

Deconvoluting cell types through scRNA-Seq

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May 2020

Abstract

This paper details how to determine what cell types are present in *Rattus norvegicus* superior cervical ganglion (SCG) cultures and how these cell types respond to different drug treatments (LY, mirin) in terms of gene expression. The SCG culture was analyzed to determine it consisted of neurons, glial, endothelial, and Schwann cells. The cultures were then separated into its respective cell type, and gene expression was analyzed to determine what genes were up and down regulated with treatment. The change in gene expression from the drug treatment affected certain pathways, that would in turn affect cell survival rate.

1 Introduction

The superior cervical ganglion is a part of the autonomic nervous system, and is the largest ganglion of the cervical sympathetic trunk. The superior cervical ganglion is composed of neurons and receive presynaptic signals from the sympathetic trunk.^(1;2) The superior cervical ganglia is the mid point between the sympathetic trunk and targets in the head and neck for the presynaptic signals.⁽²⁾

The Mre11-Rad50-Nbs1 (MRN) complex is vital in sensing DNA double-strand breaks, promoting repair, maintaining genome stability during replication, activating ATM, a protein kinase critical in maintaining genome integrity.^(3;4) MRN recruits ATM to the damaged DNA, and ATM phosphorylates substrates to coordinate cell cycle arrest and DNA repair.⁽⁵⁾ Mirin is a inhibitor of MRN, and prevents ATM activation, inhibiting the MRN-ATM pathway. Without ATM activated, there is no response to double-stranded breaks and no homology-directed repair.⁽³⁾

Another pathway, PI3K/AKT is associated with numerous cellular functions. These functions include cell metabolism, growth, proliferation, and survival.⁽⁶⁾ LY294002 (LY) is an inhibitor on the PI3K/AKT pathway, by specifically inhibiting PI3K. LY was found to successfully enhance sensitivity of cancer cells to drug-related apoptosis, because of its ability to inhibit the PI3K/AKT pathway.⁽⁷⁾

The data are single cell RNA-seq data of four *Rattus norvegicus* SCG cell cultures with two treated with DMSO, one treated with mirin, and one treated with LY. The single cell RNA-seq data came out in fastq files which had to be aligned against the *Rattus norvegicus* genome to generate counts. From there, these aligned counts could be analyzed to determine cell clusters, differentially expressed genes, and impacted pathways.

2 Results

To determine cell types within the cell culture, Seurat was employed for its graph-based clustering approach. The cultures was first integrated. Integrating the different cultures allowed identification of shared cell states across the different data sets. First, anchors had to be identified. Anchors are pairwise correspondence between cells in different data sets.⁽⁸⁾ The clusters' cell types were determined through their top conserved markers.

For example in Figure 1, S100 and SOX10 are known cell markers for Schwann cells. As, S100 and SOX10 were in the top 5 cell markers of cluster 9, cluster 9 can be defined as a Schwann cell cluster. Of the 17 different clusters, there was eight neuron, five glial, three endothelial, and two Schwann clusters. Gilal cells are support cells for neurons, and Schwann cells are a type of glial cells. The neuron and glial cells are clustered closely together, while the endothelial cells seem to be clustered on its own.

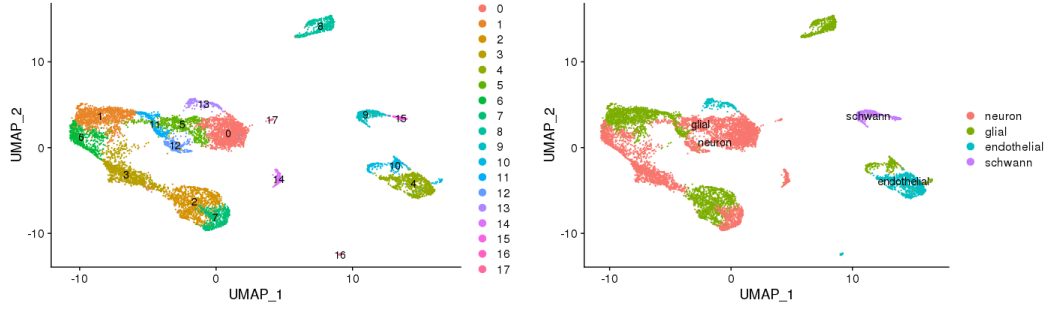
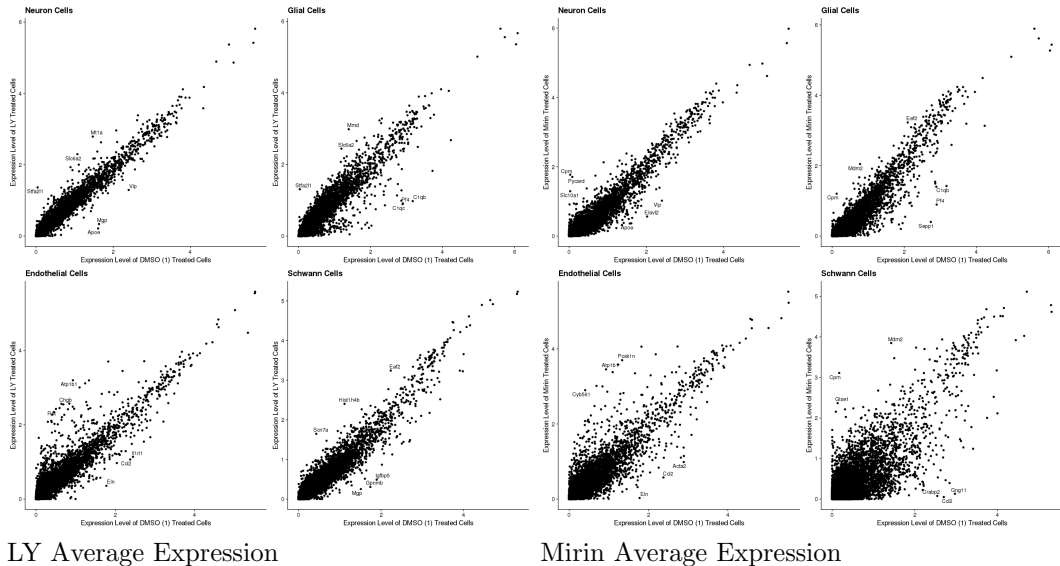


Figure 1: Clustering before and after identifying cell types through cell type markers

Experiment	Neuron	Glial	Endothelial	Schwann
DMSO Biological Replicate 1	1444	1075	437	147
DMSO Biological Replicate 2	1487	927	203	128
Treated with LY	717	638	207	87
Treated with mirin	1073	696	227	81

Table 1: Cell Numbers in Each Experiment

As shown in Table 1, compared to the first DMSO treated biological replicate, LY and mirin treated cultures had significantly less cell counts for every cell type. The second DMSO treated biological replicate also had more cells than the two drug treated cell cultures. This indicates an effect on cell counts from the drug treatment. The proportions of neuron, glial, endothelial, and Schwann cells in the control dms0 treated culture are 46%, 34%, 14%, 5%, respectively. The proportions of cells within the LY treated culture are 43%, 38%, 12%, 5% within the neuron, glial, endothelial, and Schwann cells respectively. Within the mirin treated culture the neuron cell represented 51%, the glial cells represented 33%, the endothelial cells represented 10%, and the Schwann cells represented 3% of the entire culture. The glial cells with the LY treatment were affected more harshly than those treated with mirin. However, even with the treatment of drugs, the proportions of the cell types within the cell culture generally stayed within the same range, meaning the drugs did not severely impact one cell type specifically.



LY Average Expression

Mirin Average Expression

Figure 2: Average Expression of Treated vs Control Cell Cultures, split by cell type

Seurat was also used to determine which genes were up and down regulated through the different drug treatments. Since the two cell cultures treated with DMSO are biological replicates, the one with more total cells was used as a control to determine differential expressed genes.

Experiment	Cell Type	Gene	Experiment	Cell Type	Gene
Control (DMSO)	Neuron	Tuba1a	Control (DMSO)	Neuron	Elavl2
		Tuba1b			Bri3
	Glial	Apoe		Glial	Rps27l
		Prdx1			Bri3
	Endothelial	Gapdh		Endothelial	Fam111a
		Ifitm3			Sod2
	Schwann	Npc2		Schwann	Gng11
		Prdx1			Nudt4
LY treated	Neuron	Stfa2l1	mirin treated	Neuron	Slc10a1
		Hist1h4b			Pycard
	Glial	Th		Glial	Rpl9
		Rsrp1			Eef2
	Endothelial	Ret		Endothelial	Atp1b1
		Atp1b1			Ret
	Schwann	Eef2		Schwann	Cpm
		Eef1a1			Gtse1

pathway seemed to be most commonly impacted by the LY treatment through the different cell types. Other interesting pathways to note is "negative regulation of neuron apoptotic process" and "positive regulation of neuron projection development." These pathways would be integral in a cell's ability to grow and survive. Mirin impacted "aging", and "translation" through multiple cell types. Mirin also impacted "negative regulation of neuron apoptotic process," which is interesting as both drugs impacted that pathway. Since several pathways were impacted by both LY and mirin, it could suggest that these pathways are more susceptible to drug treatment than other pathways.

3 Discussion

Through the results of this analysis, it is clear that LY and mirin affect the SCG cell cultures in a number of ways. Firstly, the cell count of cells decreased with treatment. This can be contributed to the fact that LY and mirin inhibit pathways that deal with DNA repair and survival. With these pathways inhibited, the cells are expected to have lower survival rates. Interestingly, mirin treated neuron cells did not drop decrease as much as LY treated neuron cells. This could be directly related back to the fact that the "positive regulation of neuron projection development" was impacted by LY, but not by mirin.

The different cell types found in the SCG cultures are were neuron cells, glial cells, endothelial, and Schwann cells. Since the SCG is apart of the nervous system, finding neuron cells and their support cells was to be expected. Endothelial cells are cells that line blood and lymphatic vessels, and could be found within or near the SCG. From cell clustering in Figure 1, it is clear that the neuron and glial cells are intertwined, which can be contributed to the fact that glial are support cells for neuron cells.

Pathways such as "negative regulation of neuron apoptotic process," "positive regulation of neuron projection development," and "aging" are all related to the cell's ability to grow, age, and die. With the LY treatment, these three pathways are affected, supporting the idea that the drug LY can potentially decrease cell numbers. Mirin is also shown to be affecting the same "negative regulation of neuron apoptotic process" and "aging" pathways. It is interesting to note that mirin had an effect on the "response to drug" pathway, but LY did not have that pathway in its top affected pathways.

4 Methods

Cell Ranger was first employed to align data sets to the *Rattus norvegicus* genome. The *Rattus norvegicus* genome had to first be downloaded. Cell Ranger then aligned the original fastq files and generated feature counts in a matrix.

The matrix is then imported into Seurat, which helps make sense of the raw matrix data. Seurat allowed the data sets to be integrated so that it could be comparable. Integration of the data sets required "anchors," so that the data could be "normalized" among all of the data. Seurat allowed clustering of the cells to be visualized, and the cell markers related to each of the clusters.

From the cell markers, each cluster was assigned a cell type. From here on, the analysis of the data would not only be separated by experiment but also by cell type. Through Seurat, the markers of each experiment and cell type was identified. These cell markers would be the up and down regulated genes of each cell type within each experiment.

These genes would then be inputted into DAVID, to find the pathways affected by the change in gene expression.

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