Trends in Developmental Biology
Vol. 10, 2017

Review

Cerebral cavernous malformation 3 and cerebrovascular disease

Kang Wang[1], Huanjiao Jenny Zhou[2] and Wang Min[1,2,*]

[1]Center for Translational Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China. [2]Department of Pathology and the Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT, USA.

ABSTRACT

Cerebral cavernous malformations (CCMs) are vascular lesions characterized by enlarged and irregular structure of small blood vessels in the brain, which can result in increased risk of stroke, focal neurological defects and seizures. CCMs can occur as a sporadic or familial autosomal dominant form. Three different genes, *CCM1/KRIT1*, *CCM2/MGC4607* and *CCM3/PDCD10*, have now been identified as the main targets which are involved in the CCMs' progression. These three CCM proteins have similar or unique function in maintaining the normal structure of small blood vessels. However, *CCM3* mutation results in a more severe form of the disease which may suggest we should pay more attention on the area of *CCM3*. The current research focused on the angiogenic function and mechanisms of *CCM3* including endothelial cell junction, proliferation, migration and permeability, and these findings may offer some potential targets for CCMs' therapy.

KEYWORDS: CCM3, PDCD10, cell junction, angiogenesis, GCKIII, EndMT.

1. Introduction

Cerebral cavernous malformations are collections of capillaries in the brain which are abnormal in structure. These capillaries have abnormally thin vessel walls, and they lack support tissues like elastic fibers which make them stretchy, resulting in increased risk of stroke, focal neurological defects and seizures. CCMs have been reported to affect about 0.5 percent of the population worldwide. Most CCMs occur in the central nervous system except for some that are located in the retina or skin [1]. There is no medicine available to treat CCMs yet, and the only treatment for CCMs is surgical resection for now.

CCMs may be familial or sporadic. Familial cases are caused by mutations in one of three CCM genes: *CCM1/KRIT1*, *CCM2/malcavernin*, or *CCM3/PDCD10*. These CCM patients usually develop multiple lesions in the brain. Whereas sporadic CCMs occur in people with no family history of the disorder. These individuals often present with a single lesion. Three different genes are involved in familial CCMs: *KRIT1* (Krev interaction trapped 1, also known as *CCM1*), *CCM2* (*MGC4607*) and *PDCD10* (programmed cell death 10, also known as *CCM3*) [2-4]. Loss-of-function mutations in any one of the three genes can result in the formation of CCMs, which suggests there is an essential pathway involving all three CCM proteins [5]. However, mutations in *CCM3* are often associated with higher risk of early-onset cerebral hemorrhage [6] and more severe form of the disease [7], suggesting a separate or unique role of *CCM3* compared with *CCM1* and *CCM2*.

Bergametti *et al*. [4] first identified *PDCD10* as the third CCM gene, which is located on 3q25.2-27. This locus was identified within a 22-cM

*Corresponding author: wang.min@yale.edu

interval flanked by D3S1763 and D3S1262 [8]. *CCM3* has no paralogue in the human genome; it seems to be highly conserved in vertebrates and invertebrates according to database searching [9]. The highly conserved gene encodes a 212-amino acid protein which is ubiquitously expressed. It contains an N-terminal dimerization domain and a C-terminal focal adhesion targeting-homology (FAT-H) domain [10, 11]. CCM3 involves in different pathway by the interaction of the two domains and their target proteins. CCM3/PDCD10 was originally proved to be a protein inhibiting the natural cell death of 293 cells [12]. After the relationship between PDCD10 and CCMs was elucidated, more and more functions of this protein related to CCMs have been illuminated. This review will mainly focus on the role of CCM3 in affecting cell junction, maintaining normal structure and function of vascular endothelial cells, and regulating angiogenesis, which all can be involved in the progression of CCMs.

2. CCM3 and the cerebrovascular disease

2.1. Two-hit mechanism in *CCM3* mutation

CCMs have been proved to develop through a 'two-hit' mechanism; patients with CCMs get one mutated allele of one of the CCM genes from their parents, and somatic mutation at the second allele make the complete loss-of-function of one of the CCM genes [13, 14]. More than 150 different mutations of *CCM1/CCM2/CCM3* have been found to date [15-17]. Those mutations almost all lead to a premature termination code through different mechanisms including nonsense, splice-site and frameshift mutaions [18].

2.2. The role of CCM3 in cell junction

The normal type of cell junction of endothelial cells is important to maintain the normal structure and function of blood vessels, which were often disrupted in CCM lesions. Cell-cell junction especially tight junction is essential to prevent blood-borne compounds from leaking to brain parenchyma, which further leads to inflammatory responses, endothelial injury and lesion progression [19-21]. Tight junction is built on the interactions between transmembrane tight junction proteins (claudin-5, occludin, JAM-A), scaffolding proteins (ZO-1, -2, -3, Af-6, VASP, Par3, Par6) and the actin cytoskeleton [22-23]. Stamatovic *et al*. [24] found that CCM3 regulates the integrity of brain endothelial barrier and the organization of tight junction complex. Loss of CCM3 activates ERK1/2; this induces Ser phosphorylation (pS405) of cortactin (a cortical actin ring protein), which leads to cortactin degradation. Increased cortactin degradation is associated with loss interaction of the cortical actin ring and ZO-1: actin, which is essential for providing physical support and anchoring of tight junction proteins. The reduced anchoring of ZO-1 to the actin cytoskeleton impact organization of tight junction proteins, and eventually disrupt the tight junction complex.

It has been demonstrated that *CCM2* knockdown in brain endothelial cells resulted in the activation of RhoA by dysregulating Smurf1, a ubiquitin-protein ligase (E3) that controls RhoA degradation [25-26]. Overabundant RhoA can inhibit vessel-like tube formation and increase endothelial monolayer permeability. Borikova *et al*. [27] proved that *CCM3* knockdown in endothelial cells also show RhoA overexpression and activation. Overexpressed RhoA activates ROCK, which phosphorylates several substrates like myosin light chain and LIM kinase to regulate cytoskeleton dynamics. Activated ROCK also regulates vascular permeability which is essential for the normal function of blood vessels [29]. By phosphorylating the myosin light chain, ROCK can increase the formation of actin stress fiber (microtubules) [30]; p-MLC enables myosin to bind to the actin filaments, and therefore increase the cellular contraction rate. The increased contractility in cells impact β-catenin and VE-cadherin lining the vascular walls [31], then the cell-cell adhesion of the endothelial cells is disrupted, which result in enlargement of blood vessels and unstable vessel structure, and thus leading to vascular leakage and initiation of CCM progression [25]. Faurobert *et al*. [32] proved that extracellular matrix (ECM) aberrant remolding is associated with CCM progression. CCM1-CCM2 complex directly binds to ICAP-1 [33], a negative regulator of β1 integrin, and loss of CCM1 or CCM2 will destabilize ICAP-1, and hence β1 integrin will be activated after the destabilization of ICAP-1, and then activated β1 integrin activates RhoA and ROCK pathway. β1 integrin also affect normal

FN fibrillogenesis [32]. Despite no results have proved the association between CCM3 and β1 integrin, mounting evidence suggest that the CCM progression share a common pathway, and that CCM3 can be involved in β1 integrin signaling.

The N-terminal dimerization domain and C-terminal FAT-H domain enable CCM3 to bind to different proteins including CCM2. CCM2 acts as a hub to bind both CCM1 and CCM3 [33], and hence CCM3 may affect RhoA pathway by the interaction with CCM2. CCM3 also may affect tight junction by indirectly interacting with CCM1, as CCM1 has been proved to maintain Rap-1-mediated stabilization of endothelial junction and VE-Cadherin-mediated interaction of endothelial cells [31, 34]. CCM3 associates with STRIPAK (striatin-interacting phosphatase and kinase) complex and interacts with PP2A phosphatases and GCKIII family kinase (MST4, STK24, SKT25) [35], which in turn activate RhoA inhibitor moesin by phosphorylating Thr-558 of moesin [36]. CCM3 and GCKIII family associated with striatin also interact with the CDC-42 binding kinase MRCK, which is essential for junction formation by promoting circumferential acting bundles [37].

2.3. The role of CCM3 in angiogenesis

Angiogenesis is a process that involves endothelial cells proliferation, migration and morphology remodeling. He *et al*. [38] found that *CCM3* knockdown in HUVECs significantly reduced endothelial cell proliferation and induced cell apoptosis, and also inhibited VEGF-induced endothelial cell cord formation. CCM3 specifically associates with VEGFR2 and was required for stabilization of VEGFR2, thereby maintaining the VEGF signaling pathway which is essential for angiogenesis. But there were different views about the role of CCM3 in angiogenesis. You *et al*. [39] proved that knockdown of *CCM3* in HUVECs significantly stimulates angiogenesis behaviors including proliferation, migration and sprouting. They found that silencing of *CCM3* in HUVECs significantly downregulates DLL4 expression and impaired DLL4-Notch signaling therefore activating endothelial angiogenesis. Deletion of DLL4 reduced the expression of VEGFR1 but increased VEGFR2, thereby stimulating cell proliferation, migration and sprouting [40]. These data suggest the level of DLL4 may determine endothelial angiogenesis by regulating the balance of VEGFR1 and VEGFR2. Impaired DLL4-Notch signaling also activated Erk1/2, thus affecting cell junction, and cell permeability. You *et al*. [41] also found that silencing of *CCM3* in endothelial cells significantly activates EphB4 kinase activity by up-regulating EphB4 mRNA and protein expression, accompanied by activation of Erk1/2, and hence CCM3 may regulate angiogenesis by endothelial signaling pathway of CCM3-DLL4/Notch-EphB4-Erk1/2.

CCM3 can be involved in different signaling pathways as an anchor protein which can bind to different target proteins. Hence the role of CCM3 in endothelial cells may be controversial when different pathways are coexistent. The best-characterized interaction between CCM3 and its target proteins lies in the dimerization-domain-mediated interaction with the GCKIII group of kinases, MST4/MASK, STK24/MST3 and STK25/YSK1/SOK1 [42]. Chan *et al*. [43] proved that CCM3 interaction with GCKIII is critical in lumen formation in *Drosophila*, and this result may suggest a similar function of CCM3 in human cells. But it's known that the GCKIII family is represented by a single protein in *Drosophila*, whereas a complex in mammalian, and it seems that the three kinases are functionally redundant in mammalian cells because loss of any single of the kinases doesn't affect lumen formation; only when loss of STK25 along with loss of either STK24 or MST4 will reproduce the lumen formation defects, and hence these data may suggest a more reliable and robust system in maintaining the normal lumen formation in mammalians due to the complex vasculature. Each of the GCKIII family kinases have been implicated in regulating different cellular functions [44-46]. Zhang *et al*. [47] proved that CCM3 associates with STK24 and regulates exocytosis in neutrophils. STK24 binds to UNC13D C2B domain and prevent UNC13D from binding to lipids, a step important for vesicle docking. CCM3 can stabilize the STK24 protein, and there will be a degranulation phenotype in neutrophils during loss of either CCM3 or STK24. Zhou *et al*. [48] also found that CCM3 regulates exocytosis in endothelial cells. CCM3 forms a complex with UNC13B and STK24, and inhibits UNC13B-mediated intracellular molecules exocytosis.

Thus, loss of CCM3 increases the release of ANGPT-2 from WPBs in brain endothelial cells. Increased ANGPT-2 secretion to the extracellular space disrupts the association between endothelial cells and pericytes, leading to enhanced endothelial cell spouting, and lumen formation followed by CCM lesion formation. Zheng *et al*. [49] found that loss of CCM3 or STK24 also results in actin stress fiber formation and elevated RhoA activation in endothelial cells, and hence ANGPT-2 induced excessive sprouting and lumen formation along with weakened cell adhesion and increased permeability result in an abnormal and disrupted blood vessel. CCM3-GCKIII complex also regulates Golgi assembly and cell orientation by binding to GM130, a Golgi-resident protein. Cell loss of CCM3 cannot reorient the Golgi and centrosome properly, and demonstrate impaired migration. CCM3 also stabilize the GCKIII kinases to maintain the ability to phosphorylate their substrate 14-3-3ζ, which is essential for Golgi assembly [44]. Goudreault *et al*. [35] proved that STRIPAK associates with both PP2 (protein phosphatase 2) and CCM3, which means there may be a linkage between CCM3 and PP2. It's known that PP2 can be involved in many intracellular pathways by targeting different substrates, and hence CCM3 may exhibit a more complicated function due to the linkage with PP2. Zhou *et al*. [50] found that CCM complex associated with GCKIII kinases regulates MEKK3 pathway in endocardial cells. Endocardial deletion of CCM1/CCM2/CCM3 activates MEKK3 and the downstream MEK4 and ERK5. Activated ERK5 could be transported into the nuclei and upregulate the transcription factor KLF2/4 and proteases ADAMTS4/5. Zhou *et al*. [51] also demonstrated that MEKK3-KLF2/4 signaling is critical for the CCM progression. Inhibition of MEK5 or ERK5 reversed the increase of KLF2/4, and anti-ADAMTS proteases may be more effective. CCM3 also binds to other proteins like paxillin, although the full functional significance of this interaction is not yet understood. Some evidence show paxillin takes part in MST3 autophosphorylation-dependent inhibition of cell migration [52], and hence CCM3 may regulate cell migration by the interaction with paxillin.

2.4. Potential mechanisms by which CCM3 regulates CCM progression

Endothelial-to-mesenchymal transition (EndMT) has been demonstrated with cardiac fibrosis and cancer progression, and it is characterized by the acquisition of mesenchymal and stem-cell-like features of endothelium [53, 54], The progression of EndMT leads to disrupted cell junction organization, loss of cell polarity, increased cell proliferation and migration [55]. Maddaluno *et al*. [56] found EndMT exists in *CCM1*^{ECKO} mice with disorganized VE-Cadherin and significantly up-regulated N-Cadherin, and this progress is mediated by the upregulation of endogenous BMP6, which in turn activates the TGF-β (transforming growth factor-β) and BMP (bone morphogenetic protein) signaling pathway. Inhibiting TGF-β or BMP pathway prevents EndMT and reduces CCM lesion in mice. They also found that endothelial cells from *CCM3*^{ECKO} mice present a similar phenotype to those from *CCM1*^{ECKO} mice, which means EndMT is a common feature of loss-of-function mutation-induced CCMs. Studies have proved that loss of CCM proteins induced inhibition of Notch, and upregulated KLF4 can activate BMP pathway, thus resulting in EndMT [57, 58]. Autophagy is also related to CCM formation and EndMT [59]. *CCM1*-deleted endothelial cells present suppression in autophagy with increased levels of p62 (p62 is a receptor for ubiquitinated cargoes and delivers them to autophagosome, p62 itself is degraded by autophagy) and total LC3 (autophagy protein microtubule-associated protein 1 light chain 3), the mechanism may lie in *CCM1* deletion induced up-regulation of mTOR-ULK1 pathway. They also found that down-regulation of autophagy gene *ATG7* in HUVECs (human umbilical vein endothelial cells) suppressed autophagy and was associated with EndMT progression, and down-regulation of autophagy-related protein p62 in *CCM1*-deleted endothelial cells suppressed the expression of mesenchymal markers such as *PAI1*, *Cd44* and *Id1*, and these suggest autophagy is associated with EndMT. Bravi *et al*. [60] found that Wnt-independent stimulation of β-catenin transcription activity in *CCM3*-deficient endothelial cells and β-catenin transcriptional activity promotes TGF-β/BMP signaling and consequent EndMT.

CCM2 interacts with TrkA, a receptor tyrosine kinase involved in prosurvival signaling in the nervous system, through the PTB domain [61]. CCM3-GCKIII kinase indirectly linkes with TrkA by interaction with CCM2, and hence CCM3 may regulate the cell death signaling pathway in neural cells. Louvi *et al*. [62] found that CCM3 deletion in neural cells results in a vascular phenotype that resemble human CCMs, which suggests CCM3 may affect CCM progression through cell death pathway. ROS (Reactive oxygen species) have been proved to affect tight junctions in endothelial cells by impairing the cytoskeleton and blood-brain barrier, which are related to CCMs. Fidalgo *et al*. [36] found that CCM3-GCKIII kinase (MST4) mediates ERM(ezrin/radixin/moesin) phosphorylation and cell survival after ROS stimulating, and it has been proved that disturbed Notch signaling is related to ROS accumulation [63], all of these suggesting ROS may be a factor implicated in loss of CCM3-dependent CCMs progression.

2.5. Progression in CCM therapy

The only treatment for CCM disease is surgical resection so far, although there is high risk for cerebral operation. To date, no medical therapy has been approved. Based on the researches of molecular mechanism which regulates CCM progression, some drugs which affect intracellular signaling pathway can be effective in animal trials. Administration of fasudil, a Rho-kinase inhibitor, resulted in attenuated CCM lesion in mice with CCM1 mutations [30]. Statin inhibits HMG-CoA reductase, which reduces RhoA-dependent small GTPase activation, and presents a symptomatic improvement in mouse model [25], but administration of statin was associated with increased risk of intracerebral hemorrhage [64], and hence more researches should be done before the application of statin in CCM therapy. Inhibition of TGF (transforming growth factor) signaling also presents exciting results, while administration of LY-364947, an inhibitor of TGF-β type 1 receptor, significantly inhibited EndMT transition in CCM1 mouse model [57]. Sulindac, an anti-inflammatory drug, can attenuate CCM development by suppressing β-catenin activity [61]. ANGPT2-neutralizing antibody significantly reduces CCM lesion formation in *CCM3*^{ECKO} mice [48]. TLR4 (Toll-like receptor 4) antagonists and alteration of microbiome can affect CCM formation in mice [65]. Many advances have been made, but the cure for CCM lesion is still unknown. Further study is still needed to uncover novel mechanisms regulating CCMs and possible drugs preventing the progression of CCMs.

3. Conclusion

As an anchor protein, CCM3 binds to different types of proteins, which enable it takes part in different intracellular signaling which affect cell junction, angiogenesis, apoptosis and stress action (Figure 1). When we focus on different pathways, some controversial result may exist, but we cannot ignore that CCM lesion is a comprehensive result of all different pathways in cells, not only endothelial cells. Despite the mounting knowledge about the role of CCM3 in the progression of CCMs, there are still many unclearness that needs to be clarified. We still don't know why a ubiquitously expressed CCM3 protein involves in the progression of CCMs exclusively in CNS (central nervous system), although some explanations lie in the relationship between neural cells and endothelial cells, but details are still unknown. We don't know the effect of CCM3 on translational and post-translational modification of those tight junction proteins, and how CCM3 regulates β1 integrin signaling. We don't know the detailed relationship between CCM3-UNC13B-ANGPT2-Tie2 axis and cell junction which affect CCM lesion development, and how Golgi polarization which is regulated by CCM proteins contributes to vascular defects and CCMs. Recent study uncovered a relationship between innate immune/microbiome and CCM lesion in mice with CCM1/2 deficiency, and CCM3 also may be linked to the innate immune pathway as loss of any one of the three CCM genes leads to a similar phenotype. We also should notice that there will be a possibility of other CCM-related genes, as mutations in *CCM1/CCM2/CCM3* don't cover all the familiar cases. There is no doubt that further studies are necessary to better understand the mechanism of the progression of CCMs and find a non-invasive therapy for CCM disease.

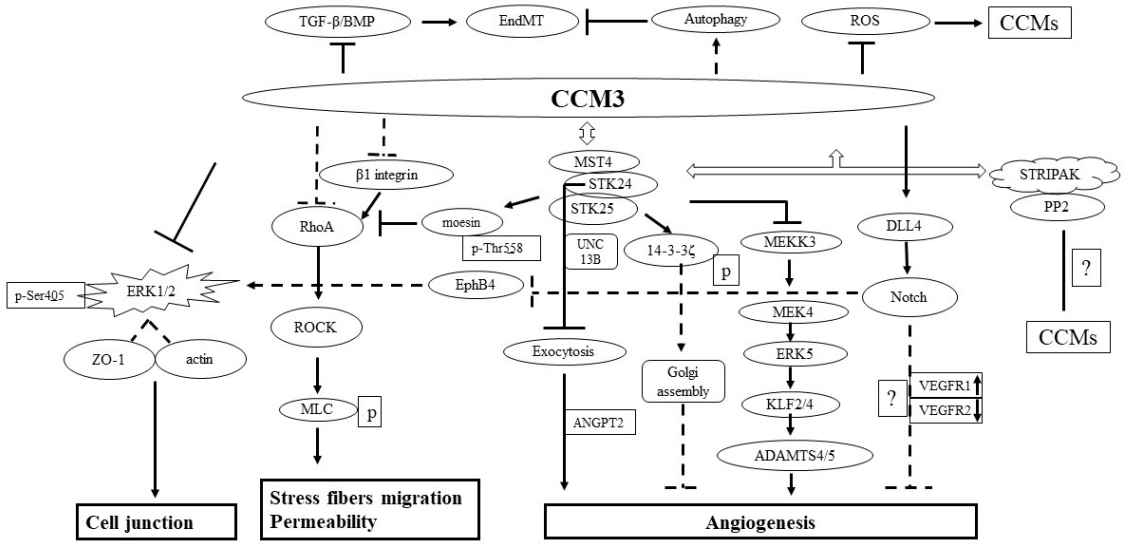

Figure 1. The possible role of CCM3 in different pathways regulating CCM formation. CCM3 maintains cell junction by inhibiting ERK1/2 phosphorylation, CCM3 inhibits stress fiber migration and endothelial permeability by inhibiting RhoA signaling, CCM3 regulates angiogenesis with or without binding to GCKIII kinases, and CCM3 also involves in EndMT transition, autophagy and ROS stimulation.

ACKNOWLEGEMENTS

This work was supported by National Natural Science Foundation of China (No. 91539110, U1601219, and 81371019), National Key Research and Development Program of China (2016YFC 1300600), Scientific Grant of Guangzhou (201604020131), and Scientific Grants of Guangdong (No. 2015B020225002 and 2015A050502018). This work was partly supported by NIH grants R01 HL109420 and HL115148.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

REFERENCES

1. Labauge, P., Denier, C., Bergametti, F. and Tournierlasserve, E. 2007, Lancet Neurology, 6(3), 237.
2. Labauge, P. 1999, European Journal of Human Genetics, 7(4), 499-504.
3. Denier, C., Goutagny, S., Labauge, P., Krivosic, V., Arnoult, M. and Cousin, A. 2004, American Journal of Human Genetics, 74(2), 326.
4. Bergametti, F., Denier, C., Labauge, P., Arnoult, M., Boetto, S. and Clanet, M. 2005, American Journal of Human Genetics, 76(1), 42.
5. Stahl, S., Gaetzner, S., Voss, K., Brackertz, B., Schleider, E. and Sürücü, O. 2008, Human Mutation, 29(5), 709-717.
6. Fauth, C., Rostasy, K., Rath, M., Gizewski, E., Lederer, A. G. and Sure, U. 2015, Clinical Neurology and Neurosurgery, 128, 41-43.
7. Shenkar, R., Shi, C., Rebeiz, T., Stockton, R. A., Mcdonald, D. A. and Mikati, A. G. 2015, Genet. Med., 17(3), 188-196.
8. Craig, H. D., Günel, M., Cepeda, O., Johnson, E. W., Ptacek, L. and Steinberg, G. K. 1998, Human Molecular Genetics, 7(12), 1851-1858.
9. Revencu, N. and Vikkula, M. 2006, Journal of Medical Genetics, 43(9), 716-21.
10. Kean, M. J., Ceccarelli, D. F., Goudreault, M., Sanches, M., Tate, S. and Larsen, B. 2011, Journal of Biological Chemistry, 286(28), 25065-75.
11. Li, X., Ji, W., Zhang, R., Folta-Stogniew, E., Min, W. and Boggon, T. J. 2011, Journal of Biological Chemistry, 286(29), 26138-47.
12. Wang, Y., Liu, H., Zhang, Y. and Ma, D. 1999, Science in China Series C: Life Sciences, 42(3), 323-329.

13. Akers, A. L., Johnson, E., Steinberg, G. K., Zabramski, J. M. and Marchuk, D. A. 2008, Human Molecular Genetics,18(5), 919-930.
14. Pagenstecher, A., Stahl, S., Sure, U. and Felbor, U. 2008, Human Molecular Genetics, 18(5), 911-918.
15. Laberge-le Couteulx, S., Jung, H. H., Labauge, P., Houtteville, J. P., Lescoat, C., Cecillon, M. and Tournier-Lasserve, E. 1999, Nature Genetics, 23(2), 189.
16. Laurans, M. S., DiLuna, M. L., Shin, D., Niazi, F., Voorhees, J. R., Nelson-Williams, C. and Scott, R. M. 2003, Journal of Neurosurgery, 99(1), 38-43.
17. Liquori, C. L., Penco, S., Gault, J., Leedom, T. P., Tassi, L., Esposito, T. and Marchuk, D. A. 2008, Neurogenetics, 9(1), 25-31.
18. Riant, F., Bergametti, F., Ayrignac, X., Boulday, G. and Tournier-Lasserve, E. 2010, The FEBS Journal, 277(5), 1070-1075.
19. Clatterbuck, R. E., Eberhart, C. G., Crain, B. J. and Rigamonti, D. 2001, Journal of Neurology, Neurosurgery and Psychiatry, 71(2), 188-192.
20. Frischer, J. M., Pipp, I., Stavrou, I., Trattnig, S., Hainfellner, J. A. and Knosp, E. 2008, Journal of Neurology, Neurosurgery and Psychiatry, 79(7), 783-788.
21. Shenkar, R., Venkatasubramanian, P. N., Zhao, J. C., Batjer, H. H., Wyrwicz, A. M. and Awad, I. A. 2008, Neurosurgery, 63(4), 782-789.
22. Paris, L., Tonutti, L., Vannini, C. and Bazzoni, G. 2008, Biochimica et Biophysica Acta (BBA)-Biomembranes, 1778(3), 646-659.
23. Mitic, L. L. and Anderson, J. M. 1998, Annual Review of Physiology, 60(1), 121-142.
24. Stamatovic, S. M., Sladojevic, N., Keep, R. F. and Andjelkovic, A. V. 2015, Acta Neuropathologica, 130(5), 731-750.
25. Whitehead, K. J., Chan, A. C., Navankasattusas, S., Koh, W., London, N. R., Ling, J. and Marchuk, D. A. 2009, Nature Medicine, 15(2), 177.
26. Crose, L. E., Hilder, T. L., Sciaky, N. and Johnson, G. L. 2009, Journal of Biological Chemistry, 284(20), 13301-13305.
27. Borikova, A. L., Dibble, C. F., Sciaky, N., Welch, C. M., Abell, A. N., Bencharit, S. and Johnson, G. L. 2010, Journal of Biological Chemistry, 285(16), 11760-11764.
28. Jaffe, A. B. and Hall, A. 2005, Annu. Rev. Cell Dev. Biol., 21, 247-269.
29. Chrissobolis, S. and Sobey, C. G. 2006, Stroke, 37(8), 2174-2180.
30. Stockton, R. A., Shenkar, R., Awad, I. A. and Ginsberg, M. H. 2010, Journal of Experimental Medicine, 207(4), 881-96.
31. Glading, A. J. and Ginsberg, M. H. 2010, Disease Models and Mechanisms, 3(1-2), 73-83.
32. Faurobert, E., Rome, C., Lisowska, J., Manet-Dupé, S., Boulday, G., Malbouyres, M. and Coll, J. L. 2013, J. Cell Biol., 202(3), 545-561.
33. Hilder, T. L., Malone, M. H., Bencharit, S., Colicelli, J., Haystead, T. A., Johnson, G. L. and Wu, C. C. 2007, Journal of Proteome Research, 6(11), 4343-4355.
34. Glading, A., Han, J., Stockton, R. A. and Ginsberg, M. H. 2007, J. Cell Biol., 179(2), 247-254.
35. Goudreault, M., D'Ambrosio, L. M., Kean, M. J., Mullin, M. J., Larsen, B. G., Sanchez, A. and Aebersold, R. 2009, Molecular and Cellular Proteomics, 8(1), 157-171.
36. Fidalgo, M., Guerrero, A., Fraile, M., Iglesias, C., Pombo, C. M. and Zalvide, J. 2012, Journal of Biological Chemistry, 287(14), 11556-11565.
37. Lant, B., Yu, B., Goudreault, M., Holmyard, D., Knight, J. D., Xu, P. and Gingras, A. C. 2015, Nature Communications, 6, 6449.
38. He, Y., Zhang, H., Yu, L., Gunel, M., Boggon, T. J., Chen, H. and Min, W. 2010, Sci. Signal., 3(116), ra26.
39. You, C., Erol Sandalcioglu, I., Dammann, P., Felbor, U., Sure, U. and Zhu, Y. 2013, Journal of Cellular and Molecular Medicine, 17(3), 407-418.
40. Suchting, S., Freitas, C., le Noble, F., Benedito, R., Bréant, C., Duarte, A. and Eichmann, A. 2007, Proceedings of the National Academy of Sciences, 104(9), 3225-3230.

41. You, C., Zhao, K., Dammann, P., Keyvani, K., Kreitschmann-Andermahr, I., Sure, U. and Zhu, Y. 2017, Journal of Cellular and Molecular Medicine, 21(9), 1848-1858.
42. Sugden, P. H., McGuffin, L. J. and Clerk, A. 2013, Biochemical Journal, 454(1), 13-30.
43. Chan, A. C., Drakos, S. G., Ruiz, O. E., Smith, A. C., Gibson, C. C., Ling, J. and Grossmann, A. H. 2011, The Journal of Clinical Investigation, 121(5), 1871-1881.
44. Wu, H. Y., Lin, C. Y., Chen, T. C., Pan, S. T. and Yuan, C. J. 2011, The International Journal of Biochemistry and Cell Biology, 43(5), 742-750.
45. Fidalgo, M., Fraile, M., Pires, A., Force, T., Pombo, C. and Zalvide, J. 2010, J. Cell Sci., 123(8), 1274-1284.
46. Lorber, B., Howe, M. L., Benowitz, L. I. and Irwin, N. 2009, Nature Neuroscience, 12(11), 1407.
47. Zhang, Y., Tang, W., Zhang, H., Niu, X., Xu, Y., Zhang, J. and Min, W. 2013, Developmental Cell, 27(2), 215-226.
48. Zhou, H. J., Qin, L., Zhang, H., Tang, W., Ji, W., He, Y. and Toomre, D. 2016, Nature Medicine, 22(9), 1033.
49. Zheng, X., Xu, C., Di Lorenzo, A., Kleaveland, B., Zou, Z., Seiler, C. and Pack, M. A. 2010, The Journal of Clinical Investigation, 120(8), 2795-2804.
50. Zhou, Z., Rawnsley, D., Goddard, L., Pan, W., Cao, X. J., Jakus, Z. and Li, D. 2015, Developmental Cell, 32(2), 168.
51. Zhou, Z., Tang, A. T., Wong, W. Y., Bamezai, S., Goddard, L. M., Shenkar, R. and Arthur, J. S. C. 2016, Nature, 532(7597), 122.
52. Lu, T. J., Lai, W. Y., Huang, C. Y. F., Hsieh, W. J., Yu, J. S., Hsieh, Y. J. and Tang, M. J. 2006, Journal of Biological Chemistry, 281(50), 38405-38417.
53. Zeisberg, E. M., Tarnavski, O., Zeisberg, M., Dorfman, A. L., McMullen, J. R., Gustafsson, E. and Neilson, E. G. 2007, Nature Medicine, 13(8), 952.
54. Medici, D., Shore, E. M., Lounev, V. Y., Kaplan, F. S., Kalluri, R. and Olsen, B. R. 2010, Nature Medicine, 16(12), 1400.
55. Kalluri, R. and Weinberg, R. A. 2009, Journal of Clinical Investigation, 119(6), 1420-1428.
56. Maddaluno, L., Rudini, N., Cuttano, R., Bravi, L., Giampietro, C., Corada, M. and Tournier-Lasserve, E. 2013, Nature, 498(7455), 492.
57. Cuttano, R., Rudini, N., Bravi, L., Corada, M., Giampietro, C., Papa, E. and Jain, M. K. 2016, EMBO Molecular Medicine, 8(1), 6-24.
58. Wüstehube, J., Bartol, A., Liebler, S. S., Brütsch, R., Zhu, Y., Felbor, U. and Fischer, A. 2010, Proceedings of the National Academy of Sciences, 107(28), 12640-12645.
59. Marchi, S., Corricelli, M., Trapani, E., Bravi, L., Pittaro, A., Delle Monache, S. and Trabalzini, L. 2015, EMBO Molecular Medicine, 7(11), 1403-1417.
60. Bravi, L., Rudini, N., Cuttano, R., Giampietro, C., Maddaluno, L., Ferrarini, L. and Dejana, E. 2015, Proceedings of the National Academy of Sciences, 112(27), 8421-8426.
61. Harel, L., Costa, B., Tcherpakov, M., Zapatka, M., Oberthuer, A., Hansford, L. M. and Avigad, S. 2009, Neuron, 63(5), 585-591.
62. Louvi, A., Chen, L., Zhang, H., Min, W. and Günel, M. 2011, Proceedings of the National Academy of Sciences, 108(9), 3737-3742.
63. Bheeshmachar, G., Purushotaman, D., Sade, H., Gunasekharan, V., Rangarajan, A. and Sarin, A. 2006, The Journal of Immunology, 177(8), 5041-5050.
64. Westover, M. B., Bianchi, M. T., Eckman, M. H. and Greenberg, S. M. 2011, Archives of Neurology, 68(5), 573-579.
65. Tang, A. T., Choi, J. P., Kotzin, J. J., Yang, Y., Hong, C. C., Hobson, N. and Cao, Y. 2017, Nature, 545(7654), 305.