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

Jasmine Hu, Zhiwei Fang, Luo Gu
Johns Hopkins University, Department of Materials Science and Engineering, Baltimore, MD

Cells have been shown to respond to the biophysical microenvironment that surrounds them. Previous phenomenological studies suggest that microenvironmental stiffness, the composition and density of adhesion ligands, and the stress relaxation properties [1, 2] can couple and interact to regulate various cell behaviors, such as morphological changes, proliferation, migration, and stem cell differentiation and fate. In order to recapitulate how cell responds to these cues, a 3D alginate culture system can be engineered to independently control the different biophysical features of the microenvironment by tuning the molecular weight distribution, the crosslinking density, and the density of cell adhesion ligands [3]. However, a deep and comprehensive understanding of how external biophysical conditions affect the cellular transcriptome which in turn regulates downstream cell responses is lacking.

RNA sequencing uses next-generation sequencing (NGS) technology to analyze the continuously changing cellular transcriptome of particular biological samples. Therefore, using RNA sequencing and bioinformatic approaches, we can identify differentially expressed genes under different cell culture environments and infer a material sensing regulatory network through enrichment analysis [4]. This could bridge the gap between biophysical microenvironmental inputs and their related cell behaviors, promoting the fundamental understanding of the genetic pathways that are associated with cell mechanotransduction, in particular, those that have not been fully studied with regards to biophysical cues, such as immunomodulation and cancer progression.

In this study, we perform RNA sequencing and data analysis on mouse mesenchymal stem cells (mMSCs) cultured in 3D alginate models with high (30kPa) and low (3kPa) stiffness, fast ($t_{1/2}$ = 35s) and slow ($t_{1/2}$ = 790s) stress relaxation, and high (1500μM) and low (200μM) adhesion ligand density. The raw data of the study comes from a published paper from Dr. David Mooney's Lab at Harvard University [5]. Using the computational packages from Galaxy platform and R, we will pre-process the raw data for quality control (FASTQC), align the RNA sequences to the annotated genome (HISAT2), quantify gene counts (StringTie), and analyze for differential gene expression (DESeq2) [6]. A pairwise comparison of each condition will be performed to identify the most significantly differentially expressed genes. Gene count heatmaps, MA plots, and PCA analysis will be used to visualize and assess our results [7]. Functional enrichment analysis will then be performed using gProfiler to link the molecular pathways to gene expression, and thus to the change in biophysical parameters. Finally, putative gene networks could be constructed using Metacore software to identify the clusters of genes analogously affected by biophysical conditions.

(2461 characters)

References

[1] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S.A. Bencherif, J.C. Weaver, et al., Hydrogels with tunable stress relaxation regulate stem cell fate and activity, *Nature Materials*. 15 (2015) 326–334. doi:10.1038/nmat4489.

[2] N. Huebsch, E. Lippens, K. Lee, M. Mehta, S.T. Koshy, M.C. Darnell, etal., Matrix elasticity of void-forming hydrogels controls transplanted-stem-cell- mediated bone formation, *Nature Materials.* 14 (2015) 1269–1277. doi:10.1038/nmat4407.

[3] M. Darnell, L. Gu, D. Mooney, RNA-seq reveals diverse effects of substrate stiffness on mesenchymal stem cells, *Biomaterials.* 181 (2018) 182–188. doi:10.1016/j.biomaterials.2018.07.039.

[4] R. Stark, M. Grzelak, J. Hadfield, RNA sequencing: the teenage years, Nature Reviews Genetics. 20 (2019) 631–656. doi:10.1038/s41576-019-0150-2.

[5] M. Darnell, A. O'Neil, A. Mao, L. Gu, L.L. Rubin, D.J. Mooney, Material microenvironmental properties couple to induce distinct transcriptional programs in mammalian stem cells, *Proceedings of the National Academy of Sciences*. 115 (2018).

[6] M. Griffith, J.R. Walker, N.C. Spies, B.J. Ainscough, O.L. Griffith, Informatics for RNA Sequencing: A Web Resource for Analysis on the Cloud, *PLOS Computational Biology*. 11 (2015). doi:10.1371/journal.pcbi.1004393.

[7] Michael I. Love, S. A. Analyzing RNA-seq data with DESeq2 (2020). Available at: http://bioconductor.org/packages/release/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#frequently-asked-questions.