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EDITORIAL

DACH1-Driven Arterialization

Angiogenic Therapy for Ischemic Heart Disease?

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Angiogenic therapies to promote myocardial perfusion and improve myocardial function have shown promise in preclinical models but have not been successfully replicated in the clinic.

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In this issue of *Circulation Research*, Raftrey et al[1] show that overexpressing the transcription factor DACH1 (dachshund family transcription factor 1) in developing or adult mouse heart endothelial cells (ECs) promotes coronary artery differentiation and improves survival after experimental myocardial infarction. Their findings identify a novel pathway driving arterial EC specification and extending arterial vessels and could form the basis for a future potential therapeutic approach to regenerate arterial blood vessels and mitigate the effects of coronary artery disease.

Ischemic heart disease (IHD) is caused by the interruption of blood supply to the cardiac muscle, resulting in inadequate myocardial oxygen and nutrient supply and demand. IHD is commonly caused by atherosclerotic plaque rupture and thrombotic vessel occlusion of the coronary arteries, resulting in malperfusion of the coronary territory. Coronary artery disease remains the leading cause of death worldwide,[2] but aortic stenosis or hypertrophic cardiomyopathy can also result in ischemic damage to the myocardium and contribute to IHD burden. Current pharmacological therapies, including β-blockers, anticoagulants, or calcium antagonists either alone, or in combination, improve survival and offer optimal treatment and/or prevention of IHD.[3] Surgical procedures such as percutaneous coronary intervention and coronary artery bypass grafting, can restore blood flow and decrease mortality for patients surviving an ischemic episode.[4] Revascularization mitigates the effects of acute hemodynamic instability and chronic maladaptive ventricular remodeling and contributes to increased patient longevity.[5] Despite these advances, there is no effective treatment for patients unfit for revascularization or for those with only partial revascularization.

Given the importance of achieving optimal myocardial collateral blood flow and microvascular perfusion, strategies targeting blood vessel repair and regeneration in the ischemic heart have gained considerable traction in preclinical and clinical research. Therapeutic angiogenesis and arteriogenesis aim to stimulate functional collateral growth and restore microvascular and macrovascular circulation to the ischemic myocardium by delivering proangiogenic factors. Angiogenic proteins, such as VEGF (vascular endothelial growth factor) and FGF (fibroblast growth factor), are the best-studied endothelial growth factors, and the only ones tested in the clinical setting. However, clinical trials employing these factors to treat IHD have had little to no success.[6]

A combination of intrinsic factors, including the existence of uncontrolled cardiovascular risk factors, the antagonistic ischemic microenvironment, the lowered secretion of factors promoting vessel sprouting and maturation, and/or decreased receptor expression on responsive cells need to be overcome.[6] Moreover, angiogenic therapy in patients with IHD may be inefficient because of the inability to deliver the angiogenic stimulus effectively. Strategies to stimulate collateral artery formation may also require consideration of the surrounding vascular smooth muscle cells and pericytes to promote vessel maturation and stabilize collateral networks.[6] Importantly, until recently, there has been a lack of recognition of

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the fundamental importance of endothelial heterogeneity and specialized functions of vascular beds, in organ development, functional maintenance, and regeneration.[7] Single-cell RNA sequencing technology has revealed extensive transcriptional heterogeneity not only in different endothelial cell systems (arterial, venous, capillary, or lymphatic) but also in neighboring ECs of the same blood vessel.[8] The early primary heart capillary plexus also contains transcriptionally distinct preartery cells that have been identified as the developmental precursors of coronary arteries.[9] These cells might be targeted for revascularization therapy in the context of strategies aimed at re-activating arteriogenic developmental processes to offer a therapeutic route for patients with chronic ischemia and no option for revascularization.

DACH1 is a nuclear protein related to the Ski/Sno family of transcription factors and co-factors discovered for its role in *Drosophila* cell fate specification and organ size control.[10] This function is conserved in mammals as DACH1 determines mouse pancreas size by supporting progenitor cell proliferation by inhibiting the cyclin-dependent kinase inhibitor P27/KIP1 (cyclin dependent kinase inhibitor 1B).[11] In human breast cancer epithelial cells, cyclin D1 is repressed by DACH1, and genetic deletion of cyclin D1 abrogates DACH1-mediated DNA synthesis inhibition, identifying cyclin D1 gene as a DACH1 functional target.[12] DACH1, therefore, acts as a tumor suppressor, and its reduced expression is associated with poor prognosis in human cancers.[13]

A previous study from K. Red-Horse's laboratory elucidated the function of DACH1 as a key mediator of arteriogenesis, artery remodeling, and endothelial cell migration against blood flow.[14] DACH1 mRNA and protein expression are associated with the immature vascular plexus and DACH1 activation regulates artery size caliber. DACH1 is decreased in mature arteries that have acquired high, uniform laminar flow rates, whereas regions of the vasculature experiencing low and/or nonuniform flow continue to express DACH1, including those in human arteries that are susceptible to vascular disease. Moreover, in vivo loss-of-function and in vitro gain-of-function experiments showed that DACH1 modulates *Cxcl12* (C-X-C motif chemokine ligand 12) expression and blocking CXCR4 (C-X-C motif chemokine receptor 4) abrogates DACH1-simulated endothelial migration against flow, identifying CXCL12 as a downstream effector. These studies showed that DACH1 interacts with mechanical signals transmitted by blood flow to shape the coronary vasculature.

Now, the study of Raftrey et al[1] highlights the potential for DACH1 to stimulate arterial regeneration and improve cardiac function after myocardial infarction. The authors develop a murine model of inducible overexpression of DACH1 in arterial ECs within the developing heart and postnatal retina. Using CRISPR-CAS9 genetic edition, the authors introduce in the *Rosa26* locus the *CAG* promoter and a *Flox-stop-Flox-Dach1-IRES-EGFP* cassette so that DACH1-IRES-EGFP expression occurs upon CRE-mediated deletion of the *Flox-Stop-Flox* sequences. This $Dach1^{OE}$ transgenic line was bred with the Apj^{CreER} tamoxifen-inducible line, expressed in ECs of developing coronary capillary plexus and veins but not in differentiated arterial ECs. Tamoxifen induction at E13.5, when arterial EC differentiation begins, led to expansion of CX40 (connexin 40)-positive arterial EC covering the heart in $Dach1^{OE}$ mice at E15.5, indicating that increasing DACH1 levels promotes preartery specification of EC within the capillary plexus. Examination of transgenic mice at E17.5 showed a 79% increase in CX40-positive arterial vessels length and a >3-fold increase in branching, suggesting that the initial increased preartery specification at E15.5 anticipates the formation of excessive distal arterial branches at E17.5. Apj^{CreER} induction at postnatal day (P) 0 leads to a marked increase in distal arterial branches at P6, without affecting the width of the main coronary artery branch. The capillary plexus- or arterial-ECs requirement for DACH1 overexpression to promote increased arterial branches was tested by breeding the $Dach1^{OE}$ mice with the arterial EC-specific $CX40^{CreER}$ line and inducing at E13.5. $CX40^{CreER}$; $Dach1^{OE}$ E15.5 mice do not show increased CX40-positive arterial ECs, indicating that DACH1 increases arterial branching acting in capillary plexus ECs.

Similar findings were obtained in the postnatal retina, indicating that DACH1 increases artery branches in both the heart and the retina. Importantly, mosaic induction with low tamoxifen dose in retinal ECs at P0 shows that DACH1 functions cell autonomously to drive EC arterialization. Analysis of EC localization in $Cdh5^{CreER}$; tdTomato+control retinas showed that at P6, most ECs were within capillary vessels and the remaining ones were equally distributed among arteries, veins, and tip cells, where prespecified arterial ECs localize. In contrast, $Cdh5^{CreER}$; $Dach1^{OE}$ cells accumulated in arteries and at the tip cell position, whereas those in veins decreased. This observation suggested that $Dach1^{OE}$ contributes to arterial prespecification also in the retina. At P9, $Dach1^{OE}$ cells accumulated in arteries, suggesting that DACH1 overexpression directs ECs arterialization cell autonomously, consistent with it being a transcriptional cofactor.

The authors combine high-resolution imaging and single-cell RNA sequencing to demonstrate that DACH1 does not widely induce the expression of known artery cell fate determinants but rather enhances arterialization of receptive capillary endothelial cell subpopulations that are normally primed for arterial specification (ie. prearterial cells). The authors then extrapolate these findings to the adult mouse heart injury and overexpress DACH1 in an experimental model of myocardial infarction, resulting in improved functional parameters, as assessed by echocardiography, and reduced fibrosis. Mechanistically, DACH1 promotes arterialization by down-regulating the expression of cell cycle (eg, *Cdkn1c*) and lipid transport (eg, *Fapb4*) genes. DACH1 is required to maintain

suppression of endothelial cell cycle, consistent with its role as a tumor suppressor.[13] Downregulation of lipid transport is an intriguing finding given that cardiac ECs are specialized to ensure that cardiomyocytes are supplied by fatty acids as the primary substrate for cardiac contraction.[15]

Taken together, the findings of Raftrey et al[1] provide important insights into how it might be possible to promote functional arterial vessels, as a therapeutic target for improving patient outcome after IHD. As aluded to above, the main issue with current angiogenic therapy remains the inability to deliver an effective and specific angiogenic stimulus to the target responsive cell (ie, pre-arterial endothelial cell in the case of DACH1). Future preclinical studies will need to focus on developing delivery techniques, including using gene transfer technology, so that the potential therapeutic benefit of DACH1 overexpression might be fully realized.

ARTICLE INFORMATION

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Disclosures

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REFERENCES

1. Raftrey B, Williams I, Rios Coronado PE, Fan X, Chang AH, Zhoao M, Roth R, Trimm E, Racelis R, D'Amato G, et al. Dach1 extends artery networks and protects against cardiac injury. *Circ Res*. 2021;129: 702–716. doi: 10.1161/CIRCRESAHA.120.318271
2. Nowbar AN, Gitto M, Howard JP, Francis DP, Al-Lamee R. Mortality from Ischemic Heart Disease. *Circ Cardiovasc Qual Outcomes*. 2019;12:e005375. doi: 10.1161/CIRCOUTCOMES.118.005375
3. Santucci A, Riccini C, Cavallini C. Treatment of stable ischaemic heart disease: the old and the new. *Eur Heart J Suppl*. 2020;22:E54–E59. doi: 10.1093/eurheartj/suaa060
4. Deb S, Wijeysundera HC, Ko DT, Tsubota H, Hill S, Fremes SE. Coronary artery bypass graft surgery vs percutaneous interventions in coronary revascularization: a systematic review. *JAMA*. 2013;310:2086–2095. doi: 10.1001/jama.2013.281718
5. Gaba P, Gersh BJ, Ali ZA, Moses JW, Stone GW. Complete versus incomplete coronary revascularization: definitions, assessment and outcomes. *Nat Rev Cardiol*. 2021;18:155–168. doi: 10.1038/s41569-020-00457-5
6. Zhang H, van Olden C, Sweeney D, Martin-Rendon E. Blood vessel repair and regeneration in the ischaemic heart. *Open Heart*. 2014;1:e000016. doi: 10.1136/openhrt-2013-000016
7. Aird WC. Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med*. 2012;2:a006429. doi: 10.1101/cshperspect.a006429
8. Stone OA, Zhou B, Red-Horse K, Stainier DYR. Endothelial ontogeny and the establishment of vascular heterogeneity. *Bioessays*. 2021;43:e2100036. doi: 10.1002/bies.202100036
9. Su T, Stanley G, Sinha R, D'Amato G, Das S, Rhee S, Chang AH, Poduri A, Raftrey B, Dinh TT, et al. Single-cell analysis of early progenitor cells that build coronary arteries. *Nature*. 2018;559:356–362. doi: 10.1038/s41586-018-0288-7
10. Mardon G, Solomon NM, Rubin GM. dachshund encodes a nuclear protein required for normal eye and leg development in Drosophila. *Development*. 1994;120:3473–3486.
11. Kalousova A, Mavropoulos A, Adams BA, Nekrep N, Li Z, Krauss S, Stainier DY, German MS. Dachshund homologues play a conserved role in islet cell development. *Dev Biol*. 2010;348:143–152. doi: 10.1016/j.ydbio.2010.09.007
12. Wu K, Li A, Rao M, Liu M, Dailey V, Yang Y, Di Vizio D, Wang C, Lisanti MP, Sauter G, et al. DACH1 is a cell fate determination factor that inhibits cyclin D1 and breast tumor growth. *Mol Cell Biol*. 2006;26:7116–7129. doi: 10.1128/MCB.00268-06
13. Popov VM, Wu K, Zhou J, Powell MJ, Mardon G, Wang C, Pestell RG. The Dachshund gene in development and hormone-responsive tumorigenesis. *Trends Endocrinol Metab*. 2010;21:41–49. doi: 10.1016/j.tem.2009.08.002
14. Chang AH, Raftrey BC, D'Amato G, Surya VN, Poduri A, Chen HI, Goldstone AB, Woo J, Fuller GG, Dunn AR, et al. DACH1 stimulates shear stress-guided endothelial cell migration and coronary artery growth through the CXCL12-CXCR4 signaling axis. *Genes Dev*. 2017;31:1308–1324. doi: 10.1101/gad.301549.117
15. Potente M, Mäkinen T. Vascular heterogeneity and specialization in development and disease. *Nat Rev Mol Cell Biol*. 2017;18:477–494. doi: 10.1038/nrm.2017.36