#12: Using spatial transcriptomics to elucidate Sash1-dependent factors driving hematopoietic stem cell generation

Gary Cole, Patrick Coulombe, Gregorios Paliouras, James Lo, Aly Karsan
Michael Smith Genome Sciences Centre & Terry Fox Laboratories, BC Cancer Research Centre, Vancouver, BC, Canada

BC CANCER RESEARCH — Provincial Health Services Authority
THE UNIVERSITY OF BRITISH COLUMBIA

Background

- Hematopoietic stem and progenitor cells (HSPCs) originate via an endothelial–to-hematopoietic transition (EHT) during embryogenesis (Fig 1).[1,2]
- EHT is regulated by an intricate mix of intrinsic and extrinsic cues. Identifying exogenous EHT-promoting factors is critical to generating HSPCs *in vitro*.[2,3]

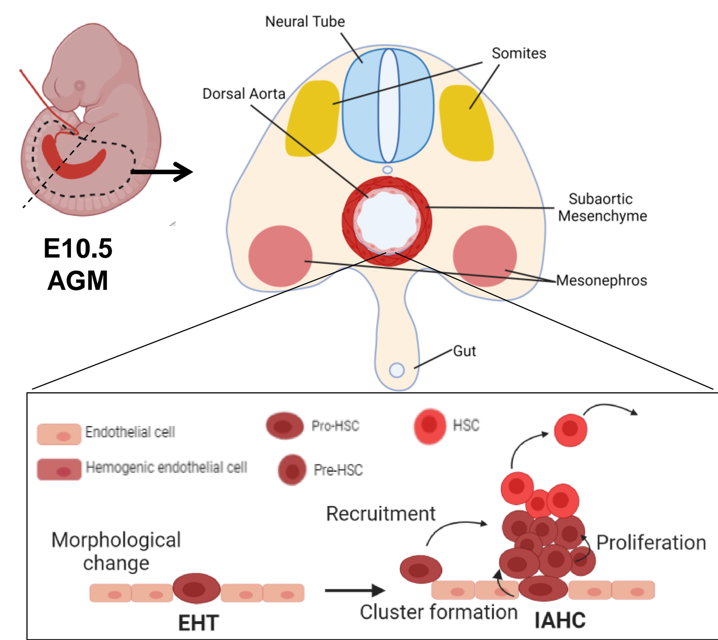


Figure 1: Schematic representation of HSC ontogeny. Specialized hemogenic endothelial cells in the AGM region undergo an EHT, forming intra-aortic hematopoietic clusters (IAHC) composed of pre-HSPCs. This trans-differentiation is driven by EHT-supportive factors produced by the AGM niche whose identity and roles remain incompletely understood.

- Loss of Sash1 compromises EHT via a cell non autonomous mechanism (Fig 2).
- We utilized single cell and spatial transcriptomics to profile Sash1-expressing tissues in the Aorta-gonad-mesonephros (AGM) microenvironment to identify novel candidate niche-derived factors that promote HSC generation

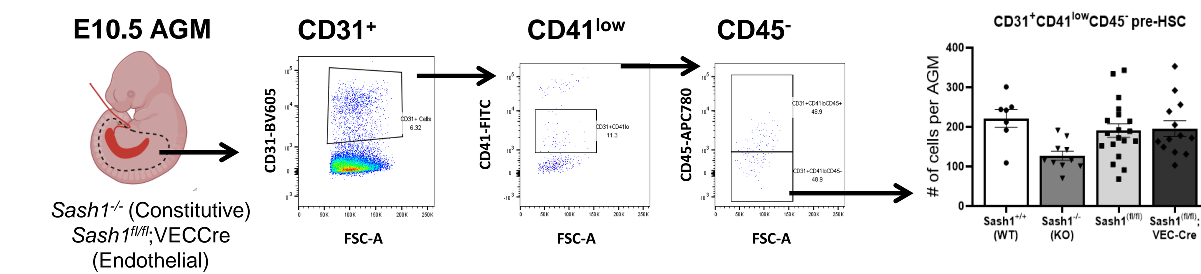


Figure 2: Loss of Sash1 impairs HSC generation via cell nonautonomous effect. Constitutive loss of Sash1 via insertion of a β-galactosidase genetrap at Intron 14 (*Sash1*$^{-/-}$) results in a loss of immunophenotypic pre-HSPCs. This is not recapitulated with endothelial specific deletion in *Sash1*-floxed embryos (*Sash1*$^{fl/fl}$;VECCre)

Methods

- Identification of Sash1-expressing tissues & candidate ligands differentially expressed within the *Sash1*-Het and –KO microenvironment via scRNA-seq (Fig 3).[4]

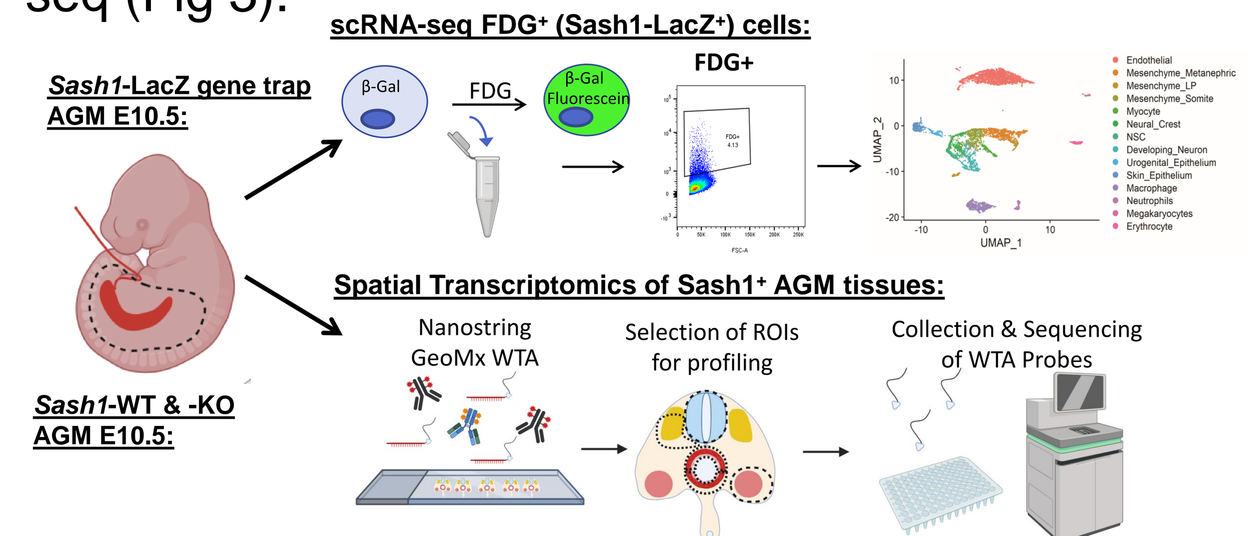


Figure 3: Strategy for profiling transcriptomes of Sash1-expressing populations in the E10.5 AGM. *Upper* - Sash1-expressing cells from *Sash1*-Het and-KO AGMs were in the were sorted and profiled with scRNA-Seq. *Sash1*-LacZ genetrap mice allow tracking of endogenous protein but lack functional Sash1 binding domains. *Lower* – GeoMx DSP WTA kit was used to profile gene expression in transverse sections of the AGM niche of Sash1-WT and –KO embryos. N= 3 litters (1 WT, 1 KO per litter)

- Profiling of dorsal and ventral tissues within the *Sash1*-WT and –KO AGM niche via Nanostring's GeoMx Whole Transcriptome Assay (WTA) (Fig 3).
- Functional follow-up in conditional and constitutive *Sash1*-KO embryos

Results

Sash1-regulated factors from the AGM mesenchyme promote EHT

- scRNA-Seq revealed widespread Sash1 expression within cells of the AGM microenvironment.
- Targeted *Sash1*-KO in each of these populations (via lineage specific Cre recombination in *Sash1*-floxed embryos) revealed loss of mesenchymal Sash1 impairs the emergence of pre-HSPCs.

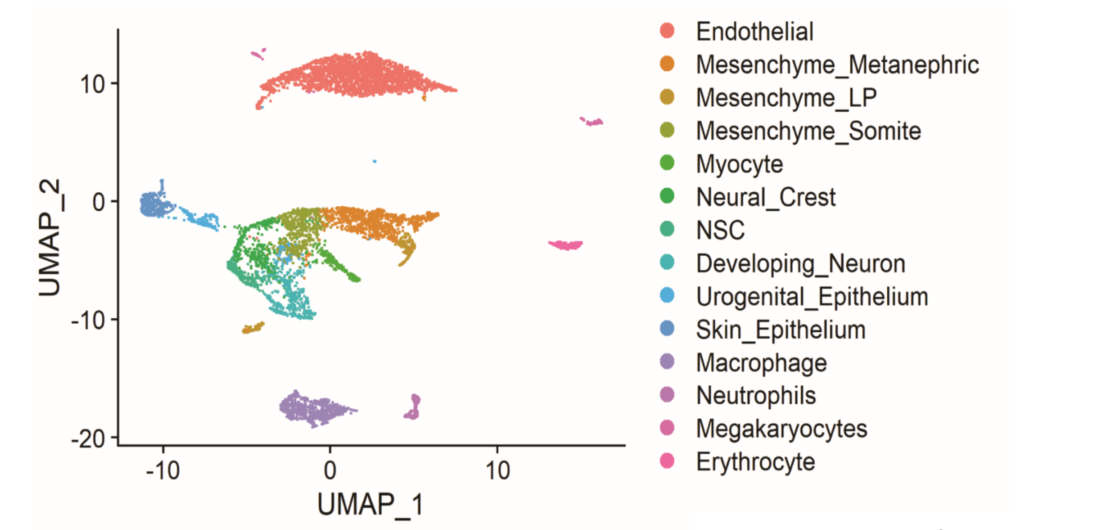


Figure 4: Identification and validation of candidate niche populations producing Sash1-dependent pro-hematopoietic factors (n = 4772 cells from 13 WT and 7 KO). *Upper:* scRNA-Seq profiling of Sash1-LacZ+ cell populations in the *Sash1*-Het and –KO AGM revealed 14 Sash1-expressing clusters. Cell populations were labeled based on distinct gene signatures, and can be classified as endothelial, hematopoietic, mesenchymal, neuronal and epithelial. *Lower:* Flow cytometry was used to examine the effect of targeted *Sash1* deletion in each of these populations on the emergence of immunophenotypic pre-HSPCs. Tamoxifen dosing was used to achieve mesenchymal restricted knockout of Sash1 in *Sash1*-floxed embryos carrying the Col1a2CreER transgene (*Sash1*$^{fl/fl}$;Col1a2CreER). Loss of mesenchymal Sash1 resulted in loss of immunophenotypic pre-HSPCs.

Identification of candidate Sash1-dependent EHT regulators

- scRNA-seq identified a high number of differentially expressed ligand-receptor pairings between mesenchymal-HEC/pro-HSPC populations related to **polarity**, **adhesion** and **motility.**

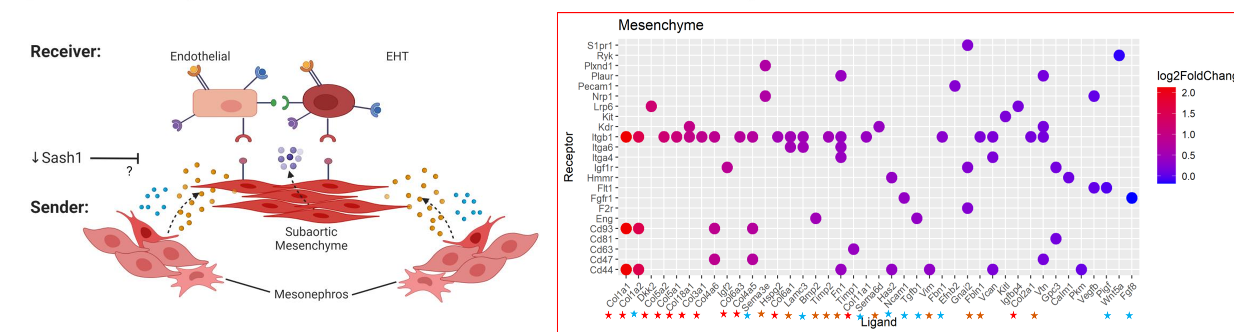


Figure 5: Identification of candidate niche-derived signals regulated by Sash1. *Left:* Sash1-expressing mesenchymal cells from *Sash1*-KO and –Het AGMs were analyzed to identify differentially expressed ligands possessing a HEC/pro-HSPC receptor. *Right:* Sash1-KO mesenchymal populations expressed significantly higher levels of collagens (red star) and other extracellular matrix components and regulators of adhesion (orange star). Other significant differentially expressed ligands included growth factors and morphogens (blue star).

Spatial transcriptomics strategy for profiling *Sash1*-KO and –WT AGM regions

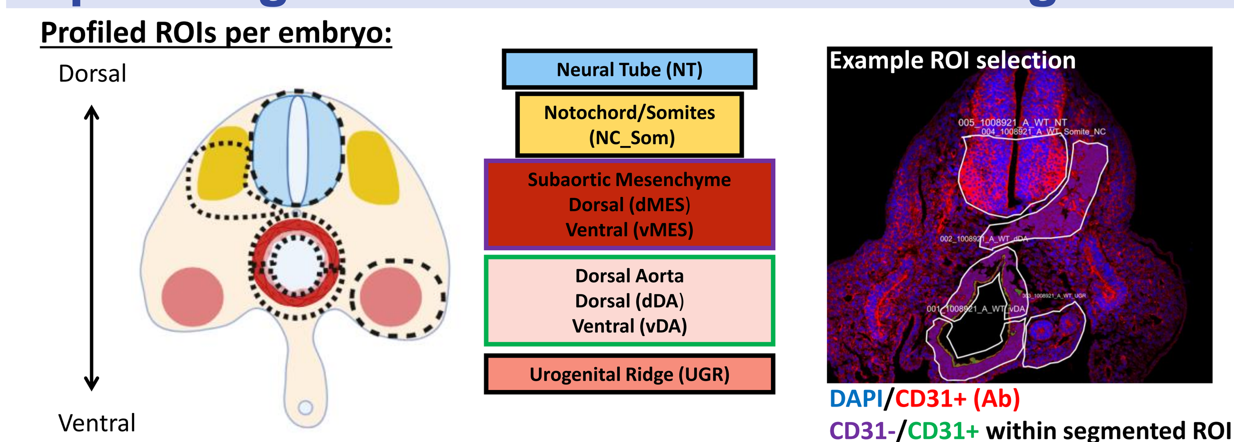
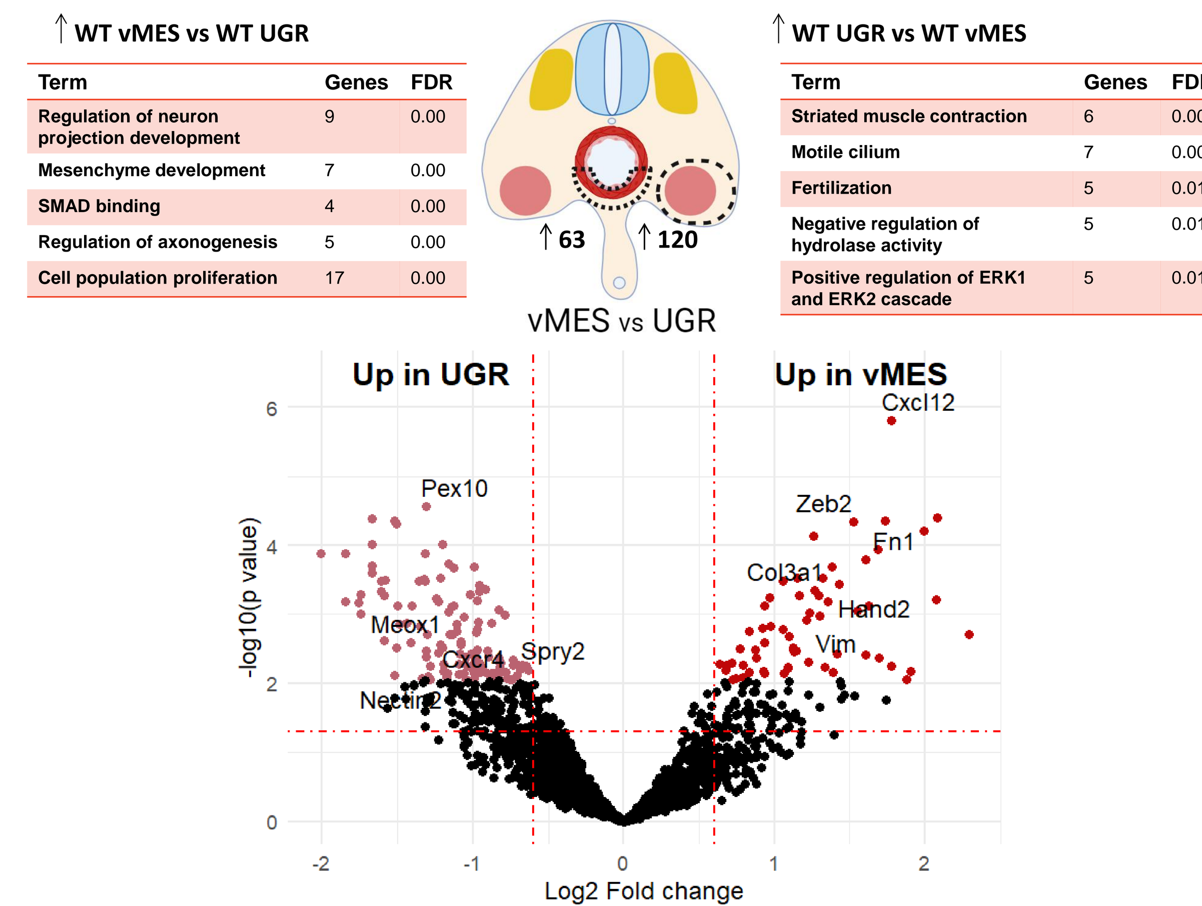


Figure 6: Spatial profiling of AGM subpopulations via GeoMx WTA. Schematic (*left*) and example (*right*) of region of interest (ROI) selection for WTA probe collection + profiling. 7 AGM subpopulations were analyzed from the AGM including the Neural Tube (NT), Notochord/Somites (NC_Som), Urogenital Ridge (UGR), the dorsal (dDA) and ventral (vDA) Dorsal Aorta & Subaortic Mesenchyme from the dorsal (dMES) and ventral (vMES) sides of the DA. To allow precise profiling of the closely associated DA (green in example image) and MES (purple in example image) populations endothelial cells were labeled with fluorescent CD31 Ab. Stain intensity was used to segment ROI around the DA into CD31- (MES) and CD31+ (DA) populations for downstream analysis.

GeoMx WTA identifies region specific DEG in the AGM microenvironment

- Spatially defined transcriptional profiling of AGM sub-populations identified differentially expressed genes (DEG) related to region identity


Transcriptional Profiling and GO term analysis of ventral AGM mesenchyme tissues:

↑ WT vMES vs WT UGR

Term	Genes	FDR
Regulation of neuron projection development	9	0.00
Mesenchyme development	7	0.00
SMAD binding	4	0.00
Regulation of axonogenesis	5	0.00
Cell population proliferation	17	0.00

↑ WT UGR vs WT vMES

Term	Genes	FDR
Striated muscle contraction	6	0.00
Motile cilium	7	0.00
Fertilization	5	0.01
Negative regulation of hydrolase activity	5	0.01
Positive regulation of ERK1 and ERK2 cascade	5	0.01

Figure 7: Validation of assay via DGE analysis of the vMES and UGR regions in WT embryos (n=3 WT). GO term enrichment analysis (tables) revealed that vMES tissue was enriched for terms related to neuron and mesenchyme development, and important processes related to EHT including "SMAD binding" and "Regulation of axonogenesis". Volcano plot depicts DEG between vMES and UGR segments. Analysis revealed region-specific upregulation of genes associated with distinct mesenchymal identities (labeled on Volcano Plot). Red dashed lines mark the FC of 1.5 on x axis and p value of 0.05 on the y axis. DEG with FDR <0.1 are colored pink (up in UGR) and red (up in vMES).

Differential signaling in the *Sash1*-KO microenvironment

- Profiling of *Sash1*-KO and –WT embryos identified 165 upregulated genes and 77 downregulated genes in the vMES AGM compartment with loss of Sash1.
- Upregulated genes were enriched for terms related to developmental processes, insulin metabolism and collagen synthesis.
- Downregulated genes were related to cellular processes, hormone secretion and signaling pathways related to serine/threonine kinase, growth factors, and TGF-β

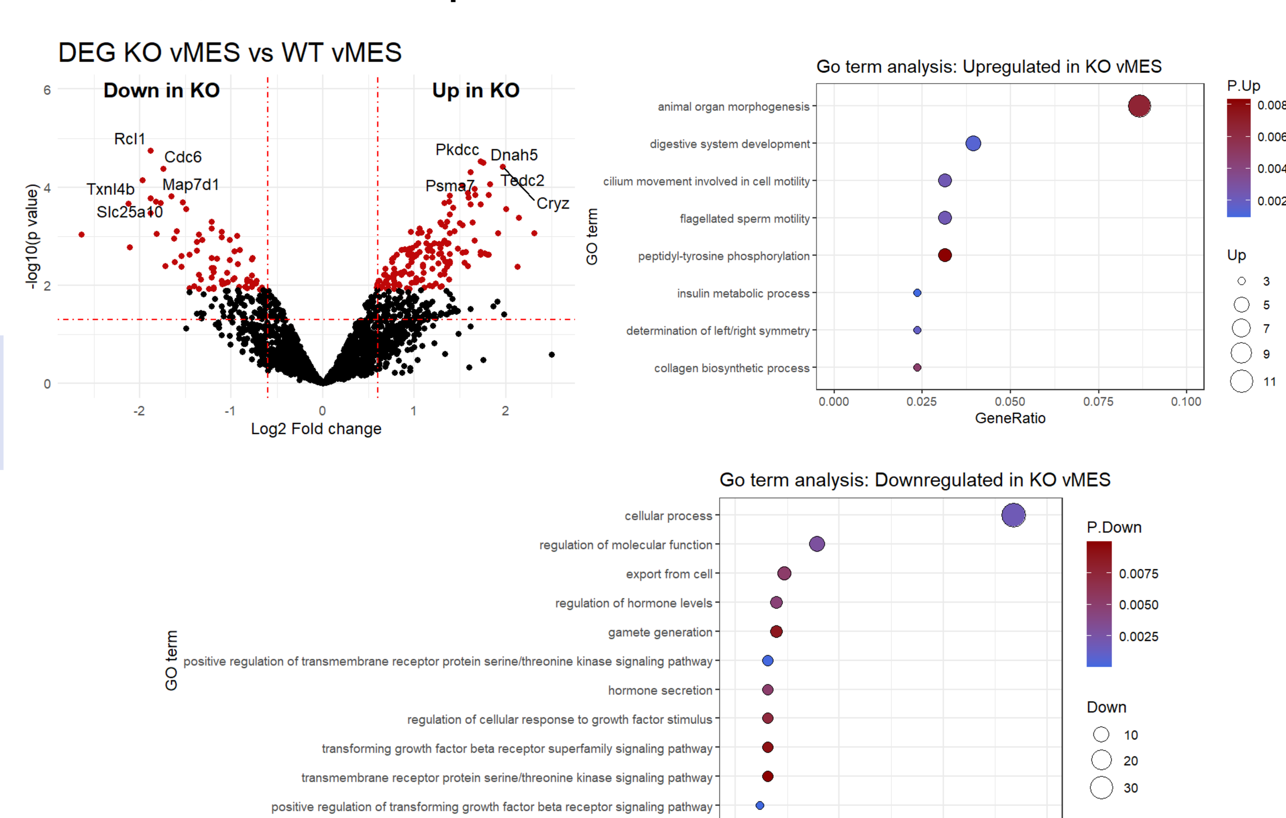
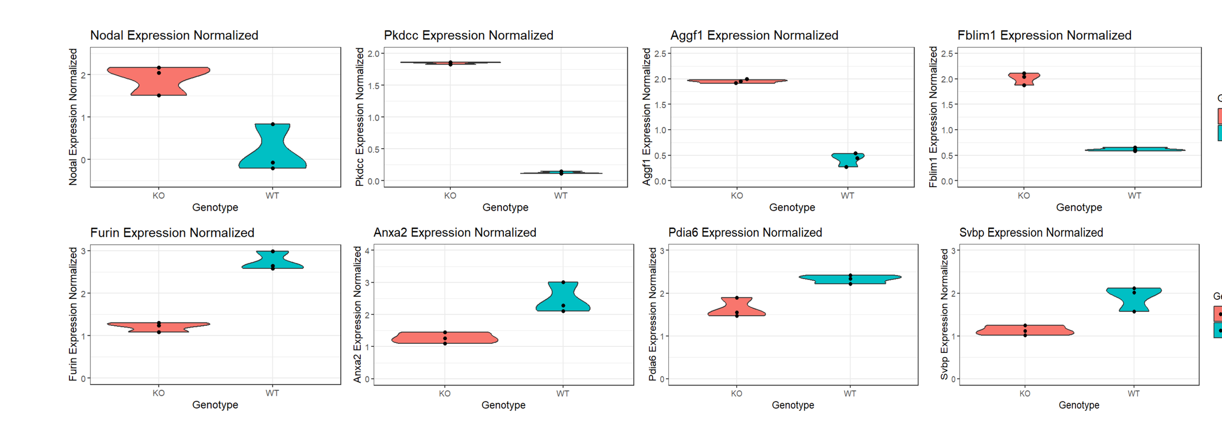


Figure 8: Signaling dynamics in the *Sash1*-KO and –WT ventral subaortic mesenchyme (vMES) (n = 3). To understand the spatial context of Sash1-regulated niche-derived signals within the AGM we performed spatial transcriptomics studies using Nanostring's GeoMx Digital Spatial Profiler platform. We used the WTA kit to profile selected ROI on transverse sections from the E10.5 AGM of 3 *Sash1*-KO and 3 *Sash1*-WT embryos. DGE and GO term enrichment analysis revealed 242 significantly dysregulated genes between *Sash1*-KO and -WT vMES regions (FDR <0.1). *Upper left*: Volcano plot depicting top 5 up- and downregulated genes in *Sash1*-KO. Red dashed lines mark FC of 1.5 on x axis and p value of 0.05 on the y axis. DEG with FDR <0.1 are colored red. *Upper right* and *Lower:* GO term enrichment analysis of significantly upregulated (Upper right) and downregulated (Lower) genes (FDR <0.1).

Sash1 deletion alters expression of niche secreted factors and cell junction proteins

- Among DEG in *Sash1*-KO vMES we identified 52 candidates potentially regulating cell-cell signaling in the AGM niche (either secreted, cell junction, or extra-cellular matrix (ECM) proteins)
- Several candidates have important roles in morphogen activation/processing, cell adhesion, ECM remodeling or embryonic development


Identification of candidate Sash1-regulated EHT-supportive factors within AGM vMES:

DEG KO vs WT vMES

Term	Genes Up in KO	Genes Down in KO
Extracellular Region (GO: 0005615) *cell-cell junction	18 - Pkdcc, Aggf1, Efemp2*, Bpifa2, Cpe, Pla1a, Pnliprp1, Serpinh1*, Hsp90b1, Lrcol1, Nodal, Ltbp1*, Tnfsf15, Rln1, Bmp2, Aspn*, Gdnf, Afm	8 - Furin, Serpinb6c, 4930503L19Rik, Anxa2*, Amy1, Svbp, Lcn10, Pdia6
Cell Junction (GO: 0030054)	20 - Psma7, Ppp2r2a, Fblim1, Igf1r, Tpbg, Rab23, Hip1r, Cpe, Ppfia1, Ctnnd1, Rgs10, Srp68, Pnkd, Rps17, Tmed20, Wasf1, Rpl36, Rpl6, Adora1, Rpl3	8 - Arpc5l, Map4k4, Snx4, Enah, Syt10, Anxa2, Rpl27, Fxr1
Extracellular Matrix (GO: 0009986)	5 - Nid, Ctnnd1, Tgc4, Iglc1, Bmp2	3- Furin, Anxa2, Ccrl2
Sum of unique genes	37	15

Figure 9: Identification of candidate vMES pro-EHT factors dependent on Sash1 signaling (n = 3 WT + 3 KO). *Upper:* We identified factors which may regulate EHT downstream of extrinsic Sash1 as those differentially expressed between Sash1-KO and –WT vMES segments and annotated by Gene Ontology (GO) terms "Extracellular Region (GO: 0005615)", "Cell Junction (GO: 0030054)" or "Extracellular Matrix (GO: 0009986)". *Lower:* Selection of factors with known roles in embryogenesis, morphogen processing or ECM modeling that are up- or down-regulated in KO vMES.

Conclusions and Future Directions

- Our findings indicate that mesenchymal Sash1 regulates early hematopoietic commitment during embryogenesis.
- By profiling the transcriptional landscape of the *Sash1*-null AGM microenvironment we identified multiple candidate EHT regulators for functional follow-up.
- These factors can be broadly classified as regulators of cell adhesion/motility, ECM composition, and morphogens/growth factors.

References

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Michael Smith Health Research BC
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