Aberrant Regulation of EndMT in Turner Syndrome: Implications for the Pathogenesis of Congenital Cardiovascular Disease

Eric Garcia Huitron[1], Xiaoying Zhang[2], Lance Babcock[2], Jane Grande-Allen[1], Siddharth Prakash[2]
[1]Rice University Department of Bioengineering, Houston, Tx
[2]University of Texas Health Science Center, Houston, Tx

UTHealth McGovern Medical School — The University of Texas Health Science Center at Houston

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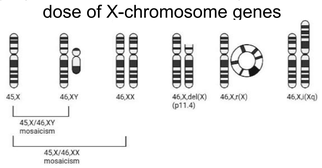
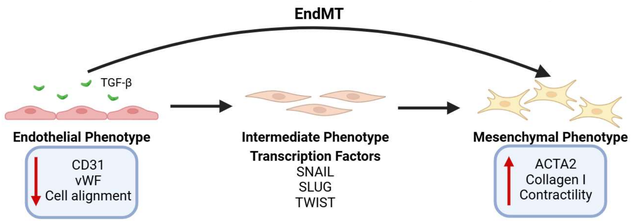
Introduction

Turner Syndrome (TS) - Condition in which biological women have a half/partial dose of X-chromosome genes

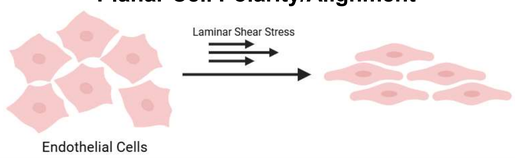


Cardiac Complication	Endothelial Cell Dysfunction	Signaling Pathways & Mechanical Regulators
Hypertension	Vascular Tone	Planar Cell Polarity/Alignment Endothelial-Mesenchymal Transition Shear Stress Extracellular Matrix
Bicuspid Aortic Valve (BAV)	Valvular Morphogenesis	None of these pathways & regulators have been explored with respect to X-chromosome dosage
Aneurysms & Dissections	Inflammation	

Endothelial-Mesenchymal Transition (EndMT)

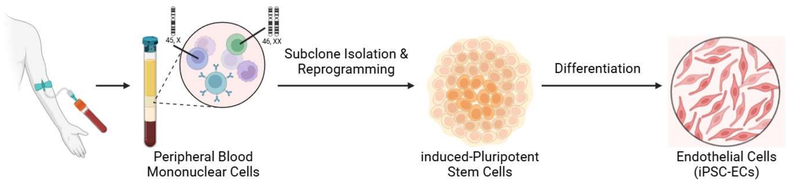


Planar Cell Polarity/Alignment



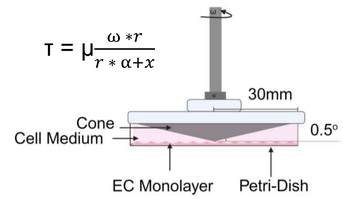
Methods

Two iPSC-EC karyotypes isolated from one mosaic patient



Uniform shear stress applied with a cone-plate bioreactor

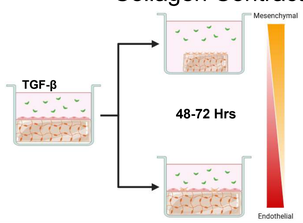
$$\tau = \mu \frac{\omega * r}{r * \alpha + x}$$



Symbol	Description
τ	Wall shear stress
μ	Dynamic viscosity
r	Radius of cone
ω	Angular velocity
α	Angle between cone and plate

Experiments

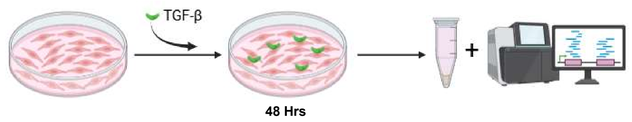
A — Collagen Contraction Assay



A – Experiment Overview
- Rat tail collagen is crosslinked before iPSC-ECs are seeded on the scaffold and TGF-β is added for 48-72 hrs
- Gels were separated from the walls of the well to allow cells at a mesenchymal state to contract the gel

A.1 (Right Panel) – Results and Implications
- Images were taken from the top of the gels and compared to their control as percent contraction
- 45,X iPSC-ECs contracted gels the most at all time timepoints after TGF-β exposure
- 46,XX iPSC-ECs comparably contract gels as commercially available Human Aortic ECs (HAECs)

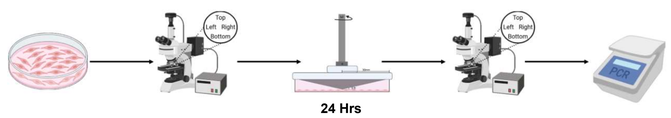
B — RNA-sequencing



B – Experiment Overview
- iPSC-ECs (46,XX & 45,X subclones) were exposed to TGF-β for 48Hrs before being sent for RNA-sequencing

B.1 (Right Panel) – Results and Implications
- Via gene ontology enrichment analysis, KDM5C, ZEB2, SNAIL and SLUG were upregulated in 45,X cells exposed to TGF-β compared to 46,XX

C — Laminar Shear Stress & rt-qPCR

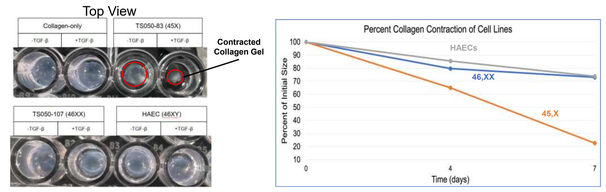


C – Experiment Overview
- iPSC-ECs (46,XX & 45,X subclones) were exposed to uniform shear stress (0, 15 & 25 dynes/cm²) with a cone-plate bioreactor for 24Hrs
- Brightfield images were taken before and after shear stress from 4 locations per biological replicate
- RNA was isolated from the same samples for rt-qPCR

C.1 (Right Panel) – Results and Implications
- The average cell orientation distribution before (Blue) and after (Red) shear stress was depicted in histograms and their standard deviations as regions of their respective color
- 46,XX iPSC-ECs increase in alignment after each shear stress condition while 45,X cells do not align with fluid flow
- Rt-qPCR suggests EndMT activation in 45,X iPSC-ECs by shear stress while 46,XX increase transcription of endothelial CD31

Results

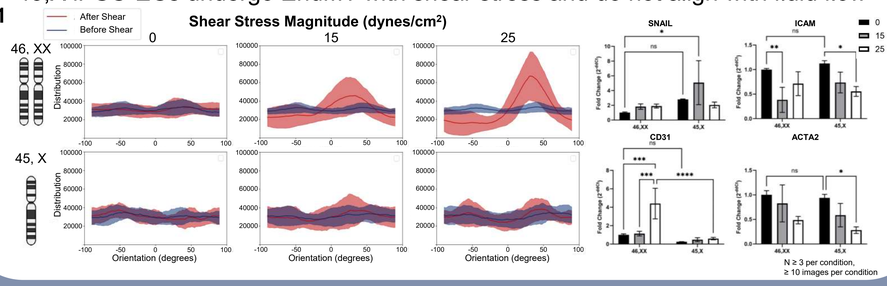
A.1 — 45,X iPSC-ECs readily transition to a mesenchymal phenotype with TGF-β



B.1 — TGF-β promotes phenotypic plasticity in 45,X iPSC-ECs by autosomal and X-chromosome transcriptional regulators

Expression Pattern	Gene(s)	Description	Role
Upregulation of EndMT in 45,X vs 46,XX	ZEB2 SNAIL SLUG	Autosomal transcription factors driving mesenchymal phenotype	Vasculature Development, Cell Morphogenesis
Upregulation of epigenetic regulators in 45,X vs 46,XX	KDM5C	X-linked demethylase - increases transcriptional activity	Tube morphogenesis, Tissue Development

C.1 — 45,X iPSC-ECs undergo EndMT with shear stress and do not align with fluid flow



Hypothesis

We hypothesize that disregulation of EndMT during cardiac development, at least partially due to abnormal sensing of shear stress by ECs, predisposes to congenital heart and vascular diseases in TS.

Conclusions

X-chromosome dosage on isogenic iPSC-ECs regulates:
- Epigenetic factors (KDM5C)
- TGF-β and shear stress induced EndMT (SNAIL, SLUG, ZEB2 & CD31)
- Shear stress induced cellular polarity & anti-inflammatory response

X-chromosome dosage on pathogenesis in Turner Syndrome
- Aberrant EndMT & epigenetic regulation by TGF- β in 45,X ECs may drive BAV formation
- Aberrant polarity & EndMT by shear stress in 45,X ECs may drive aneurysms/dissections

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References