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When bigger is better: utilizing large animal models in vein graft surgery to gain insights into endothelial-to-mesenchymal transition

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This editorial refers to 'Inhibition of endothelial-to-mesenchymal transition in a large animal preclinical arteriovenous fistula model leads to improved remodelling and reduced stenosis', by Y. Xu et al., <https://doi.org/10.1093/cvr/cvae157>.¹

Despite recent advances in therapies, the rise in cardiometabolic diseases has resulted in more vein graft surgeries for atherosclerotic disease. Unfortunately, these grafts often fail, with up to 42% showing levels of failure within 12–18 months post-surgery.² Similarly, the increasing incidence of end-stage kidney disease necessitates the creation of arteriovenous fistulas (AVFs) for haemodialysis, which also suffer from high failure rates.³ Failures in both vein grafts and AVFs result from thrombosis, adverse vein remodelling, and neointimal hyperplasia. A major contributor to these processes is endothelial-to-mesenchymal transition (EndMT), a phenomenon where endothelial cells (EC) progressively transform into multi-potent mesenchymal stem cells (MSCs), compromising their normal functions. In response to tissue- and organ-specific signals, MSC can further differentiate into myofibroblasts and other mesodermal derivatives (Figure 1). EndMT involves significant changes in cell morphology, molecular structure, and function. It has been described in both developmental processes (i.e. cardiac valves formation and maturation of pulmonary arteries) and cardiovascular diseases (i.e. atherosclerosis, pulmonary arterial hypertension, and cerebral cavernous malformations), making it a potential therapeutic target. However, challenges remain to accurately define and characterize EndMT-intermediates or EndMT-derived cells due to the process's dynamic nature and the plasticity and heterogeneity of ECs. Additionally, thus far, EndMT inhibition has been achieved by targeting upstream signal transduction pathways, both biochemical and biomechanical, which are not only responsible for EndMT (see Figure 1). The TGF- β superfamily, encompassing the TGF- $\beta_{1,2}$ isoforms, bone morphogenetic proteins (BMPs), activins, and related proteins, is pivotal in regulating EndMT. TGF- β_1 and TGF- β_2 promote EndMT through receptor complexes composed of type I [e.g. activin receptor-like kinase (ALK) 5] and type II receptors [e.g. TGF- β receptor type 2 (TGF β R2)], which activate the SMAD2/3 transcription factors upon phosphorylation. Replicating tissue microenvironment cues in cell culture systems often fails to fully capture the *in vivo* dynamics of EndMT.

The group of Jason C. Kovacic has made significant contributions to the field of EndMT,⁴ particularly in understanding its role in atherosclerotic lesions,⁵ and TGF- β -induced EndMT in vein graft models.⁶ In this issue of *Cardiovascular Research*, Xu et al. investigate the contribution of

TGF- β -induced EndMT to neointima formation in a novel large animal model of AVF. They employ a sophisticated approach involving the genetic knock-down of SMAD3 locally through lentiviral-mediated gene silencing. Inhibiting SMAD3 is sufficient to ameliorate EndMT, thereby preventing the occlusion of the lumen, limiting neointima formation, and reducing stenosis severity. Furthermore, SMAD3 inhibition enhances endothelialization and increases eNOS expression, underscoring the therapeutic potential of this strategy. Noteworthy, *in vitro* studies have demonstrated the necessity of both SMAD3 and SMAD2 for TGF- β -induced EndMT in EC.⁷ However, this study shows that inhibition of SMAD3 alone is sufficient to prevent EndMT almost completely in a large animal model of AVF. It remains to be explored whether the relative functions of SMAD2 vs. SMAD3 along with other components of the pathway may differ between small and large animal models of cardiovascular disease.

While some progress has been made over the years to develop large animal models for EndMT (i.e. a sheep model for valve regurgitation⁸), there remains a notable scarcity, particularly for vein graft surgery studies. This study by Xu et al. marks an important advancement within the broader landscape of EndMT studies, particularly in large animal models, as they facilitate the translation to a clinical setting. Another highlight of this research is the innovative approach of creating a preclinical pig model with intra-vessel injection of the lentiviral constructs containing shRNA against SMAD3 or scrambled control, followed by *in situ* dwelling of the virus, with subsequent flushing of residual viral article with saline solution. This method differs from previous approaches⁹ and circumvents the need for systemic administration, thereby reducing potential side effects and minimizing additional surgical training requirements. Furthermore, this approach is highly adaptable for clinical applications, which enables direct implementation in clinical settings, particularly in AVFs and vein graft surgeries.

In this *in vivo* study, EndMT was confirmed with immunostaining to identify cells co-expressing previously reported endothelial and mesenchymal markers. Similar to cell fate mapping strategies in rodents, implementation of endothelial lineage tracking system in pigs (like the tamoxifen-inducible end.SclCreER^T mouse strain⁵) would assist in verifying the functional involvement of EndMT in cardiovascular disease, under conditions closely resembling human pathophysiology. In this system, endothelial-specific Cre expression induces continuous yellow-fluorescent protein expression, regardless of cell phenotype changes. This method would allow for more precise tracking of ECs as they undergo EndMT. While this study

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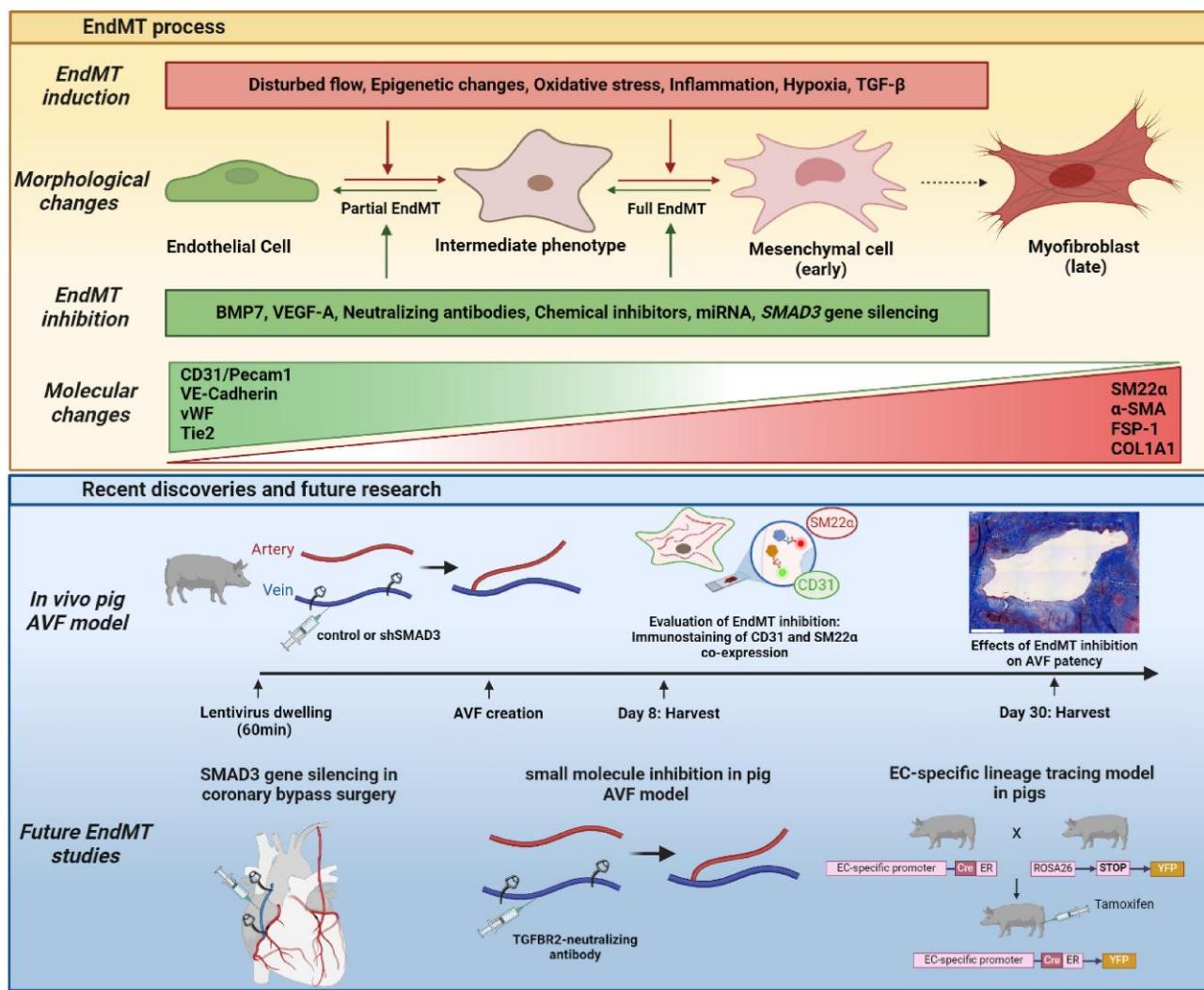


Figure 1 Schematic overview of EndMT and approaches to inhibit EndMT. EndMT involves the transition of ECs to a mesenchymal phenotype via cell intermediates, in response to disturbed flow, epigenetic changes, oxidative stress, inflammation, hypoxia, or TGF- β signalling. Factors described to inhibit EndMT are SMAD3 gene silencing, BMP7, vascular endothelial growth factor-A, TGF- β -neutralizing antibodies, chemical inhibitors, and miRNA. During EndMT, endothelial markers (i.e. CD31, VE-cadherin, vWF, and Tie2) are lost, while mesenchymal markers [i.e. SM22 α , α -smooth muscle actin (α -SMA), fibroblast-specific-1 (FSP-1), and collagen 1] are increased. In this study, EndMT was targeted by local SMAD3 knock-down in a preclinical AVF model. EndMT was assessed through the reduction in the number of cells co-expressing the endothelial marker (CD31) and mesenchymal marker (SM22 α) through immunostaining. Finally, the impact of SMAD3 inhibition on AVF patency was assessed by luminal dimension, neointimal formation, and stenosis severity. Future studies may use SMAD3 gene silencing in bypass graft surgery and compare the local inhibition of SMAD3 to the effects of small molecule TGF- β inhibitors and the implementation of endothelial lineage tracing system in pigs, to proof the functional involvement of EndMT in cardiovascular diseases. Created with Biorender.com.

demonstrates significant success in inhibiting EndMT in surgeries like AVF and vein graft surgery, where local application of SMAD3 knock-down is feasible, the approach may face challenges in other 'EndMT diseases'. The feasibility of a local SMAD3 knock-down in less accessible organs for local lentiviral delivery needs further exploration and future research should investigate how a local, endothelial-specific knock-down could be achieved and what the implications of such an approach are. Additionally, while the authors investigated the role of EndMT inhibition on haemodialysis fistulas as a model for vein graft surgeries, it would be interesting to extend those findings using other models of vein graft disease, such as bypass graft surgery, one of the most commonly performed revascularization techniques for occlusive arterial disease.¹⁰

Overall, this study bridges an important research gap in large animal models for drug testing. Future studies may apply a similar model to compare the local inhibition of SMAD3, as described here, with the effects of small molecules that inhibit TGF- β signalling, such as losartan,¹¹ to assess their efficacy in inhibiting EndMT locally. Xu *et al.*'s study provides a significant advancement in our understanding of the contribution of EndMT and its potential therapeutic applications in cardiovascular diseases. Their work underscores the value of large animal models in translating preclinical findings to clinical settings, particularly for AVF and vein graft surgeries. As research continues to unravel the complexities of EndMT, the potential to prevent and treat a broader range of cardiovascular diseases through targeted interventions becomes increasingly promising.

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