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Embryonic Stem Cell-like Population within Venous Malformation Expresses the Renin–Angiotensin System

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Background: We have recently demonstrated the presence of a NANOG$^+$/pSTAT3$^+$/OCT4$^+$/SOX2$^+$/SALL4$^+$/CD44$^+$ embryonic stem cell (ESC)-like subpopulation localized to the endothelium and a NANOG$^+$/pSTAT3$^+$/SOX2$^+$/CD44$^+$ subpopulation outside of the endothelium, within subcutaneous VM (SCVM) and intramuscular VM (IMVM). We have also shown the expression of components of the renin-angiotensin system (RAS): (pro)renin receptor (PRR); angiotensin converting enzyme (ACE), angiotensin II receptor 1 (ATIIR1) and angiotensin II receptor 2 (ATIIR2), in both SCVM and IMVM. This study investigated whether the ESC-like subpopulations within SCVM and IMVM expressed the RAS.

Methods: Formalin-fixed paraffin-embedded sections of two representative samples from each of the seven SCVM and seven IMVM patients included in our previous studies underwent dual immunofluorescence (IF) immunohistochemical (IHC) staining for ESC marker OCT4 or SOX2 with PRR, ACE, ATIIR1, and ATIIR2.

Results: IF IHC staining demonstrated the expression PRR by the OCT4$^+$ cells on the endothelium and outside the endothelium in SCVM and IMVM. ACE was expressed by the SOX2$^+$ cells, predominantly in the endothelium in SCVM and IMVM. ATIIR1 was expressed by the SOX2$^+$ cells on the endothelium and outside the endothelium in SCVM and IMVM. ATIIR2 was expressed by the OCT4$^+$ endothelium and outside the endothelium in SCVM and IMVM.

Conclusions: Components of the RAS are expressed by ESC-like subpopulations within both SCVM and IMVM. These primitive subpopulations may be a novel therapeutic target by manipulation of the RAS using existing medications.*(Plast Reconstr Surg Glob Open 2019;7:e2170; doi: 10.1097/GOX.0000000000002170; Published online 2 April 2019.)*

Venous malformation (VM) consists of a network of thin-walled ectatic venous channels with deficient media,[1] and TIE2 and PIK3CA mutations have been demonstrated.[2] Management of VM remains unsatisfactory.[2]

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There are 2 embryonic stem cell (ESC)-like subpopulations within subcutaneous VM (SCVM) and intramuscular VM (IMVM): one on the endothelium expressing the ESC markers NANOG, pSTAT3, OCT4, SOX2, SALL4, and CD44; and another outside the endothelium expressing NANOG, pSTAT3, SOX2, and CD44.[2] Both SCVM and IMVM express components of the renin–angiotensin system (RAS).[1] This study investigated whether the primitive subpopulations within SCVM and IMVM express the RAS.

Four-micrometer-thick formalin-fixed paraffin-embedded sections of 2 representative samples each from 7 SCVM (mean age 22.9 years) and 7 IMVM (mean age 21.1 years) patients included in our previous studies,[1,2] underwent immunofluorescence (IF) immunohistochemi-

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cal (IHC) staining with primary antibodies OCT4 (1:30; cat#MRQ-10, Cell Marque, Santa Cruz, Calif.), SOX2 (1:200, cat#PA1-094, Thermo Fisher Scientific, Waltham, Mass.), (pro)renin receptor (PRR) (1:2000; cat#AB40790, Abcam, Cambridge, Mass.), ACE (1:40; cat#3C5, Serotec, Raleigh, N.C.), angiotensin II receptor 1 (ATIIR1) (1:25; cat#ab9391, Abcam), and angiotensin II receptor 2 (ATIIR2) (1:2000; cat#NBPI-77368, Novus Biologicals, Littleton, Colo.). Vectafluor Excel anti-rabbit 594 (ready-to-use; cat#VEDK-1594, Vector Laboratories, Burlingame, Calif.) and Vectafluor Excel anti-mouse (ready-to-use; cat#VEDK2488, Vector Laboratories) secondary antibodies combinations, were used for IF IHC detection.

IF IHC-stained slides were viewed and imaged using an Olympus FV1200 biological confocal laser-scanning microscope, and the images were deconvoluted using cellSens Dimension 1.11 software 2D deconvolution algorithm (Tokyo, Japan).

(Pro)renin receptor (red) was expressed by the $OCT4^+$ (green) cells on the endothelium and pericytes in SCVM (Fig. 1A) and IMVM (**see figure, Supplemental Digital Content 1**, http://links.lww.com/PRSGO/B49, which displays representative IF IHC-stained sections of IMVM, demonstrating expression of PRR (A, red) by the $OCT4^+$ [A, green] cells on the endothelium and cells outside of the endothelium, and ACE [B, green] and ATIIR1 [C, green] by the $SOX2^+$ [B and C, red] cells on the endothelium and cells outside the endothelium. Angiotensin II receptor 2 [D, red] was expressed by the $OCT4^+$ [D, green] cells on the endothelium and cells outside the endothelium. Cell nuclei were counterstained with 4′, 6′-diamindino-2-phenylindole [A–D, blue]. Original magnification: 600×, http://links.lww.com/PRSGO/B23). ACE (green) was expressed by the $SOX2^+$ (red) cells, predominantly in the endothelium in SCVM (Fig. 1B) and IMVM (**see figure, Supplemental Digital Content 1**, http://links.lww.com/PRSGO/B49). Angiotensin II receptor 1 (green) was

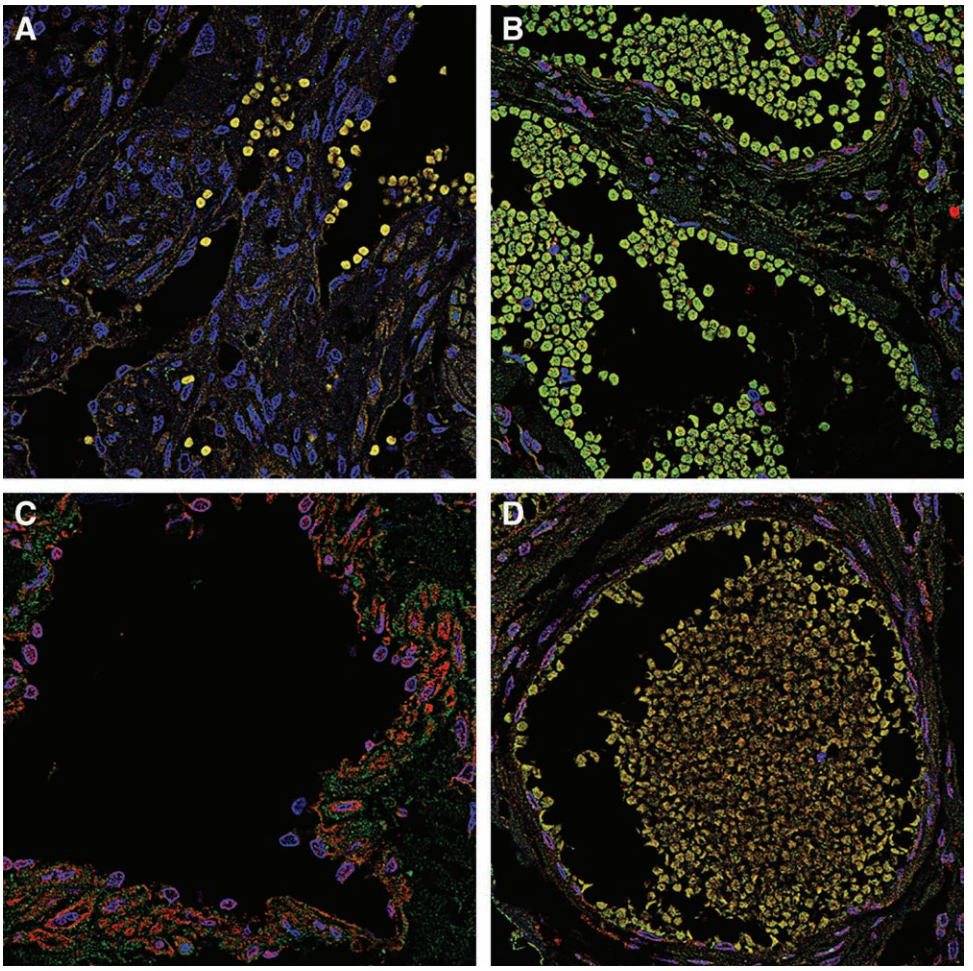

Fig. 1. Representative IF IHC-stained sections of SCVM, demonstrating expression of PRR (A, red) by the $OCT4^+$ (A, green) cells on the endothelium and cells outside of the endothelium, and ACE (B, green) and ATIIR1 (C, green) by the $SOX2^+$ (B and C, red) cells on the endothelium and cells outside the endothelium. Angiotensin II receptor 2 (D, red) was expressed by the $OCT4^+$ (D, green) cells on the endothelium and cells outside the endothelium. Cell nuclei were counterstained with 4′, 6′-diamindino-2-phenylindole (A–D, blue). Original magnification: 600×.

expressed by the $SOX2^+$ (red) cells on the endothelium and pericytes in SCVM (Fig. 1C) and IMVM (**see figure, Supplemental Digital Content 1**). Angiotensin II receptor 2 (red) was expressed by the $OCT4^+$ (green) endothelium and pericytes in SCVM (Fig. 1D) and IMVM (**see figure, Supplemental Digital Content 1**, http://links.lww.com/PRSGO/B49). Negative controls for IF IHC staining for SCVM and IMVM samples demonstrated minimal staining (data not shown; **see figure, Supplemental Digital Content 1**, http://links.lww.com/PRSGO/B49).

We hypothesize that the ESC-like subpopulations may give rise to cells in VM, and that the RAS may sustain these ESC-like subpopulations. The novel finding of the expression of components of the RAS by the ESC-like subpopulations within SCVM and IMVM suggests that these primitive cells may be a novel therapeutic target by manipulation of the RAS using medications such as β-blockers and ACE inhibitors. In vitro and in vivo functional studies are needed to determine the effects of administration of RAS modulators.

Serendipitous observation of reduced rectal bleeding and anemia in a case of rectosigmoid junction VM following propranolol and celecoxib treatment[3] could be explained by the inhibitory effects of a β-blocker and COX2 inhibitor on the RAS. (Pro)renin receptor, expressed on the endothelium of VM, signaling through the Wnt-β-catenin pathway[4] to maintain pluripotency of ESCs[5] may be the mechanism by which the RAS regulates the ESC-like subpopulations within VM.

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REFERENCES

1. Siljee S, Keane E, Marsh R, et al. Expression of the components of the renin-angiotensin system in venous malformation. *Front Surg.* 2016;3:24.
2. Tan EMS, Siljee SD, Brasch HD, et al. Embryonic stem cell-like subpopulations in venous malformation. *Front Med (Lausanne).* 2017;4:162.
3. Abematsu T, Okamoto Y, Nakagawa S, et al. Rectosigmoid colon venous malformation successfully treated with propranolol and celecoxib. *J Pediatr Surg Case Reports.* 2015;3:331–333.
4. Nguyen G. Renin, (pro)renin and receptor: an update. *Clin Sci (Lond).* 2011;120:169–178.
5. Sato N, Meijer L, Skaltsounis L, et al. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med.* 2004;10:55–63.