Single cell RNA sequencing- Kim Lab

**Cells:**

332X: T-cell acute lymphoblastic leukemia (T-ALL)

LAX7R: B-cell acute lymphoblastic leukemia (B-ALL)

**Samples:**

YMK1: 332X Control

YMK2: 332x Tegavivint treated 6 hours

YMK3: 332x Tegavivint treated 24 hours

YMK4: LAX7R Control

YMK5: LAX7R Tegavivint treated 6 hours

YMK6: LAX7R Tegavivint treated 24 hours

Control vs 6h: Look for immediate-early genes, Control vs 24h

6h vs 24h: Identify sustained vs transient transcriptional changes

**Project Summary:**

TBL1X (Transducin Beta Like 1 X-Linked) plays a role in the Wnt signaling pathway to help activate the pathway by binding to beta-catenin (CTNNB1 gene) to turn on downstream Wnt genes. The Wnt signaling pathway plays a role in regulating cell fate, self-renewal, proliferation, migration, and body axis formation. This pathway is upregulated in leukemia cells which is why it is important to inhibit Wnt signaling in this pathway by inhibiting TBL1 with Tegavivint, a small molecule inhibitor of TBL1. Overall, we see that Tegavivint causes leukemia cell death and sensitizes leukemia cells to chemotherapy.

**What to look for:**

Cluster changes

* Does the proportion of specific leukemia subpopulations shift after treatment? (e.g., stem-like vs proliferative vs differentiated clusters).

For T-ALL (332x) cells: Leukemia initiating cells (LIC) markers: LIC phenotype we have been focusing on are cells that are positive for CD7 and negative for CD1a (CD7+CD1a-). Can we see of the cells that are CD7+CD1a-, what genes are differentially expressed in this population compared to all others? Can we also try this with CD34+CD38-

Other Phenotypes:

B-ALL phenotypes:

CD34+/CD10-/CD19−/CD38−

CD133+/CD19−

CD133+/CD38−

CD34+/CD38-/CD19+

CD34+/CD38+/CD19+

T-ALL LIC phenotypes:

CD34+/CD4−

CD34+/CD7+

other differentiation markers:

T cells (332x samples) CD34, CD38, CD44, CD4, CD8, CD3

B cells (LAX7R samples) CD19, CD20, CD24

Note: Everything with CD (cluster of differentiation) in front of it is a surface marker on the cells, so I am not sure if we can look at this with scRNA sequencing? Maybe instead of relying just one CD7 marker transcripts we need combine sets of TFs + markers to define the population (for ex. does this cluster have the signature of early T progenitors (TCF7, NOTCH1, CD7, LEF1)) or early B cell (RAG1/2, Pax-5, CD19, CD34)

Here are some other genes we can look at for differentiation: RUNX1, PROM1, c-Kit, LGR5, HOX, LMO2, TCF, TCR, Bcl11b, GATA3, E2A, EBF1, and PAX5, RAG, PAX5, EZH2, DOT1L, MLL, SUV420H1/2, DNMT3A, TET2, BCL6, IL7R, FOXP3, PTCRA

Differential Gene expression:

Wnt signaling pathway genes to look out for: (Protein name in bold, Gene name in parenthesis)

Upstream: **FZD** (Frizzled), **LRP5/6** (Low-density lipoprotein receptor-related protein), Dishevelled (**DVL**), **Axin**, **APC** (Adenomatous Polyposis Coli), **GSK-3β** (Glycogen Synthase Kinase-3β), **CK1** (Casein Kinase I),

Downstream: **TBL1X** (Transducin Beta Like 1 X-Linked), **TBLR1** (Transducin Beta-Like 1X-Related Protein 1), **Beta-catenin** (CTNNB1 gene), **MYC**, **Suvivin** (BIRC5), **Cyclin D1** (CCND1), **AXIN2**, **TCF** (Transcription Factor), **LEF1** (Lymphoid Enhancer Binding Factor 1), **CBP** (CREB binding protein), **P300** (EP300), **DKK1** (Dickkopf Wnt Signaling Pathway Inhibitor 1), **BCL2** (B-Cell Lymphoma 2), **P53**, (tumor protein 53), **SIAH1** (Siah E3 Ubiquitin Protein Ligase 1), Cyclin-Dependent Kinase (**CDK**), **c-Jun** (JUN), **CDH1** (cadherin 1).

Other pathways:

* **NFkB** (nuclear factor-κB) pathway: **P65** (RELA)
* SMRT/NCoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoic acid and thyroid hormone receptors): **HDAC3** (Histone deacetylase 3), **GPS2** (G Protein Pathway Suppressor 2)
* SCF complex: Cullin 1 (**Cul1**), **Skp1**, and a **RING** protein (**Rbx1/Roc1**).
* **PI3K** (phosphoinositide 3-kinase), **AKT** (AKT Serine/Threonine Kinase 1), **PTEN** (phosphatase and tensin homolog)
* **MTOR** (Mechanistic Target Of Rapamycin Kinase), mTORC1
* **MAPK** (mitogen-activated protein kinase) : ERK, JNK, p38, and ERK5
* **TNF** (Tumor necrosis factor), **Interleukins** (IL), **Interferon** (IFN)
* **NOTCH** (Neurogenic locus notch homolog protein 1)
* **JAK-STAT** (Janus kinases)/ (Signal Transducers and Activators of Transcription), **VEGF** (vascular endothelial growth factor)
* DNA repair: **PARP1, PPP1R15A, RAD51, BRCA1/2**
* apoptotic signaling **BAX, BIM, PUMA, MCL1**
* drug efflux pumps ATP- binding cassette (ABC) **ABCB1/MDR1, ABCC1, ABCG2 (**multidrug resistance protein 1)