Mapping the Spatial Proteome of Leukemia Cells Undergoing Fludarabine Treatment

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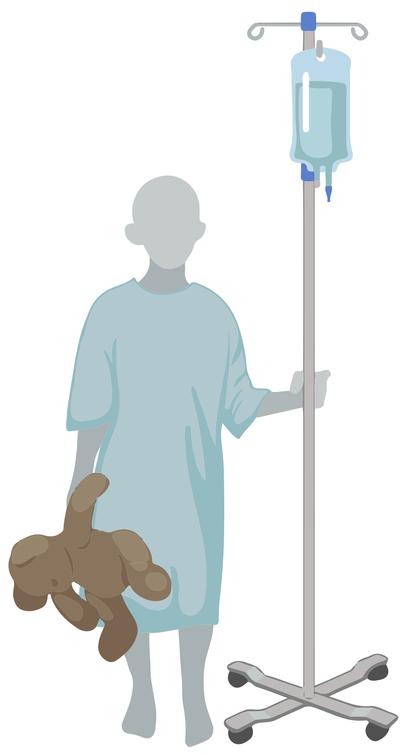
1. Introduction



Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children worldwide. ALL develops in the bone marrow from an uninhibited proliferation of immature B or T cells. Fludarabine, a purine nucleotide analogue, disrupts DNA synthesis and is a critical component of preparatory regimes for both chimeric antigen receptor T-cell and allogenic stem cell therapies for refractory and relapsed ALL patients. Despite fludarabine's essential role in the preparatory regimes prior to immunotherapies, it's molecular impact on leukemia cells and the factors influencing it's eficacy remain limited.

We recently showed that fludarabine elicited a strong transcriptional response in the Reh cell-line, a widely used *in vitro* model of ALL. With three single-cell RNA-sequencing methods we found that genes related to the P53 signaling pathway were dysregulated in Reh cells after fludarabine treatment (1). Here we explore the effect of fludarabine on Reh cells using a novel single-cell spatial proteomics method.

2. Project aims

The aim of this study is to evaluate the protein expression profile of Reh cells after fludarabine treatment with Molecular Pixelation (2), a single-cell spatial proteomics platform that quantifies protein abundance, spatial distribution, and colocalization of targeted proteins.

3. Study design and methods

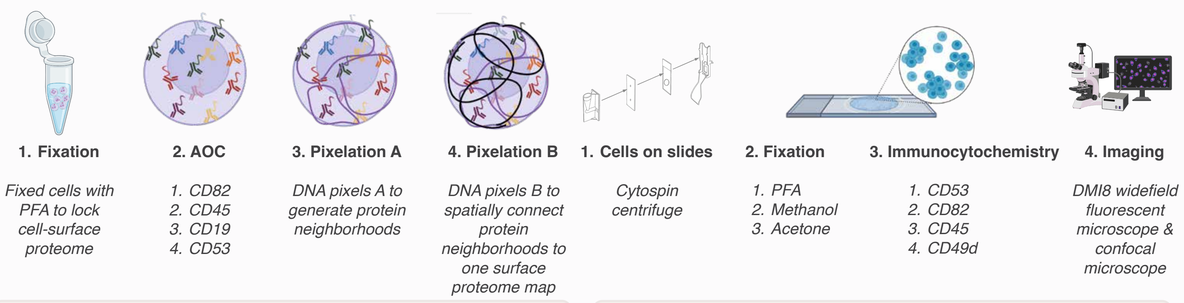
Treated Reh cells with: 0.56 μM Fludarabine or 0.3% DMSO (control) for 72 hrs

Molecular Pixelation

1. Fixation
2. Antibody-oligo conjugates (AOC, 80-plex)
3. Pixelation A
4. Pixelation B

Immunocytochemistry

1. Add cells to slides
2. Fixation
3. Immynocytochemistry
4. Fluorescence imaging



1. Fixation — *Fixed cells with PFA to lock cell-surface proteome*

2. AOC — *1. CD82 2. CD45 3. CD19 4. CD53*

3. Pixelation A — *DNA pixels A to generate protein neighborhoods*

4. Pixelation B — *DNA pixels B to spatially connect protein neighborhoods to one surface proteome map*

1. Cells on slides — *Cytospin centrifuge*

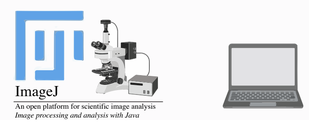
2. Fixation — *1. PFA 2. Methanol 3. Acetone*

3. Immunocytochemistry — *1. CD53 2. CD82 3. CD45 4. CD49d*

4. Imaging — *DMI8 widefield fluorescent microscope & confocal microscope*

Library preparation, sequencing, and data analysis

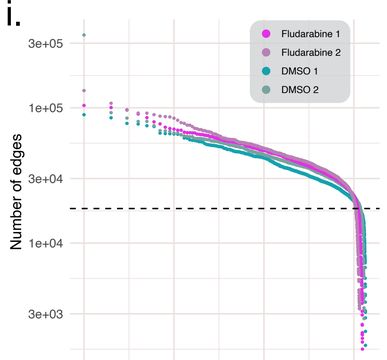
Image analysis



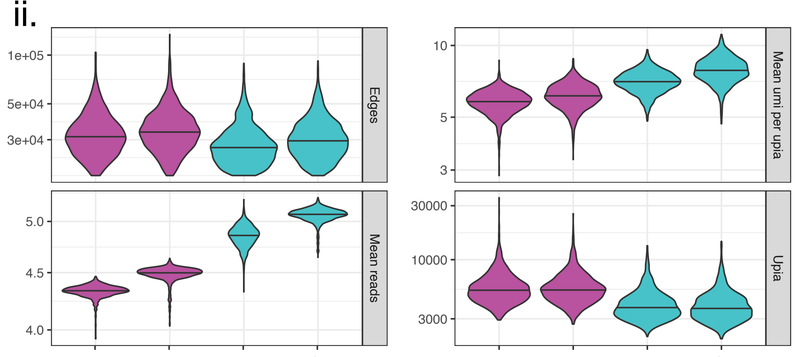
4. Results

A. Quality control of the data: fludarabine treated cells were larger and had fewer reads, more A pixels, and less dense A pixels

i.

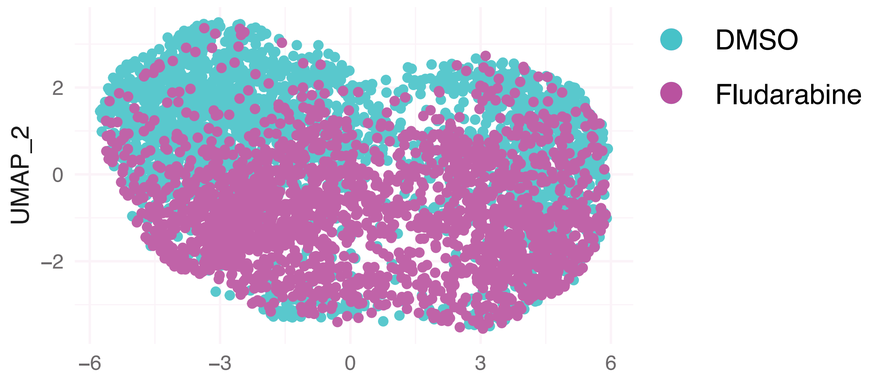


ii.



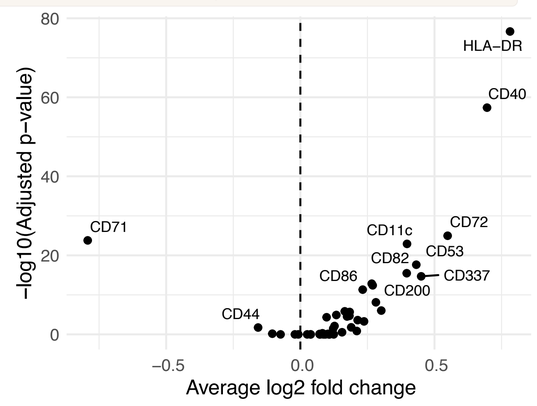
B. Dimensionality reduction showed that there was a difference between fludarabine and DMSO control cells

i.

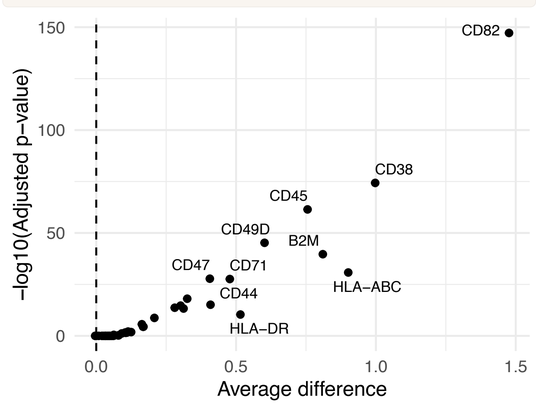


C. Multiple membrane proteins are differentially expressed in abundace, polarity, and/or colocalization between DMSO and fludarabine treated cell

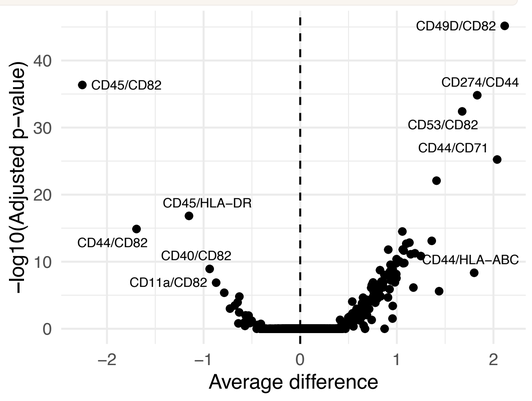
i. Differential protein abundance



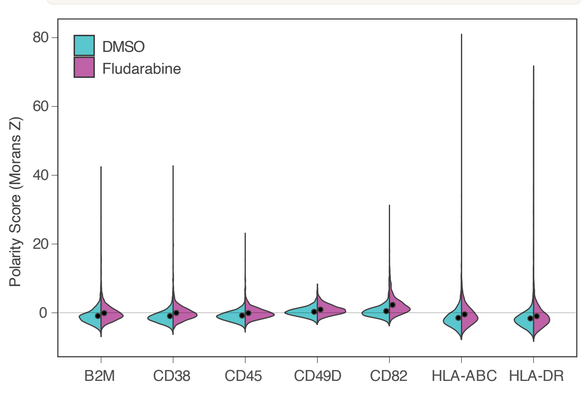
ii. Differential protein polarity



iii. Differential co-localization of proteins



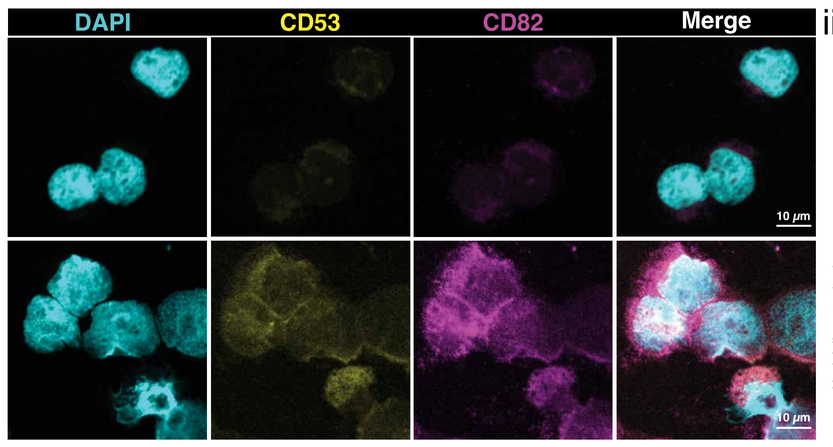
iv. Selected proteins that increase in polarity



Wilcox Rank sum test on protein (i) abundance, (ii) polarity, and (iii)colocalization of fludarabine vs DMSO control cells.
Results were considered significant when adj p-value < 0.05

D. Immunocytochemistry validated the increased protein expression of CD53 and CD82 in Reh cells after fludarabine treatment.

i.

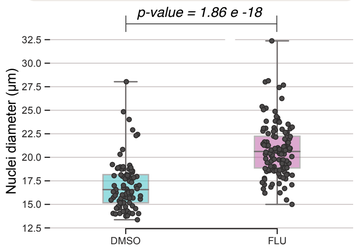


ii.

Fludarabine treated Reh cells have a larger nuclear diameter compared with DMSO control cells

n=85 DMSO and n=124 fludarabine

Each datapoint represents one single cell and the bars represent the standard deviation. The difference between the two groups was determined with a t test.



E. The upregulation of CD82 may be specific to fludarabine

i.

Transcript levels of *CD82* are upregulated in Reh cells after treatment with fludarabine. This finding is consistent with the Molecular Pixelation data

Log normalized data from Gezelius *et al.*, 2024 *NAR Genom Bioinform.*

5. Key points

A.Molecular Pixelation showed that fludarabine treated cells were larger and had fewer reads, more A pixels, and less dense A pixels

B. Dimensionality reduction showed that there was a difference between fludarabine and DMSO treated cells

C. Molecular Pixelation identified several proteins as differentially expressed in abundacne, polarity and/or colocalization between DMSO and fludarabine treated Reh cells

D. The upregulation of CD53 and CD82 was validated with immunocytochemistry

E. The upregulation of CD82 may be specific to fludarabine

6. Ongoing experiments and future perspectives

A. Continue validating the differentially expressed proteins identified by Molecular Pixelation

B. Perform Molecular Pixelation on Reh cells treated with additional cytototoxic/cytostatic drugs

B. Perform Molecular Pixelation on additional ALL cell lines

C. Investigate the biological relevance of the identified proteins in the context of ALL

7. References

1. Gazelius *et al.*, 2024 *NAR Genom Bioinform.*
2. Karlsson *et al.*, 2024 *Nature Methods*

8. Aknowledgements

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Illustrations created with BioRender.
Molecular Pixelation image adapted from Pixelgen, cytospin technique image adapted from ThermoFisher and Image J image adapted from Image J

9. Have a question?

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MPM molecular precision medicine