Identifying Therapeutic Targets for Improved Bone Regeneration

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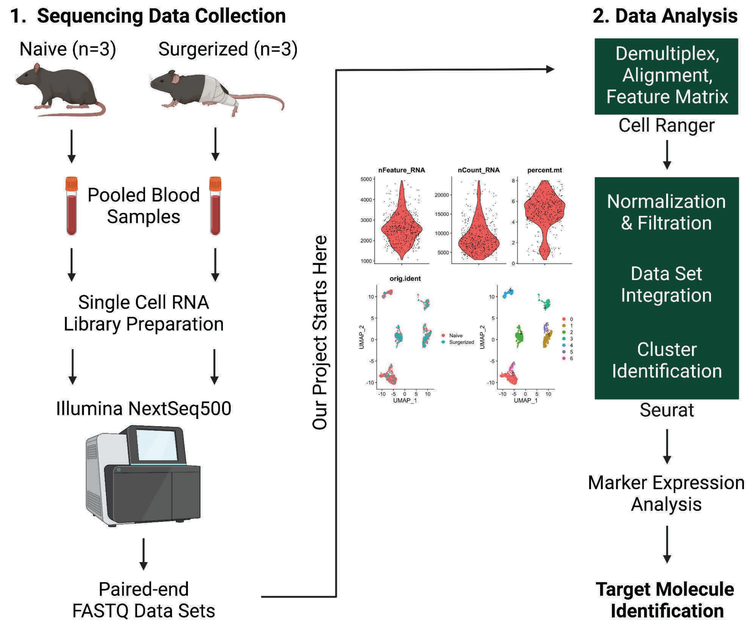
1. Background

Myeloid-derived suppressor cells (MDSCs) are an immature immune cell-type associated with poor bone regeneration following severe musculoskeletal trauma, increasing the likelihood of bone non-unions and serious infections. Therefore, **MDSC depletion may lead to improved bone regeneration** and clinical outcomes.

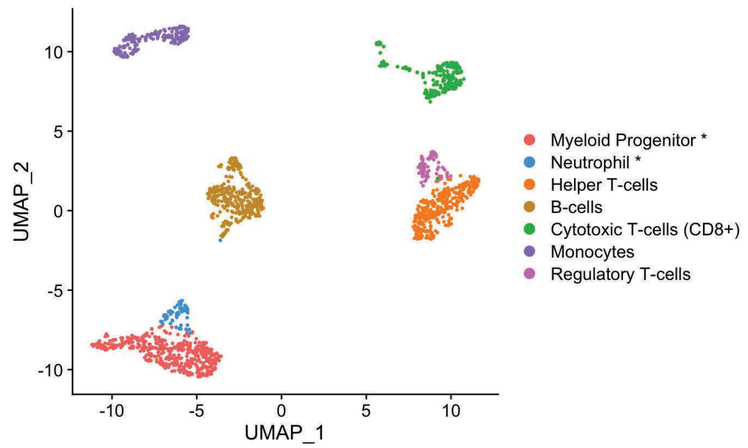
A previous study targeted S100A8/A9 surface proteins of MDSCs using synthetic nanoparticle antibodies (SNAbs). This resulted in depletion of one MDSC subtype and proliferation of another–resulting in a net increase in MDSC abundance.

The goal of this project was to **analyze single-cell RNA sequencing data from naïve and surgerized rats to identify a new target molecule** for SNAbs. Ideally, this target molecule would be a surface protein highly expressed in both MDSC subtypes and lowly expressed in other immune cells.

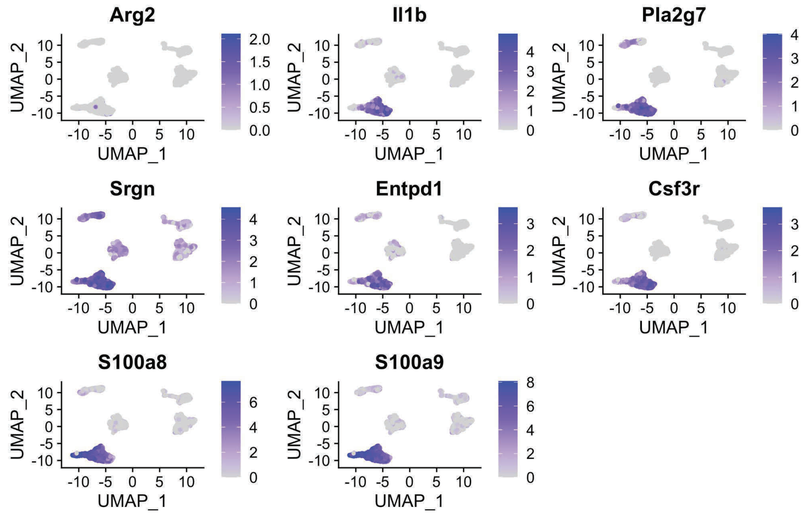
2. Workflow



3. Cluster Identification



UMAP of all immune cell clusters. We identified two MDSC subclusters, divided between myeloid progenitor cells and neutrophil cells, marked with *. These two MDSC clusters were then isolated to be analyzed for differentially expressed genes.



Feature plots displaying expression levels of known MDSC markers in UMAP clusters. All known MDSC markers displayed high expression (purple) in the same two clusters, suggesting two MDSC subtypes.

4. Marker Expression Analysis

	DEVOC	Avg. log2 fold change	% expressed in MDSC clusters	% expressed in all other clusters
Cxcr2	2.51e6	3.58	70.1%	< 1e-4%
Slpi	2.35e6	3.35	70.1%	< 1e-4%
Steap4	1.70e6	2.59	65.7%	< 1e-4%
G0s2	1.59e6	2.67	59.7%	< 1e-4%
HCar2	1.49e6	2.37	62.7%	< 1e-4%
ENSRNOG00000064524	1.35e6	1.93	70.1%	< 1e-4%
Nfam1	1.17e6	1.61	73.1%	< 1e-4%
ENSRNOG00000068554	1.17e6	3.56	32.8%	< 1e-4%
Clec4d	1.15e6	1.98	58.2%	< 1e-4%
Lpcat2	1.01e6	1.73	58.2%	< 1e-4%

Top ten differentially-expressed genes in MDSC subclusters. We used a custom ranking score named "Differentially Expressed Versus Other Clusters (DEVOC)" to calculate differential expression of markers in the MDSC clusters using the formula:

$$DEVOC = \frac{\%\ expressed\ in\ MDSC\ clusters}{\%\ expressed\ in\ all\ other\ clusters} \times avg.\ log_2\ fold\ change$$

The higher the DEVOC score, the better the target molecule, making Cxcr2 the most promising target for future SNAbs.

5. Conclusions

- Two clusters were isolated and characterized as MDSCs based on their expression profiles on known MDSC markers, which match previously observed subtypes
- Within these clusters, shared, differentially-expressed genes were identified as biomarkers for both MDSC subtypes and ranked by the custom ranking score DEVOC
- Of these shared markers, the surface protein **Cxcr2** was identified as the most promising therapeutic target for future SNAbs and the depletion of both MDSC subtypes

6. Future Directions

- Employ pseudo-time analysis to predict terminal MDSC differentiation in both subtypes
- Perform pathway enrichment analysis to further investigate MDSC biomarkers
- Find additional targets if Cxcr2 proves to be unviable

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SCAN ME

REFERENCES