RNA Sequencing and Data Analysis of the Effects of Biophysical Cues on Cellular Transcriptome

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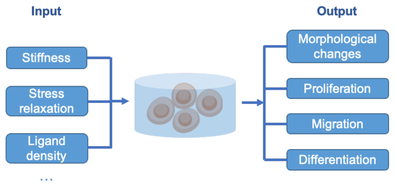
Background

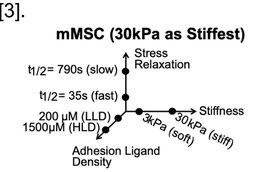
- Biophysical microenvironmental factors have shown to couple and interact to regulate various cell behaviors [1,2].



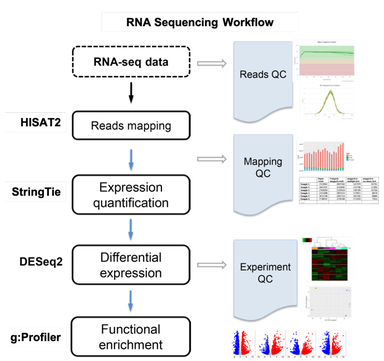
- Although plenty of phenomenological work has been done on different cell types to investigate microenvironmental sensing. A deep and comprehensive understanding of how biophysical conditions affect the cellular transcriptome is lacking.
- In this study, we harness **RNA sequencing** as a computational tool to thoroughly screen the response of the cellular transcriptome of **mouse mesenchymal stem cells** (mMSCs) to different combinations of biophysical cues, including **stiffness**, **stress relaxation** and **adhesion ligand density**.

Methods & Design

- The raw data of the study comes from a published paper from Dr. David Mooney's lab at Harvard University [3].
- A 3D alginate culture system was engineered to independently control biophysical parameters. mMSCs were encapsulated in eight conditions: low or high values of stiffness, stress relaxation, and ligand density.



- A pairwise comparison of each condition was performed to identify the most significantly differentially expressed genes.

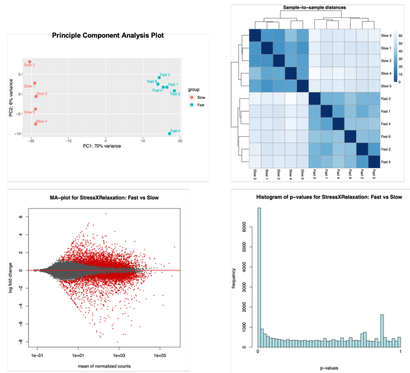


- Using computational packages from the Galaxy platform [4], we aligned the sequences to the annotated genome (HISAT2), quantified gene counts (StringTie), and analyzed significant changes in gene expression between groups (DESeq2).
- Functional enrichment analysis (g:Profiler) maps genes to their related biological processes and functional pathways [5].

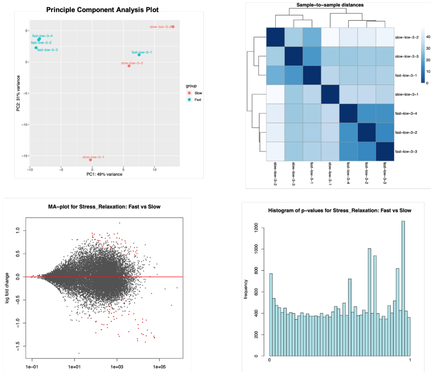
Results

For each pairwise comparison, four plots were used to visualize the distribution of gene counts. Clear separation of clusters in principle component analysis, large sample-to-sample distance, high frequencies of p-values lower than 0.001, and high log fold changes compared to mean normalized counts (genes labeled in red) indicate larger numbers of and higher extent of differentially expressed genes between the two conditions. The below two comparisons show drastically different behaviors.

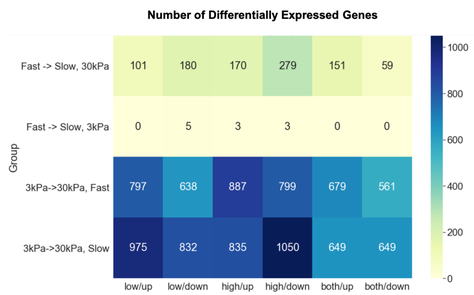
Fast vs. Slow relaxation, 30kPa, High ligand Density



Fast vs. Slow relaxation, 3kPa, Low ligand Density



The below heatmap shows **a large discrepancy in the number of differentially expressed genes and their up/down regulation** under different parameter conditions. **Stiffness > Stress Relaxation > Adhesion Ligand Density** in influencing #DE genes at the conditions of this study.

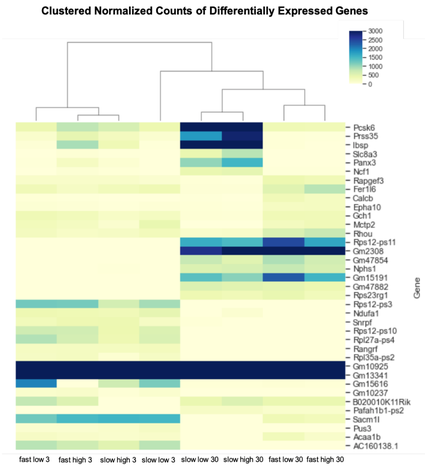


The result is also indicative of **the context-dependence of biophysical sensing**. At the conditions of this study, changes in stress relaxation induce larger number of DE genes at high stiffness but few DE genes at low stiffness. Changes in stiffness drive more DE genes at a lower stress relaxation. Higher ligand density generally leads to more DE genes. We can conclude that **certain matrix properties can modulate the response of cells to other matrix properties**, which is consistent with previous studies [1,6].

Group/Regulation	Affected Pathways
Fast → Slow, 30kPa, Down	collagen degradation/formation/chain trimerization; blood vessel development; biomineralization; cartilage development; extracellular matrix organization/structural constituent; neuron development; cell-cell adhesion; Wnt signaling pathway
Fast → Slow, 3kPa, Down	positive regulation of T cell proliferation; cell adhesion; Mmu-miR-203-5p
3kPa → 30kPa, Fast, Up	large/small ribosomal structural constituent; cytoplasmic ribosomal proteins; SRP-dependent co-translational proteins; Cap-dependent translation initiation; COVID-19
3kPa → 30kPa, Slow, Down	extracellular matrix organization/degradation/receptor interactions; collagen degradation/chain trimerization/ formation; assembly of collagen fibrils; pulp stones

Results

Clustered Normalized Counts of Differentially Expressed Genes



We identified and functionally analyzed the most significant DE genes of mMSCs under different combinations of biophysical cues. A list of DE genes was extracted and visualized for every pairwise comparison.

Overall, the results agree with the conclusions of the original paper [3]. We attribute the discrepancy of results to the following factors. 1) Different analysis tool and settings, 2) Sample variations, 3) Different gene annotation files used.

The above clustered heatmap shows the average normalized counts of 5-10 most DE genes in each group (>3 samples per group).

Future Directions

- Further functional enrichment analysis could be done using Metacore to construct putative gene networks and identify clusters of genes analogously affected by biophysical conditions.
- Various hypothesis linking cell function to biophysical cues may be formulated from the results of the functional enrichment analysis. However, one must be mindful of the cell-type and biophysical parameter range dependence of cellular behaviors.
  - PCSK6 is upregulated in human atherosclerotic plaques associated with extracellular matrix remodeling [7]. Mechanistic studies could be done to investigate how stress relaxation regulates intimal hyperplasia progression.
  - IBSP is a cancer gene that promotes tumor metastasis through epithelial mesenchymal transition (EMT) [8]. 3D hydrogel models capitulating cancer cell relaxation conditions could be constructed to study EMT and screen for anti-cancer therapeutics.

References

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