation–induced diarrhea in vivo. J Clin Invest 115:2702–2715. https://doi.org/10.1172/JCI24970
Hicks K, O’Neill RG, Dubinsky WS, Brown RC (2010) TRPC-mediated actin-myosin contraction is critical for BBB disruption following hypoxic stress. Am J Phys Cell Phys 298:C1583–C1593. https://doi.org/10.1152/ajpcell.00458.2009

Huppert J, Closden D, Croxford A, White R, Kulig P, Pietrowski J, Closhen D, Croxford A, White R, Kulig P, Pietrowski J, Closhen D (2015) The blood–brain barrier. Cold Spring Harb Perspect Biol 7:a020412. https://doi.org/10.1101/cshperspect.a020412

Fazal F, Bijli KM, Murrill M, Leonard A, Minhasuddin M, Anwar KN, Finkelstein JN, Watterson DM, Rahman A (2013) Critical role of non-muscle myosin light chain kinase in thrombin-induced endothelial cell inflammation and lung PMN infiltration. PLoS One 8:e59965. https://doi.org/10.1371/journal.pone.0059965

Gaceb A, Vergori L, Martinez MC, Andriantsitohaina R (2016) Activation of endothelial pro-resolving anti-inflammatory pathways by circulating microvesicles from non-muscular myosin light chain kinase-deficient mice. Front Pharmacol 7:322. https://doi.org/10.3389/fphar.2016.00322

Gilbert S, Zhang R, Denson L, Moriggl R, Steinbrecher K, Shroyer N, Lin J, Han X (2012) Enterocyte STAT5 promotes mucosal wound healing via suppression of myosin light chain kinase-mediated loss of barrier function and inflammation. EMBO Mol Med 4:109–124. https://doi.org/10.1002/emmm.201100192

Guo M, Yuan SY, Frederich BJ, Sun C, Shen Q, McLean DL, Wu MH (2012) Role of non-muscle myosin light chain kinase in neutrophil-mediated intestinal barrier dysfunction during thermal injury. Shock 38:436–443. https://doi.org/10.1097/SHK.0b013e3182868c731

Hicks K, O’Neil RG, Dubinsky WS, Brown RC (2010) TRPC-mediated actin-myosin contraction is critical for BBB disruption following hypoxic stress. Am J Phys Cell Phys 298:C1583–C1593. https://doi.org/10.1152/ajpcell.00458.2009

Huppert J, Closden D, Croxford A, White R, Kulig P, Pietrowski J, Beichman I, Beicher B, Luhmann HJ, Haig C, Waisman A, Kuhlmann CR (2010) Cellular mechanisms of IL-1β-induced blood-brain barrier disruption. FASEB J 24:1023–1034. https://doi.org/10.1096/fj.09-141978

Janelidze S, Hertz J, Nägga K, Nilsson K, Nilsson C, Swedish BioFINDER Study Group, Wennström M, van Westen D, Blennow K, Zetterberg H, Hansson O (2017) Increased blood-brain barrier permeability is associated with dementia and diabetes but not amyloid pathology or APOE genotype. Neurobiol Aging 51:104–112. https://doi.org/10.1016/j.neurobiolaging.2016.11.017

Khapchaev AV, Shirinsky VP (2016) Myosin light chain kinase MYLK1: anatomy, interactions, functions, and regulation. Biochem Mosc 81:1676–1697. https://doi.org/10.1134/S000629791613006X

Kuhlmann CRW, Tamaki R, Gamerdinger M, Lessmann V, Behl C, Kempski OS, Luhmann HJ (2007) Inhibition of the myosin light chain kinase prevents hypoxia-induced blood-brain barrier disruption. J Neurochem 103:501–507. https://doi.org/10.1111/j.1471-4159.2007.04506.x

Lorentz CA, Liang Z, Meng M, Chen CH, Yoseph BP, Breed ER, Mittal R, Klingensmith NJ, Farris AB, Burd EM, Koval M, Ford ML, CooperSmith CM (2017) Myosin light chain kinase knockout improves gut barrier function and confers a survival advantage in polymicrobial sepsis. Mol Med 23:155–165. https://doi.org/10.2119/molmed.2016.00256

Luh C, Feiler S, Frauenknecht K, Meyer S, Lubomirov LT, Neulen A, Thal SC (2018) The contractile apparatus is essential for the integrity of the blood-brain barrier after experimental subarachnoid hemorrhage. Transl Stroke Res Nov 23. https://doi.org/10.1007/s12975-018-0677-0

Luh C, Kuhlmann CR, Ackermann B, Timaru-Kast R, Luhmann HJ, Behl C, Werner C, Engelhard K, Thal SC (2010) Inhibition of myosin light chain kinase reduces brain edema formation after traumatic brain injury. J Neurochem 112:1015–1025. https://doi.org/10.1111/j.1471-4159.2009.06514.x

Mirzapaoazova T, Moitra J, Moreno-Vinasco L, Sammuni S, Turner JR, Chiang ET, Evenoski C, Wang T, Singleton PA, Haung Y, Lussier YA, Watterson DM, Dudek SM, Garcia J (2011) Non-muscle myosin light chain kinase isoform is a viable molecular target in acute inflammatory lung injury. Am J Respir Cell Mol Biol 44:40–52. https://doi.org/10.1165/rcmb.2009-0197OC

Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, Harrington MG, Chui HC, Law M, Zlokovic BV (2015) Blood-brain barrier breakdown in the aging human hippocampus. Neuron 85:296–302. https://doi.org/10.1016/j.neuron.2014.12.032

Nighot M, Al-Sadi R, Guo S, Rawat M, Nighot P, Watterson DM, Ma TY (2017) Lipopolysaccharide-induced increase in intestinal epithelial tight permeability is mediated by toll-like receptor 4/myeloid differentiation primary response 88 (MyD88) activation of myosin light chain kinase expression. Amer J Pathol 187:2698–2710. https://doi.org/10.1016/j.ajpath.2017.08.005

Ohlmann P, Tese A, Loichot C, Ralay Ranaivo H, Roul G, Philippe C, Watterson DM, Haiech J, Andriantsitohaina R (2005) Deletion of MLCK210 induces subtle changes in vascular reactivity but does not affect cardiac function. Am J Physiol Heart Circ Physiol 289:H2342–H2349. https://doi.org/10.1152/ajpheart.00511.2004

Price B, Wilcock DM, Weekman EM (2018) Hyperhomocysteinemia as a risk factor for vascular contributions to cognitive impairment and dementia. Front Aging Neurosci 10:350. https://doi.org/10.3389/fnagi.2018.00350

Ralay Ranaivo H, Carusio N, Wangensteen R, Ohlmann P, Loichot C, Tese A, Chalupsky K, Lobysheva I, Haiech J, Watterson DM, Andriantsitohaina R (2007) Protection against endotoxin-induced increase in intestinal epithelial tight permeability is mediated by toll-like receptor 4/myeloid differentiation primary response 88 (MyD88) activation of myosin light chain kinase expression. Am J Pathol 187:2698–2710. https://doi.org/10.1016/j.ajpath.2017.08.005

Recoquillon S, Carusio N, Lagrue-Lakhal A-H, Tuai-Chalot S, Filippelli A, Andriantsitohaina R, Martinez C (2015) Interaction in endothelium of non-muscular myosin light-chain kinase and the NF-κB pathway is critical to lipopolysaccharide-induced vascular hyporeactivity. Clin Sci (Lond) 129:687–698. https://doi.org/10.1042/CS20140625
Rempe RG, Hartz A, Bauer B (2016) Matrix metalloproteinases in the brain and blood–brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab 36:1481–1507. https://doi.org/10.1038/jcbfm.2016.45

Reynoso R, Perrin RM, Breslin JW, Daines DA, Watson KD, Watterson DM, Wu MH, Yuan S (2007) A role for long chain myosin light chain kinase (MLCK-210) in microvascular hyperpermeability during severe burns. Shock 28:589–595. https://doi.org/10.1097/SHK.0b013e31804d41ff

Rigor RR, Shen Q, Pivetti CD, Hu MH, Yuan SY (2013) Myosin light chain kinase signaling in endothelial barrier dysfunction. Med Res Rev 33:911–933. https://doi.org/10.1002/med.21270

Rossi JL, Todd T, Bazan NG, Belayev L (2013) Inhibition of myosin light-chain kinase attenuates cerebral edema after traumatic brain injury in postnatal mice. J Neurotrauma 30:1672–1679. https://doi.org/10.1089/neu.2013.2898

Rossi JL, Velentza AV, Steinhorn DM, Watterson MS (2007) MLCK210 gene knockout or kinase inhibition preserves lung function following endotoxin-induced lung injury in mice. Amer J Physiol: Lung Cell Mol Physiol 292:L1327–L1334. https://doi.org/10.1152/ajplung.00380.2006

Sudduth TL, Powell DK, Smith CD, Greenstein A, Wilcock DM (2013) Induction of hyperhomocysteinemia models vascular dementia by induction of cerebral microhemorrhages and neuroinflammation. J Cereb Blood Flow Metab 33:708–715. https://doi.org/10.1038/jcbfm.2013.1

Sudduth TL, Weekman EM, Brothers HM, Braun K, Wilcock DM (2014) β-amyloid deposition is shifted to the vasculature and memory impairment is exacerbated when hyperhomocysteinemia is induced in APP/PS1 transgenic mice. Alzheimers Res Ther 6:1–11. https://doi.org/10.1186/alztr262

Sudduth TL, Weekman EM, Price BR, Gooch JL, Woolums A, Norris CM, Wilcock DM (2017) Time-course of glial changes in the hyperhomocysteinemia model of vascular cognitive impairment and dementia (VCID). Neuroscience 341:42–51. https://doi.org/10.1016/j.neuroscience.2016.11.024

Sun C, Wu MH, Yuan S (2011) Nonmuscle myosin light-chain kinase deficiency attenuates atherosclerosis in apolipoprotein E-deficient mice via reduced endothelial barrier dysfunction and monocyte migration. Circulation 124:48–57. https://doi.org/10.1161/CIRCULATIONAHA.110.88915

Usatuyk PV, Singleton PA, Pendyala S, Kalari SK, He D, Gorshkova IA, Camp SM, Moitra J, Dudek SM, Garcia JGN, Natarajan V (2012) A novel role for non-muscle myosin light chain kinase (MLCK) in hyperoxia-induced recruitment of cytoskeletal proteins, NADPH oxidase activation, and reactive oxygen species generation in lung endothelium. J Biol Chem 287:9360–9375. https://doi.org/10.1074/jbc.M111.294546

Vignali DA, Kuchroo VK (2012) IL-12 family cytokines: immunological playmakers. Nat Immun 13:722–728. https://doi.org/10.1038/nri.2366

Wainwright MS, Rossi J, Schavocky J, Crawford S, Steinhorn D, Velentza AV, Zasadzki M, Shirinsky V, Jia Y, Haiech J, Van Eldik LJ, Watterson DM (2003) Protein kinase involved in lung injury susceptibility: evidence from enzyme isofrom genetic knockout and in vivo inhibitor treatment. Proc Natl Acad Sci U S A 100(10):6233–6238. https://doi.org/10.1073/pnas.1031595100

Wang T, Mathew B, Wu X, Shimizu Y, Rizzo AN, Dudek SM, Weichselbaum RR, Jacobson JR, Hecker L, Garcia JGN (2016) Nonmuscle myosin light chain kinase activity modulates radiation-induced lung injury. Pulm Circ 6:234–239. https://doi.org/10.1080/20489032.2016.1180525

Wang T, Moreno-Vinasco L, Ma S-F, Zhou T, Shimizu Y, Sammani S, Epshtein Y, Watterson DM, Dudek SM, Garcia JG (2014) Nonmuscle myosin light chain kinase regulates murine asthmatic inflammation. Am J Respir Cell Mol Biol 50:1129–1135. https://doi.org/10.1165/rcmb.2013-0434OC

Weekman EM, Woolums AE, Sudduth TL, Wilcock DM (2017) Hyperhomocysteinemia-induced gene expression changes in the cell types of the brain. ASN Neuro 9:1759091417742296. https://doi.org/10.1177/1759091417742296

Wilcock DM, Rojiani A, Rosenhal A, Subbarao S, Freeman MJ, Gordon MN, Morgan D (2004) Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. J Neuroinflammation 1:24. https://doi.org/10.1186/1742-2094-1-24

Wu L-L, Peng W-H, Kuo W-T, Huang C-Y, Ni Y-H, Lu K-S, Turner JR, Yu LCH (2014) Commensal bacterial endocytosis in epithelial cells is dependent on myosin light chain kinase-activated brush border fanning by interferon-γ. Am J Pathol 184:2260–2274. https://doi.org/10.1016/j.ajpath.2014.05.003

Xiong Y, Wang C, Shi L, Wang L, Zhou Z, Chen D, Wang J, Guo H (2017) Myosin light chain kinase: a potential target for treatment of inflammatory diseases. Front Pharmacol 8:292. https://doi.org/10.3389/fphar.2017.00292

Xu C, Zhu H, Shen R, Feng Q, Zhou H, Zhao Z (2018) IL-35 is a protective immunomodulator in brain ischemic injury in mice. Neurochem Res 43:1454–1463. https://doi.org/10.1007/s11064-018-2560-5

Zahs A, Bird MD, Ramirez L, Turner JR, Choudhry MA, Kovacs EJ (2012) Inhibition of long myosin light-chain kinase activation alleviates intestinal damage after binge ethanol exposure and burn injury. Am J Physiol Gastrointest Liver Physiol 303:G705–G712. https://doi.org/10.1152/ajpgi.00157.2012

Zhang HF, Li TB, Liu B, Lou Z, Zhang JJ, Peng JJ, Zhang XJ, Ma M, Wang T, Moreno-Vinasco L, Ma S-F, Zhou T, Shimizu Y, Sammani S, Epshtein Y, Watterson DM, Dudek SM, Garcia JG (2014) Nonmuscle myosin light chain kinase regulates murine asthmatic inflammation. Am J Respir Cell Mol Biol 50:1129–1135. https://doi.org/10.1165/rcmb.2013-0434OC

Weekman EM, Woolums AE, Sudduth TL, Wilcock DM (2017) Hyperhomocysteinemia-induced gene expression changes in the cell types of the brain. ASN Neuro 9:1759091417742296. https://doi.org/10.1177/1759091417742296

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