

## Vascular Disease in a Dish All the Right Ingredients?

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The emerging field of stem cell medicine offers unprecedented opportunities, among which the ability to model genetic diseases in vitro is one of the most exciting. In particular, disease modeling using stem cells promises to deliver a deeper understanding of disease mechanisms and to provide a test bed for novel therapies within a relatively short timeframe. The seminal discovery by Shinya Yamanaka only 6 years ago that somatic adult cells, such as fibroblasts taken from the skin, could be reprogrammed into cells that look and behave remarkably like embryonic stem cells<sup>1</sup> has greatly accelerated progress toward these goals. These induced pluripotent stem cells (iPSCs), in common with their embryonic counterparts, possess the ability to self-replicate indefinitely yet also have the capacity to differentiate into almost any somatic cell type. Importantly, if derived from patients with overt disease, iPSCs will possess both the disease-causing mutation as well as the permissive genetic background that in many cases is required for full expression of the disease phenotype. In this issue of *Circulation*, Qyang and colleagues<sup>2</sup> use this technology to increase our understanding of the vascular smooth muscle cell (SMC) pathology in supra-valvular aortic stenosis (SVAS) and Williams-Beuren Syndrome (WBS)—conditions associated with reduced elastin expression and incorporation into the vascular wall.

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Elastin is the predominant extracellular protein in elastic arteries, accounting for  $\approx 33\%$  of the dry weight of the aorta.<sup>3</sup> It has key roles in conferring tensile and elastic properties and in regulating vascular SMC function. Nonsyndromic SVAS is caused by *ELN* mutations that increase SMC proliferation and lead to occlusive vascular disease in the aorta and other major arteries as a result of haploinsufficiency.<sup>4,5</sup> In WBS, segmental chromosomal deletions that include the *ELN* gene locus lead to a wide range of abnormalities in other systems, but the cardiovascular manifestations are very similar to SVAS.<sup>6</sup> Together, these conditions have been referred to as elastin arteriopathies and are characterized by low levels of elastin leading to severe vascular disease with high morbidity and mortality.

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Genetically modified mice have been used to model the effects of loss of elastin.<sup>7</sup> However, the heterozygous-null animal only develops minor vessel wall thickening without the occlusive features seen in humans. A more aggressive phenotype is seen in the homozygous-null mouse, which dies soon after birth and so the adult animals cannot be studied. These differences between mice and humans may in part be attributed to differences in wall tension and blood pressure. However, at the genetic level there are also interspecies differences in splicing of the *ELN* gene product that are poorly understood and could account for some of the phenotypic variation<sup>8</sup> underlining the need for a human model.

Qyang and colleagues<sup>2</sup> generated iPSC lines from a patient with SVAS and another with WBS. SMCs derived from these pluripotent cells produced reduced amounts of elastin and recapitulated the cellular abnormalities known to be a feature of SVAS, namely a reduction in actin polymerization, increased proliferation, and increased migration of patient-derived cells compared with wild-type controls. Next, in keeping with results from murine studies,<sup>9</sup> they showed that the abnormality in actin polymerization in SVAS-derived SMCs could be rescued by the addition of exogenous tropoelastin or expression of ras homolog gene family, member A (RhoA). Having validated the iPSC-derived model, the authors investigated the cause of the SMC hyperproliferation associated with SVAS. They found that signaling through extracellular signal-regulated kinase 1/2 was increased in the diseased cells and that blocking this signaling pathway normalized SMC proliferation. These findings are exciting and encouraging. In addition to the establishment of a unique human model of SVAS that can be used to identify perturbed signaling pathways that may have a central role in the pathology of SVAS, such as extracellular signal-regulated kinase 1/2, the system may also be used to test new therapeutic approaches for this disease. However, much remains to be done in using this model to understand SVAS and other elastin arteriopathies. The mechanisms by which lack of elastin leads to the disease phenotype need to be further delineated. Also, given the different levels of elastin expression in the SVAS SMCs versus the WBS cells, the contribution of gene dosage to phenotype needs further study, as Qyang and colleagues<sup>2</sup> themselves acknowledge. Additionally, recent studies have identified genetic modifiers of the vascular phenotype in mice,<sup>10</sup> and the iPSC-derived model will facilitate complementary studies in a human system.

A key factor in any disease modeling studies using iPSCs will be the ability to faithfully differentiate the relevant cell types in vitro. Qyang and colleagues<sup>2</sup> generated SMCs by aggregating clumps of iPSCs into aggregates known as embryoid bodies with serum-assisted differentiation. How-

ever, it is unclear whether the resultant SMCs were vascular or not and likely comprised a heterogeneous mixture of types. Another important consideration is that, despite a generalized arteriopathy in SVAS and WBS, severe focal lesions occur characteristically at discrete anatomic sites such as the proximal aorta just above the sinotubular junction and the pulmonary trunk and branches.<sup>11</sup> Because different vascular territories have distinct embryonic origins, which may influence disease localization,<sup>12</sup> then it may be beneficial for future studies to use more defined in vitro differentiation protocols that have been shown to generate vascular SMCs arising from specific embryonic lineages.<sup>13</sup>

Using iPSCs for disease modeling raises other crucial questions that still need to be addressed by the field. Multiple clonal iPSC lines are typically generated during reprogramming of somatic cells. However, there may be significant differences in the properties of individual iPSC lines, including differences in the ability to differentiate into specific somatic cells even though all lines originate from one individual and possess the same genotype.<sup>14</sup> This raises the question of which line(s) should then be used for the disease modeling studies. Similarly, which of the many lines isolated from a wild-type donor should be used as negative controls? Qyang and colleagues<sup>2</sup> generated 2 iPSC lines from each patient and each wild-type control. Findings are reported predominantly from one line from each patient, and although the authors state that the paired lines behaved similarly, only limited comparative data are provided and it is unclear as to the basis on which the lines used for detailed analyses were selected.

Further challenges involved in using wild-type iPSC controls go beyond the line-to-line variability observed from a single source. Qyang and colleagues<sup>2</sup> matched the source tissue and sex of the wild-type donors to the SVAS and WBS patients. However, the ideal wild-type control iPSCs would be genetically matched to the patient-derived cells apart from the disease-causing mutation. Nonaffected sibling controls would be a reasonable match, but there may be practical or ethical issues in obtaining such samples. An alternative approach would be to correct the genetic mutation in the patient-derived iPSCs themselves using homologous recombination,<sup>15</sup> thus providing direct proof that the mutation in question causes the disease phenotype. Building on this, a variety of disease-related mutations could be introduced into a wild-type iPSC. These types of approaches using genetic manipulation in iPSCs would then open the door to extensive future studies on genotype–phenotype correlation and help us to further understand the mechanisms underlying variable disease penetrance and the role of genetic modifiers. Despite the challenges ahead, the confluence of iPSC technology with an understanding of vascular biology promises great advances in our understanding and treatment of human genetic vascular diseases.

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## Disclosures

None.

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