PP-02

내피세포 및 오가노이드 모델을 이용한 동정맥기형의 비정상적 혈관신생 모델링과 약물치료 가능성 연구

(Modeling Aberrant Angiogenesis in Arteriovenous Malformations Using Endothelial Cells and Organoids for Pharmacological Treatment)

경북대학교 의과대학 성형외과학교실[1]

경북대학교 의과대학 성형외과학교실 세포 및 기질 연구소[2]

경북대학교 의과대학 창의미래인재양성을 위한 KNU 의생명과학 융합 교육연구단 [3]

강명재[1], 오은정[2], 김현미[3], 곽수인[2], 정호윤[1,2,3,*]

Purpose: Arteriovenous malformations (AVMs) are congenital vascular anomalies characterized by abnormal direct connections between arteries and veins. This study investigates the therapeutic potential of thalidomide, U0126, and rapamycin in modeling aberrant angiogenesis and identifying pharmacological targets using patient-derived endothelial cells (ECs) and organoids.

Methods: Thirty human samples were examined, comprising 10 normal vascular samples, 10 AVM-derived vascular samples, and 10 AVM skin samples for hiPSC reprogramming. ECs were isolated and treated with thalidomide (10 μM), rapamycin (10 nM), and U0126 (10 μM) for 24 h. Cellular morphology, immunofluorescence (CD31, VEGF, ANG2), qRT-PCR for angiogenesis-related genes (VEGF, ANG2, FSTL1, MARCKS, CSPG4), TaqMan assay for miR-135b-5p, and tube formation assays were performed. AVM blood vessel organoids were generated from hiPSCs and analyzed by whole-mount immunofluorescence after pharmacological treatment.

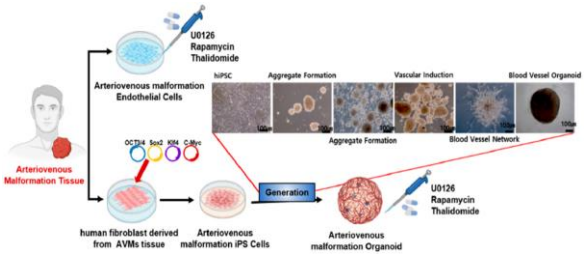

Fig 1. Generation of AVM organoids and analysis of response to pharmacological treatment. The generation and analysis of AVM organoids derived from human fibroblasts obtained from AVM tissue. Human dermal fibroblasts were reprogrammed into induced pluripotent stem cells (iPSCs), followed by differentiation, during which organoid structures were formed, mimicking the vascular architecture associated with AVMs. The AVM ECs and AVM organoids were treated with pharmacological inhibitors (thalidomide, rapamycin, and U0126) to assess drug responses.

Results: Pharmacological treatments reduced the proliferation of AVM ECs and downregulated miR-135b-5p. Expression levels of VEGF, ANG2, FSTL1, and MARCKS decreased, whereas CSPG4 was upregulated. Tube formation was significantly suppressed. In AVM organoids, treatment resulted in reduced expression of CD31 and α-SMA and disruption of vascular network integrity. Among the tested agents, thalidomide demonstrated the most pronounced anti-angiogenic effect.

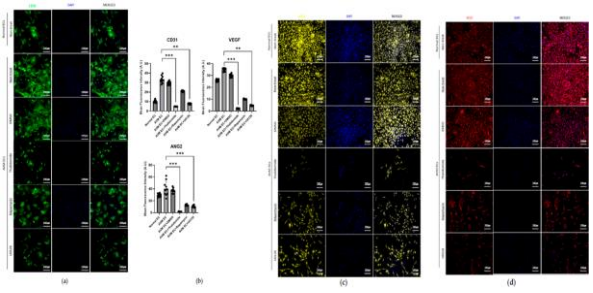

Fig 2. Immunofluorescence image of AVM ECs treated with thalidomide (10 μM, 24 h), rapamycin (10 nM, 24 h), and U0126 (10 μM, 24 h) and stained for CD31 (green), VEGF (red), ANG2 (yellow), and nuclei (DAPI, blue). (a) CD31; (b) Quantification of fluorescence intensity following pharmacological treatment, analyzed using Fiji ImageJ software 1.54h (National Institutes of Health, Bethesda, MD, USA); (c) ANG2; (d) VEGF. Scale bar: 200 μm, ** p ≤ 0.01, *** p ≤ 0.001.

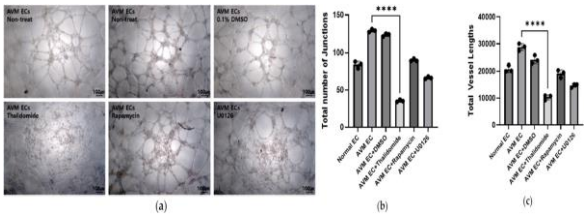

Fig 3. Pharmacological inhibition suppresses tube formation in AVM ECs. Image of AVM EC tube formations: untreated and DMSO-treated controls and those treated with thalidomide (10 μM, 24 h), rapamycin (10 nM, 24 h), or U0126 (10 μM, 24 h), (a) Representative images of ECs tube formation assay after 24 h exposure to pharmacological treatment (×10, scale bar: 100 μm). (b) Quantification of total number of junctions using Fiji ImageJ software 1.54h (National Institutes of Health, Bethesda, MD, USA), (c) Quantification of total vessel lengths using Fiji ImageJ software 1.54h (National Institutes of Health, Bethesda, MD, USA); **** p < 0.0001.

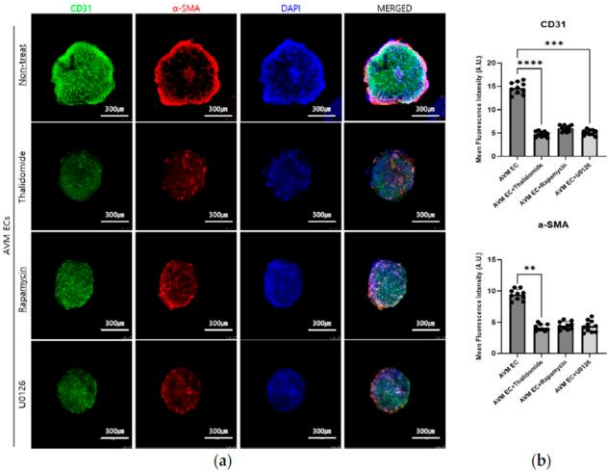

Fig 4. Pharmacological treatment reduces the expression of CD31 (green), α-SMA (red), and DAPI (blue) in AVM blood vessel organoids. Scale bar: 300 μm, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001. Whole-mount immunofluorescence image of the AVM blood vessel organoids (BVOs) treated with thalidomide (10 μM, 24 h), and rapamycin (10 nM, 24 h), U0126 (10 μM, 24 h), and stained with CD31 (green), α-SMA (red), and DAPI (blue), (a) Representative confocal z-stack images of AVM BVOs, (b) Quantification of fluorescence intensity.

Conclusion: Thalidomide, rapamycin, and U0126 exert anti-angiogenic effects in AVM ECs and organoids, with thalidomide showing the most significant efficacy. The establishment of AVM blood vessel organoids offers a physiologically relevant in vitro model for disease characterization and drug screening and may support the development of personalized therapies for AVM patients.