DOI: https://doi.org/10.53350/pjmhs2023172645

ORIGINAL ARTICLE

Delta-Like Factor 1 Negatively Regulates Angiogenesis as a Target Gene of MIR-126-5P after Indirect Revascularization Surgery in Patients with Moyamoya Disease

LT COL AYYAZ HUSSAIN AWAN TI(M)[1], HUSSNAIN MOHAMMAD BUKSH[2], ALI ZAIN UL ABIDIN[3]
[1]*Associate Professor Ophthalmology, Akhtar Saeed Medical College Lahore*
[2]*Medical Officer Lahore General Hospital*
[3]*Assistant Professor King Edward Medical University*
Corresponding author: Lt Col Ayyaz Hussain Awan TI(M), Email: ayyazhussainawan3@gmail.com

ABSTRACT

Introduction: Moyamoya disease (MMD) is a chronic cerebral hypoperfusion state with increased narrowing of the intracranial internal carotid artery.

Objectives: The main objective of the study is to analyses the delta-like factor 1 negatively regulates angiogenesis as a target gene of miR-126-5p after indirect revascularization surgery in patients with moyamoya disease.

Material and methods: This retrospective study was conducted in Akhtar Saeed Medical College Lahore during 2020 to 2021. First, we compared the DLK1 expression in DM tissues from patient with moyamoya disease (n = 8) and unruptured aneurysms (n = 8, control group). DM samples (1.0 × 0.5 cm) were collected from patients with moyamoya disease during revascularization procedures. DM samples were harvested from the temporal part of the head and stored for further use.

Results: EPC markers CD31, VE-cadherin, and VEGFR-2 were strongly expressed in cultured cells, while hematopoietic marker CD133 did not display remarkable expression, suggesting highly purified EPC isolation.

Practical implication: This study will be helpful in EC proliferation and angiogenesis.

Conclusion: It is concluded that DLK1, a target gene of miR-126-5p, negatively regulates EC proliferation and angiogenesis and that downregulating DLK1 expression potentially promotes angiogenesis in chronically ischaemic brains.

Keywords: DLK1, EC, Proliferation, Angiogenesis

INTRODUCTION

Moyamoya disease (MMD) is a chronic cerebral hypoperfusion state with increased narrowing of the intracranial internal carotid artery. Treatments such as direct and indirect revascularization surgery are used for patients with MMD. Encephalo-myo-synangiosis (EMS) is a simple, indirect revascularization surgery for younger patients with MMD, yet EMS can provide inadequate collateral flow with a possible case of an ischemic stroke[1 1]. To enhance angiogenesis in the brain cortex, EMS should be combined with gene therapy. A previous study analyzed the results from an indirect revascularization surgery combined with vascular endothelial growth factor (VEGF) gene in the temporal muscle of a rat with chronic cerebral hypoperfusion. In addition, the current study examined the effect of combined gene therapy with VEGF plus apelin during indirect revascularization surgery[2 2]. EMS showed indirect bypass surgery for the hypoperfusion state after bilateral common carotid artery ligation. Thus, angiogenesis in the brain cortex progressed when EMS worked with gene therapy. Other treatments for chronic hypoperfusion involve the use of limb remote ischemic conditioning (LRIC). This method is neuroprotective for white matter lesions after ischemia, but the means it protects after chronic cerebral hypoperfusion is unknown[33]. A recent study did find that PTEN/Akt/mTOR signaling pathways were activated after LRIC.

Delta-like factor 1 (DLK1) is encoded by a paternally imprinted gene on human chromosome 14 and mouse chromosome 12 and is highly expressed during embryonic development. DLK1 is synthesized as a membrane-bound precursor that has a signal peptide, six epidermal growth factor (EGF)-like extracellular repeats, a juxta-membrane region and a short intracellular tail. Numerous of studies have demonstrated that DLK1 plays a regulatory role in a series of mesoderm differentiation processes, including adipogenesis, myogenesis and osteo blastogenesis[4]. However, a few reports recently suggested that DLK1 negatively regulates EC proliferation. Given that 1) both our previous study and studies from other scholars demonstrated that microRNA-126-5p (miR-126-5p) promotes EC proliferation) bioinformatics analysis (both TargetScan and miRDB) indicated the existence of a potential binding site between miR-126-5p and DLK1, and 3) a relatively authoritative study suggested that miR126-5p and DLK1 interact, we hypothesized that DLK1 inhibits EC proliferation and angiogenesis in patients with moyamoya disease as a target gene of miR-126-5p[5].

Objectives: The main objective of the study is to analyses the delta-like factor 1 negatively regulates angiogenesis as a target gene of miR-126-5p after indirect revascularization surgery in patients with moyamoya disease.

MATERIAL AND METHODS

This retrospective study was conducted in Akhtar Saeed Medical College Lahore during 2020 to 2021. First, we compared the DLK1 expression in DM tissues from patient with moyamoya disease (n = 8) and unruptured aneurysms (n = 8, control group). DM samples (1.0 × 0.5 cm) were collected from patients with moyamoya disease during revascularization procedures. DM samples were harvested from the temporal part of the head and stored for further use. All fresh samples were used for quantitative real-time polymerase chain reaction (qRT–PCR), frozen in liquid nitrogen and stored at -80°C. Quantitative PCR (qRT–PCR). TRIzol reagent (Invitrogen, Guangzhou, China) was used to isolate total RNA from HUVECs or DM tissues, and the Advantage RT-for-PCR Kit (TaKaRa, Otsu, Japan) was used to perform reverse transcription with U6 serving as the endogenous control. Briefly, sections were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 5 min at room temperature, permeabilized, and blocked for 30 min with 0.1% Triton X-100 and 1% bovine serum albumin.

Statistical Program for Social Science (SPSS) version 22.0 was used for all statistical analyses. The data are presented as the means ± standard deviations (SDs).

RESULTS

EPC markers CD31, VE-cadherin, and VEGFR-2 were strongly expressed in cultured cells, while hematopoietic marker CD133 did not display remarkable expression, suggesting highly purified EPC isolation.

The overall mortality rate after the surgery was 9.89% (9/91). As we observed that both EMS and EMS combined with EPC treatment resulted in amelioration of microcirculation impairment after BICAL, we further performed the rotarod test to assess functional outcome in these 2 groups after BICAL.

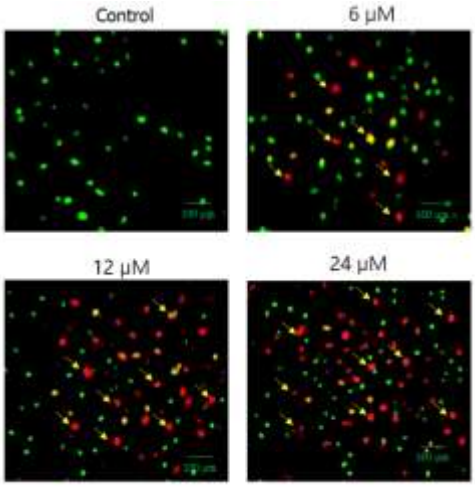

Figure 1: Characterization of human umbilical cord blood-derived late EPCs. A Late EPCs expressed endothelial markers CD31, VE-cadherin, and VEGFR-2, but did not express hematopoietic marker CD133 by immunofluorescence staining. The cell nuclei were counterstained with Hoechst 33,342. A negative control was incubated with a secondary antibody only.

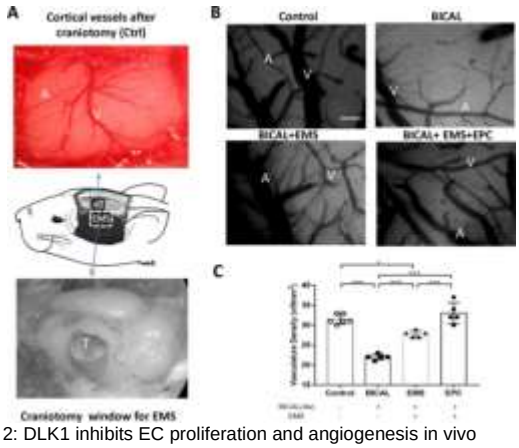

Figure 2: DLK1 inhibits EC proliferation and angiogenesis in vivo

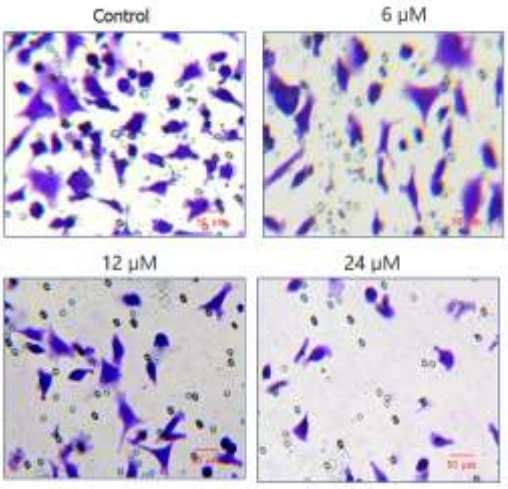

Figure 3: Representative TEM images of vacuoles and TJs in each group. Red arrows indicate vacuoles, green arrowheads indicate intact TJs, and blue arrowheads indicate abnormal TJs. Scale bar = 1 μm. Quantitation of the number of vacuoles per 10 mm of vascular endothelium in each group.

DISCUSSION

DLK1 can negatively regulate anastomosis formation after indirect revascularization surgery. We previously discovered that performing the EMS procedure on 2VO rats could successfully induce angiogenesis in the ischaemic brain covered by TM tissue (the same mechanism as the EMS effect on patients with moyamoya disease) and that the angiogenesis induced in the rats peaked in the 4th week after the EMS procedure[7]. Therefore, we used the 2VO+EMS rat model to determine whether DLK1 regulates angiogenesis in chronically ischaemic brains after indirect revascularization surgery (i.e., EMS)[8]. Four weeks after establishment of the rat model and TM transfection, TM-covered brain tissue was analysed; compared to the control group, the Lv-DLK1 group had significantly higher expression of DLK1 and significantly lower expression of both eNOS and CD31 (an EC marker). These in vivo expression patterns of the abovementioned cytokines were consistent with the in vitro findings regarding the DLK1/eNOS pathway[9]. In addition, the TEM results revealed that DLK1 exerted a negative effect on EC repair and angiogenesis (more vacuoles and fewer intact TJs in the Lv-DLK1 group). Furthermore, the MWM test was performed to determine whether the increase in angiogenesis induced by DLK1 downregulation could improve the impaired cognitive function caused by chronic ischaemia[10]. The cognitive improvement in the shDLK1 group was significantly more extensive than that in the control group. Therefore, we can infer that the DLK1/eNOS pathway, which is downstream of miR126-5p, negatively regulate anastomosis formation in the ischaemic brain after indirect revascularization surgery[11-13].

CONCLUSION

It is concluded that DLK1, a target gene of miR-126-5p, negatively regulates EC proliferation and angiogenesis and that downregulating DLK1 expression potentially promotes angiogenesis in chronically ischaemic brains.

REFERENCES

1. Ando T, Shimada Y, Fujiwara S, Yoshida K, Kobayashi M, Kubo Y, et al. Revascularisation surgery improves cognition in adult patients with moyamoya disease. J Neurol Neurosurg Psychiatry. 2020;91(3):332-4. https://doi.org/10.1136/jnnp-2019-321069.
2. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, et al. MicroRNA126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med. 2014;20(4):368-76. https://doi.org/10.1038/ nm.3487.
3. Nakamura M, Imai H, Konno K, Kubota C, Seki K, Puentes S, et al. Experimental investigation of encephalomyosynangiosis using gyrencephalic brain of the miniature pig: histopathological evaluation of dynamic reconstruction of vessels for functional anastomosis. Laboratory investigation. J Neurosurg Pediatr. 2009;3(6):488-95. https://doi. org/10.3171/2008.6.PEDS0834.
4. Rodríguez-Roiz, J.M., Sastre-Solsona, S., Popescu, D. *et al.* The relationship between ACL reconstruction and meniscal repair: quality of life, sports return, and meniscal failure rate—2- to 12-year follow-up. *J Orthop Surg Res* 15, 361 (2020). https://doi.org/10.1186/s13018-020-01878-1
5. Sarraj M, Coughlin RP, Solow M, Ekhtiari S, Simunovic N, Krych AJ, MacDonald P, Ayeni OR. Anterior cruciate ligament reconstruction with concomitant meniscal surgery: a systematic review and meta-analysis of outcomes. Knee Surg Sports Traumatol Arthrosc. 2019 Nov;27(11):3441-3452. doi: 10.1007/s00167-019-05389-3. Epub 2019 Feb 4. Erratum in: Knee Surg Sports Traumatol Arthrosc. 2019 Mar 13;: PMID: 30719542.
6. Wang, KC., Yang, LY., Lee, JE. *et al.* Combination of indirect revascularization and endothelial progenitor cell transplantation improved cerebral perfusion and ameliorated tauopathy in a rat model of bilateral ICA ligation. *Stem Cell Res Ther* 13, 516 (2022). https://doi.org/10.1186/s13287-022-03196-1
7. Shear A, Nishihiro S, Hishikawa T, Hiramatsu M, Sugiu K, Yasuhara T, Date I. Cerebral circulation improves with indirect bypass surgery combined with gene therapy. Brain Circ. 2019 Sep 30;5(3):119-123. doi: 10.4103/bc.bc_33_19. PMID: 31620658; PMCID: PMC6785951.
8. Li S, Zhao W, Han C, Rajah GB, Ren C, Xu J, et al. Safety and efficacy of remote ischemic conditioning in pediatric moyamoya

disease patients treated with revascularization therapy. *Brain Circ.* 2017;3:213–8.
9. Lei C, Lin S, Zhang C, Tao W, Dong W, Hao Z, et al. Effects of high-mobility group bo×1 on cerebral angiogenesis and neurogenesis after intracerebral hemorrhage. *Neuroscience.* 2013;229:12–9.
10. Ishii Y, Tanaka Y, Momose T, Yamashina M, Sato A, Wakabayashi S, et al. Chronologic evaluation of cerebral hemodynamics by dynamic susceptibility contrast magnetic resonance imaging after indirect bypass surgery for moyamoya disease. *World Neurosurg.* 2017;108:427–35
11. So Y, Lee HY, Kim SK, Lee JS, Wang KC, Cho BK, et al. Prediction of the clinical outcome of pediatric moyamoya disease with postoperative basal/acetazolamide stress brain perfusion SPECT after revascularization surgery. *Stroke.* 2005;36:1485–9.
12. Pianta S, Lee JY, Tuazon JP, Castelli V, Mantohac LM, Tajiri N, et al. A short bout of exercise prior to stroke improves functional outcomes by enhancing angiogenesis. *Neuromolecular Med.* 2019 Epub ahead of print.
13. . Nahirney PC, Reeson P, Brown CE. Ultrastructural analysis of blood-brain barrier breakdown in the periinfarct zone in young adult and aged mice. J Cereb Blood Flow Metab. 2016;36(2):413-25. https://doi. org/10.1177/0271678X15608396.