Highlight

Sequencing the landscape of cerebellar gene expression

The cerebellum plays critical roles in motor performance and non-motor functions such as cognition and emotion (Koziol et al., 2014; Baumann et al., 2015). These functions are encoded by canonical microcircuits that are composed of diversified cell types distributed in the cerebellar cortex and cerebellar nuclei (Buckner, 2013). The abnormality and dysfunction in cerebellar microcircuits cause a number of neurological and psychiatric symptoms, such as ataxia, tremor, autism spectrum disorder, and schizophrenia. Extensive morphologic and electrophysiological evidence has demonstrated the functional machinery of cerebellar microcircuits under physiological and pathological conditions. However, the gene expression framework for cerebellar development and dysfunction is poorly understood.

The cerebellar cytostructure emerges mainly from ventricular zone and the rhombic lip during early embryonic stage (Hatten and Heintz, 1995). Purkinje cells, for example, are derived from ventricular zone of mouse at E10–E13 (Wang and Zoghbi, 2001), followed by radial migration to form a monolayer configuration after birth (Marzban et al., 2014). Granule cells, for another example, are derived from rhombic lip in mice at E12.5–E17.5, followed by tangentially migration to form the external germinal layer. At birth, granule precursors exit the cell cycle, differentiate into mature, and migrate along the radial fibers of Bergmann glia to populate at granular cell layer (Adams et al., 2002). Both Purkinje cells and granule cells experience dynamic morphological changes during their maturation: Purkinje cells form a highly elaborated dendritic configuration that is flattened within the sagittal plane, meanwhile granule cells project an ascending axon that bifurcates at molecular layer to form parallel fibers. Many signaling pathways are suggested to be involved in the development of cerebellar neurons, however, the dynamic gene expression networks that orchestrate cell fate trajectory and functions are unclear. Several studies have presented transcriptional profiles of the developmental cerebellum (Carter et al., 2018; Gupta et al., 2018; Rosenberg et al., 2018), but they mainly focus on the expression of transcription factors associated with the fate determination of granule cells. The dynamic molecular changes at the transcriptome level governing fate determination and maturation in the cerebellum remain yet unclear.

Single-cell RNA sequencing (scRNA-seq) has been proven to be a powerful tool to reflect specific changes at the transcriptome level of individual cells. This technique is particularly useful to detect the expressions of molecules that determine the fate of diversified cerebellar neurons. By using the droplet-based scRNA-seq (Figure 1), Peng et al. (2019) analyzed transcriptome profiles of 21119 single cells of postnatal mouse cerebellum at P0 and P8 ages. For analyzing 58994 unique molecule identifiers (UMIs) and a median of 2615 genes, they used a series of known cerebellar cell lineage markers to annotate each cell cluster, such as granule cell precursor, granule cells, Purkinje cells, astrocytes, interneurons, oligodendrocytes, and endothelial cells. In general, they found that different cell types were distinct in the size of cell body, while the numbers of genes and UMIs of each cell type were similar. Interestingly, the population of mitochondrial transcripts in Purkinje cells was significantly larger than that in other cell types, suggesting that the mitochondria in Purkinje cells may exert essential roles. Peng et al. (2019) analyzed temporal mechanisms underlying the development of mouse Purkinje cells. They identified 618 differentially expressed genes (DEGs) by comparing P0 and P8 stages. Interestingly, they showed that many DEGs were involved in mitochondrial and ATPase biological processes, suggesting that mitochondrial pathway may contribute to morphological development of Purkinje cells. They also found 20 divergent genes between Purkinje cells and other cell types, which may play non-autonomous roles in cerebellar development and circuit formation. In addition to known marker genes, they found several novel genes solely expressed in Purkinje cells, whose functions remain to be investigated in the development of the cerebellum and its related diseases.

Given by a number of ataxia risk genes (Ashizawa et al., 2018), susceptible cell types in the cerebellum remain unclear. Peng et al. (2019) analyzed 28 ataxia risk genes and found that the majority of them were highly expressed in Purkinje cells, in consistent with the current hypothesis that the dysfunction of Purkinje cells is closely related with the occurrence of ataxia. They showed that most significantly enriched genes were related with organizations of organelle and mitochondrial inner membrane and were largely consistent with DEGs of Purkinje cells between P0 and P8, suggesting that mitochondrial function is highly correlated with the development of Purkinje cells and motor behavior disorders.

Granule cells account for ~80% of all neurons in the cerebellum. Differentiated granule precursors migrate into granule cell layer and mature into functional granule cells around postnatal week 3. Upon

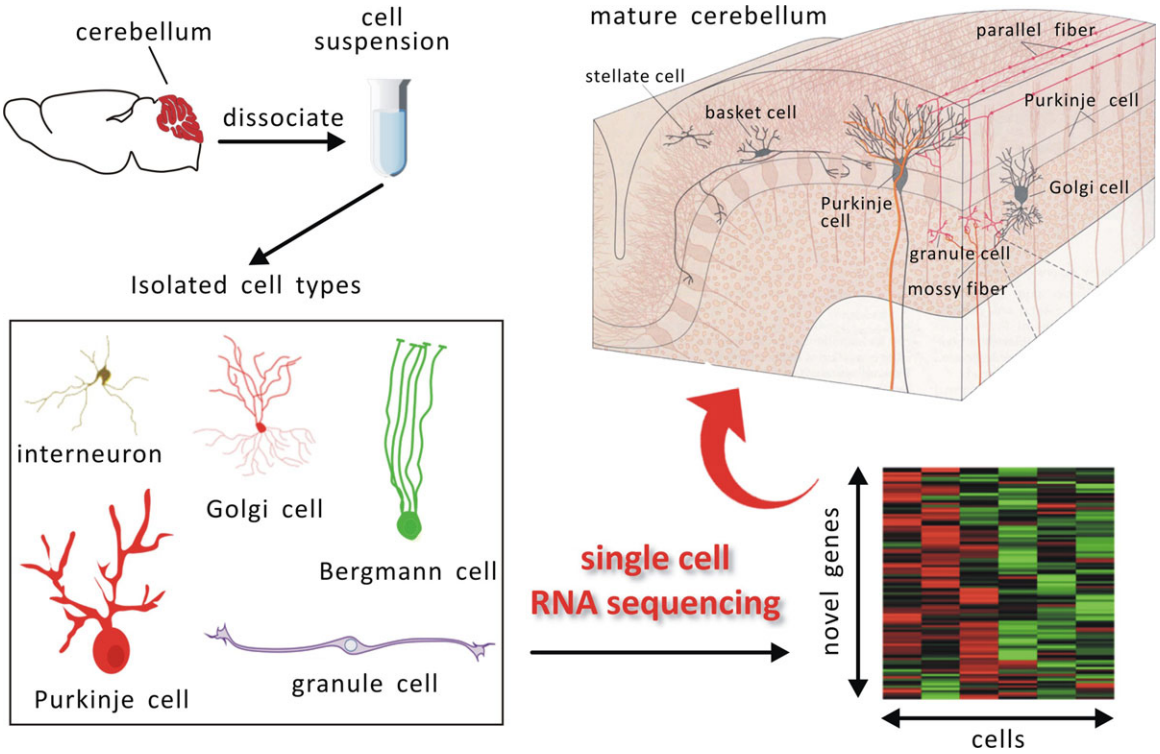

Figure 1 A schematic illustration summaries the major technical route used in Peng et al. (2019). The cartoon of the cerebellum in the upper right panel was modified from Kandel et al. (2000).

the investigation of a total of 17160 granule cells and granule precursors, Peng et al. (2019) found that Naca showed a persistent high-level expression in all stages, whereas some other differentiation- and proliferation-related genes exhibited gradual down-regulation, demonstrating a temporal and dynamic transcriptional program in the continuous process of granule cell trajectory.

The current work also shed new insights toward the implications of interneurons and glia in the development of the cerebellum (Peng et al., 2019). On one hand, they investigated a total of 2034 interneurons, including basket/stellate cells, Golgi cells, unipolar brush cells, and their progenitors. They found that Erh and Hmgb3 were the candidate core transcription factors regulating the differentiation of basket/stellate/Golgi cells, and the distinct transcription factor co-expression network suggests a new subtype of inhibitory neuron lineage derived from rhombic lip. On the other hand, they identified 233 DEGs From a total of 2826 glia cells (2034 astrocytes, 455 oligodendrocytes, and 337 microglia). They found that a series of genes encoding chemokine ligands were expressed in microglia, suggesting their roles in cerebellar development.

The exciting findings of Peng et al. (2019) present a systematic landscape of cerebellar gene expression in defined cell types and a general gene expression framework for cerebellar development and dysfunction. Their work raises many interesting questions and suggests several avenues of future investigation. For example, what genetic factors specify characteristic morphology of Purkinje cells? How do molecular cascades finely tune neurogenesis and migration of granule cells? What factors determine specific synaptic connections in the cerebellum? How do core transcription factors correlate with interneuron differentiation? How do secretary proteins in glia cells participate in neuronal modulation? Functional validation of newly found genes in defined cell types will provide deeper insights into cellular mechanisms of related diseases. In summary, this elegant study by Peng et al. (2019) adds to the repertoire of development and disease-related genes in defined cerebellar cell types, accelerates our understanding of molecular and cellular mechanisms of cerebellar circuitry and diseases, and may help to design specific interventions in future.

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References

Adams, N.C., Tomoda, T., Cooper, M., et al. (2002). Mice that lack astrotactin have slowed neuronal migration. Development *129*, 965–972.

Ashizawa, T., Oz, G., and Paulson, H.L. (2018). Spinocerebellar ataxias: prospects and challenges for therapy development. Nat. Rev. Neurol. *14*, 590–605.

Baumann, O., Borra, R.J., Bower, J.M., et al. (2015). Consensus paper: the role of the cerebellum in perceptual processes. Cerebellum *14*, 197–220.

Buckner, R.L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. Neuron *80*, 807–815.

Carter, R.A., Bihannic, L., Rosencrance, C., et al. (2018). A single-cell transcriptional atlas of the developing murine cerebellum. Curr. Biol. *28*, 2910–2920.

Gupta, I., Collier, P.G., Haase, B., et al. (2018). Single-cell isoform RNA sequencing characterizes isoforms in thousands of cerebellar cells. Nat. Biotechnol. *36*, 1197–1205.

Hatten, M.E., and Heintz, N. (1995). Mechanisms of neural patterning and specification in the development cerebellum. Annu. Rev. Neurosci. *18*, 385–408.

Kandel, E.R., Schwartz, J.H., and Jessell, T.M. (2000). Principles of Neural Science (4th edn). New York: McGraw-Hill.

Koziol, L.F., Budding, D., Andreasen, N., et al. (2014). Consensus paper: the cerebellum's role in movement and cognition. Cerebellum *13*, 151–177.

Marzban, H., Del Bigio, M.R., Alizadeh, J., et al. (2014). Cellular commitment in the developing cerebellum. Front. Cell Neurosci. *8*, 1–26.

Peng, J., Sheng, A., Xiao, Q., et al. (2019). Single-cell transcriptomes reveal molecular specializations of neuronal cell types in the developing cerebellum. J. Mol. Cell Biol. doi:10.1093/jmcb/mjy089.

Rosenberg, A.B., Roco, C.M., Muscat, R.A., et al. (2018). Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. Science *360*, 176–182.

Wang, V.Y., and Zoghbi, H.Y. (2001). Genetic regulation of cerebellar development. Nat. Rev. Neurosci. *2*, 484.