Modeling Neurodegeneration with Single Cell RNA Sequencing

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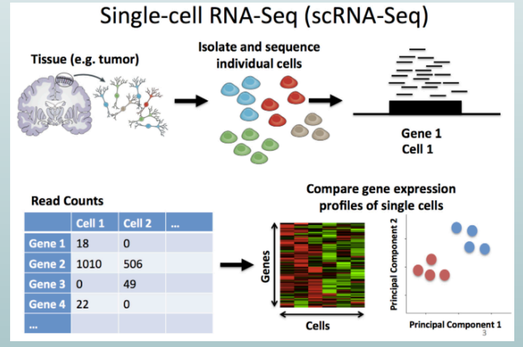
Background / Motivation

- Single Cell RNA Sequencing: **transcriptional profiling** of thousands of **individual cells**



	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

- Our goal is to **model neurodegeneration** in brain organoids by single cell RNA sequencing
- We aim to understand the **different types of cells** present in brain organoids, and how they differ
- We aim to compare **differences in gene expression** between **control** and **mutant** brain organoids, and **analyze** which processes they affect

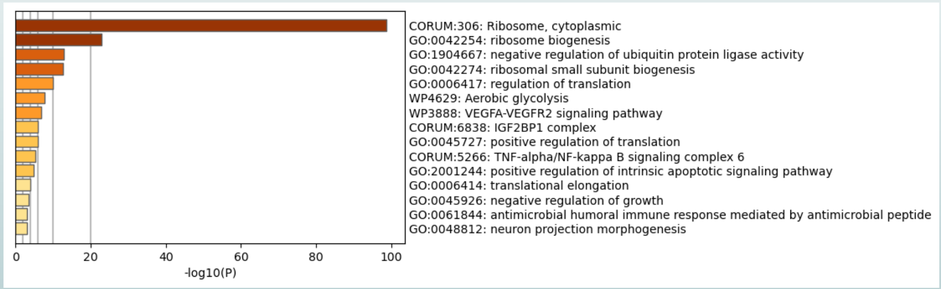
Data

- **Filtered** single-cell expression dataset given to us by our sponsor
- Organoids **vary** across different cell lines **control vs. mutant** and **age**
- **Quality control** performed to remove low quality cells, but batch effects are still present
- Size: ~76k cells, ~22k genes
- **Sparse**: many genes are rarely registered

Samples and # of cells:

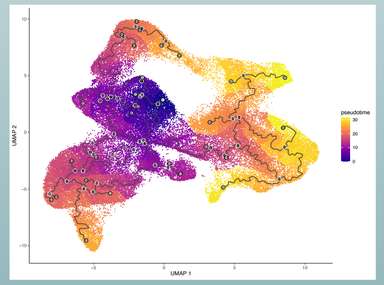
	R406W mutation					V337M mutation					
	donor1: F11362 "406RW1"		donor2: F11421 "406RW2"		donor3: GIH143 "406WW1"	donor1: ND32951A "V337M1"		donor2: GIH6 "V337M2"		donor3: GIH7 "V337M3"	
line / age	RW (F11362.1, ΔP10)	RR (F11362.1, ΔC11)	RW (F11421.12 parent)	RR (F11421.12 ΔA07)	WW (GIH143-C3)	VM (ND32951A.15 ΔZB09)	VV (ND32951A15 Δ1B06)	VM (GIH6-1-C1 ΔA02)	VV (GIH6-1-C1 ΔE11)	VM (GIH7-C2 ΔZA01)	VV (GIH7-C2 ΔZB12)	VV (GIH7-C2 ΔZF02)
1 mo			997	1098								
2 mo	1768	1506	1400	997	868 / 1047	1684	1252	1170	987	975	1333	1085
3 mo	917	1095	1551	1510	2093 / 1509	1078	1207	1327	1165	1260	1160	1586
4 mo	1181	1351			783	932 / 1087 / 1264	868 / 940 / 1110	1085 / 1202	1226 / 992	1257	1372	897
6 mo	1421 / 1308	1060 / 1331			1275	1399/1445	1876 / 1083	1540 / 967	1738 / 1268	1150	1046	1234
8 mo	1378	1421										

Results



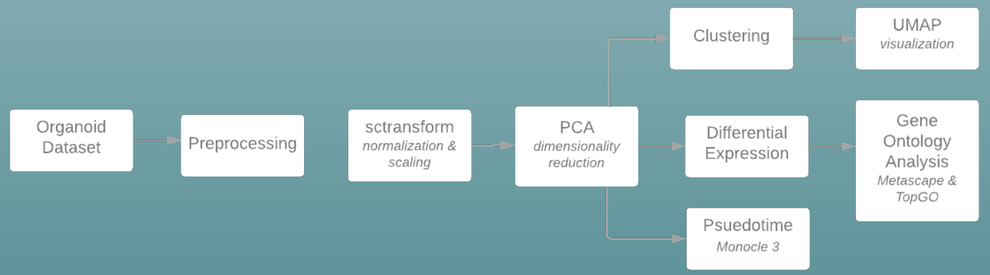
Downregulated ontologies: two-line mutation

- Key ontologies **upregulated** in in the mutant line were neuron projection development, regulation of nervous system development, positive regulation of neuron death, and negative regulation of microtubule polymerization or depolymerization.
- **Downregulated** ontologies included translation and ribosome function, protein folding

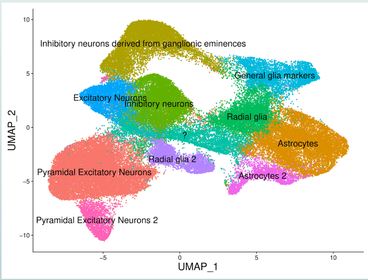


Cell Trajectory analysis with partitions

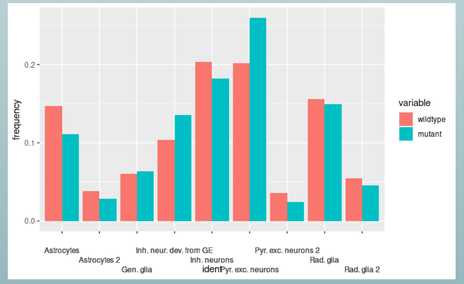
Methodology



Exploratory Analysis/ Preprocessing



Cell clusters labelled with cell types corresponding to marker genes they differentially express



Relative frequencies of cell types in control vs. mutant

Conclusion

- **Cell identification**: We labeled most clusters with likely cell types and found almost all expected types of brain cells
- **Ontology analysis**: Found gene ontologies impacted in the mutant group which align with existing research and suggest other effects.
- **Cell Trajectory (Pseudotime) analysis**: Visualized arrangement of cells according to their developmental stage in biological processes, and found that organoids continue to develop through 8 months

Acknowledgments

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