Multi-omics insights into neuronal regeneration and re-innervation

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The regeneration of peripheral nervous system and central nervous system (CNS) neurons after injury remains challenging. We have come a long way since the identification of a 37 kDa protein specific for regenerating peripheral nervous system and CNS nerves (Muller et al., 1985). However, peripheral nervous system neurons still remain more regeneration compliant than CNS neurons. A few decades of scientific progress has led to the discovery of intrinsic (neuron specific) and extrinsic (environment specific) factors (Tedeschi and Bradke, 2017). These factors have become tightly ensconced in our understanding of neuronal development and adult neuroregeneration. Regenerative biology focuses on ways the damaged cells and tissues can be repaired or rejuvenated in the adult and may borrow plans from the patterns of connectivity during embryonic and early postnatal life (Levitan and Kaczmarek, 2015). Many of the molecular signals present during development are down-regulated in the adult. Transient induction of these molecules in adulthood may enable functional re-connectivity (Tomassy et al., 2010), for example, work on amblyopia has helped deduce the concept of plasticity breaks (Morishita et al., 2010), with the identification of Ly-6/neurotoxin-like protein 1 (Lynx1) as molecular break of plasticity. Lynx1 is largely absent during highly plastic phase of postnatal days P0–P5. The Lynx1 level in mice then increases and stabilizes with robust levels at age P60 in mouse leading to a near complete loss of plasticity. Lynx1 thus negatively modulates the plastic environment in the adult CNS of mice. Transient down-regulation of Lynx1 is thus an opportunity to confer a plastic environment in the adult CNS, allowing plasticity and regeneration of the axons possible. A CNS region optic nerve where regeneration of axons of the retinal ganglion cell (RGC) may be rendered permissive by this approach.

Our recent multi-omics analysis combined developmental and adult regenerative data to identify common molecular entities between these two disparate regimes. We combined two different aspects of axonal growth: growth cone expansion in development and axon growth in adult neuroregeneration (Chauhan et al., 2020). We combined transcriptomic, proteomic, and lipidomic data from developmental growth cones with similar datasets from regenerating adult optic nerves after crush injury. Using this combined approach, we discovered common molecules, interaction networks, and pathways that are important for neuronal development and regeneration after axonal injuries. Despite this substantial advancement in the understanding of neural development and neuroregeneration, much remains to be understood and achieved in order to reach functional re-innervation of CNS neurons.

The genome and transcriptome bear the message that is translated into proteome and functional lipidome and water-soluble repertoire of metabolome. In terms of actual amount, diversity (for example posttranslationally modified proteome) of the later omes (proteome, lipidome and metabolme) are greater in magnitude than genome and transcriptome. They are also expected to show greater correlation with functional aspects than genome or transcriptome. Advances in techniques, particularly mass spectrometry has undergone tremendous advances in the last decade. Advances in methods such as isotopic ratio outlier analysis (Stupp et al., 2013), bioinformatics and computational tools have paved the way to make multi-omics, ontogenic and computational analyses much more effective.

We propose in this Perspective a powerful multi-omics approach for the identification of molecules that will aid in regeneration and re-innervation in adults. Combined with conditional genetic ablation, we propose a biochemical fractionation based approach for cell separation and cell culture of the optic nerve *in vitro*, which we term the fractionation as "tweezer" approach, across varying models of regeneration. This will provide multi-omics great power to identify intrinsic and extrinsic regenerative promoting molecules in adults. The identified short-listed molecules can then be tested in model systems for reductionist as well as additive experimental approaches for functional restoration. Of course, no magical "tweezers" exists for proposed fractionation, but current biochemical and biophysical fractionation techniques will be a close approximation to tweezers that can be deployed for cell separation. In **Figure 1A**, we present an overview of how an ideal fractionation-tweezer approach will enable isolation of RGC from the environment in the optic nerve and the tract. This tweezer approach is conceptually similar to the one that enabled Luis Pasteur to separate tartaric acid enantiomers. Such isolation will pave the way for multi-omic analysis of neurons, their environment, and residing cell bodies in the retina.

In **Figure 1B**, we provide a different aspect of this perspective. Adult genetic ablation will enable at least partial determination of the contribution of specific supporting non-RGC types. A similar conceptual "tweezer" approach will enable multi-omics analysis of what is present in these cells. While such isolation will complement information derived from selective ablation, it will still not provide the secretome of specific supporting cells. It is possible that some molecules captured as intrinsic to RGCs may be from the direct and rapid transport from supporting cells. The culture of supporting cells, as well as isolated RGCs, will allow the capture of the cellular secretome, albeit in a completely artificial environment, which may have limited mimicry to the native environment. However, taken together this approach will provide a much greater insight into extrinsic and intrinsic factors determining regeneration.

Characterizing the extrinsic and intrinsic factors influencing regeneration in varying models of optic nerve regeneration is only a step towards better understanding. We still need to determine the factors responsible for innate plasticity breaks during development in the CNS. As stated earlier, Lynx1 identification has shown that P0–P5 developmental stages are plasticity permissive, while P6–P20 stages are non-permissive (**Figure 1C**). Multi-omic analysis of these plasticity categories, whether fractionated as different cells and environment or as a whole tissue, may help identify the molecular markers of plasticity breaks that are expected to be tremendously upregulated during later phases. Overcoming innate plasticity breaks during development will lead to much larger regeneration capacity as opposed to focusing on regenerating alone.

In **Figure 1D**, we summarize mega-multi-omics analyses combining approaches presented in **Figure 1A**–**C** that will identify (1) intrinsic and (2) extrinsic growth promoters and growth inhibitors. They will also help identify (3) plasticity breaks and (4) neuronal/RGC deflectors. The upregulation of growth promoters and removal of growth inhibitors as well as plasticity breaks is expected to promote regeneration, whereas promotion of regeneration together with deflection will enable re-innervation.

Functional re-innervation of optic nerve neurons necessitates long distance growth of both ipsilateral eyes and contralateral RGCs. Contralateral RGCs will need to undergo deflections at the optic chiasm in order to reach appropriate targets in the lateral geniculate nucleus.

Misdirection and turning back of regenerating neurons in the peripheral nervous system has been well known since 1883 (Howell and Huber, 1892). This has now been well realized for regenerating CNS neurons (Bray et al., 2017). A comprehensive multi-omic analysis may shed new light and help in ameliorating misdirection of neuronal growth. At the same time, such insight may enable desired turning, re-innervation and functional recovery. Re-innervation will also necessitate programmed and targeted growth cone collapse and synaptogenesis. Multi-omics may help analyze factors mediating these processes. Biochemical factors such as lysolipids (Cheng et al., 2016) and physical factors such as optical tweezers or non-invasive optical guidance (Mondal et al., 2014) have been shown to enable controlled deflections and growth cone collapse (**Figure 1E** and **F**).

This combinatorial multi-omics analysis with other approaches will greatly advance our understanding and insight. For example, with advances in bioinformatics, we will be able to integrate intrinsic and extrinsic molecular factors across varying stages of development, models of adult regeneration, and injury for the creation of interaction networks and pathways that define a growth permissive and growth non-permissive state. This will lead to

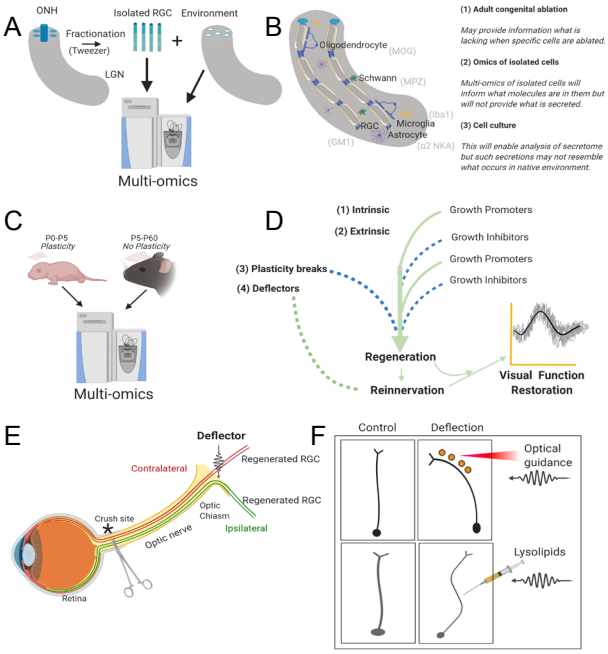

Figure 1 | **Strategies for fractionation of optic nerve cells and residual tissue and for targeted deflection resulting in re-innervation.**
(A–D) A schematic diagram to depict outcome from multiple multi-omics analyses of optic nerve cells and residual tissue. The idea is to obtain clean fractionation of cells and the rest of the tissue environment and perform their multi-omics analyses. (A) The approach of fractionation of isolated retinal ganglion cells (RGCs) from the residual environment and the subsequent multi-omics of both, separately. An ideal approach will be as if a tweezer that could just isolate and pick up RGCs for analysis. As described in the text of the perspective there is no such tweezer or ideal fractionation currently available. However, careful longitudinal sectioning of the optic nerve cylinder and use of a scalpel under microscope to separate fibers to the extent possible, followed by a collagenase treatment may loosen the fibers. After fiber loosening, the cholera toxin B coupled beads binding of RGCs and biochemical approaches to isolate or fractionate bead-bound RGCs will be a step towards this direction. (B) The cross-sectional view of the optic nerve to depict major cell types. A surface marker for each cell type has been shown in grey: myelin-oligodendrocyte glycoprotein (MOG), myelin protein P0 (MPZ), Ionized calcium-binding adaptor molecule 1 (Iba1), α2 isoform of $Na^{(+)}$, $K^{(+)}$ ATPase (α2 NKA) and lipid GM1 for oligodendrocyte, Schwann, microglia, astrocyte and RGC cell types respectively, as indicated. The idea is to fractionate each cell type and subject them to analyses. Three distinct strategies are detailed to complement information: (1) to use conditional adult genetic ablation of different cell types (RGCs, as noted in panel A, astrocytes, microglia, Schwann cells and oligodendrocytes). The analysis of fractionated cells and tissues for each ablation type may inform the changes in omics specially lipidome and metabolome changes. (2) The fractionation of all gross cell types using potential surface marker binding-beads and analyzing them. This will primarily inform non-secretome content of the cells. (3) The analysis of cultured secretome of the isolated cells (as in 2 above). This will inform their secretome albeit in an artificial environment. (C) The fractionated analysis of RGCs and residual environment in P0–P5 (plasticity permissive) and higher time points (plasticity non-permissive). (D) The outcome of multi-omics is likely to inform comprehensively about intrinsic and extrinsic factors, plasticity breaks and about potential deflectors to test in animal models of regeneration and re-innervation to restore functional vision. (E, F) The targeted controlled deflection for re-innervation. The idea is to enable targeted deflection of the long distance regenerated RGCs for re-innervation. (E) The depiction of the need for targeted deflection, while some long distance regenerated neurons may travel a straight path, others, may need to be deflected towards ipsilateral (or contralateral as it may be) side. A convoluted arrow represents the deflectors. (F) The depiction of deflection using laser spots generating heat (optical guidance) or using fine-tuned concentration of specific lysolipids such as lysophospatidic acid (LPA).

the identification of growth promoters and growth inhibitors of CNS regeneration. Machine learning algorithms will allow us to create molecular models of regeneration taking into account a multitude of factors (Datta, 2013). Database creation and user-friendly tools will allow us to make the data widely available and accessible for researchers across multiple domains.

The discovery of molecules that play a role in developmental plasticity states, together with intrinsic/extrinsic growth factors and inhibitors, will enable us to fully utilize machine learning approaches, create models of regeneration permissive and non-permissive states (**Figure 1C** and **D**). Combined with targeted deflections of ipsilateral and contralateral RGCs (**Figure 1E** and **F**), we can promote directed re-innervation of long distance regenerated neurons and render the restoration of visual function in adults a tangible reality. The breakthroughs in functional restoration of damaged optic nerves is likely to go beyond the visual system. These discoveries may provide insights into mechanisms of CNS regeneration that have implications in the reversal of a multitude of neurodegenerative diseases outside of ocular pathology.

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References

Bray ER, Noga M, Thakor K, Wang Y, Lemmon VP, Park KK, Tsoulfas P (2017) 3D visualization of individual regenerating retinal ganglion cell axons reveals surprisingly complex growth paths. eNeuro doi: 10.1523/ENEURO.0093-17.2017.
Chauhan MZ, Arcuri J, Park KK, Zafar MK, Fatmi R, Hackam AS, Yin Y, Benowitz L, Goldberg JL, Samarah M, Bhattacharya SK (2020) Multi-omic analyses of growth cones at different developmental stages provides insight into pathways in adult neuroregeneration. iScience 23:100836.
Cheng J, Sahani S, Hausrat TJ, Yang JW, Ji H, Schmarowski N, Endle H, Liu X, Li Y, Böttche R, Radyushkin K, Maric HM, Hoerder-Suabedissen A, Molnár Z, Prouvot PH, Trimbuch T, Ninnemann O, Huai J, Fan W, Visentin B, et al. (2016) Precise somatotopic thalamocortical axon guidance depends on LPA-mediated PRG-2/Radixin signaling. Neuron 92:126-142.
Datta S (2013) Feature selection and machine learning with mass spectrometry data. Methods Mol Biol 1007:237-262.
Howell WH, Huber GC (1892) A physiological, Histological and clinical study of the degeneration and regeneration in peripheral nerve fibers after severance of their connections with the nerve centers. J Physiol 13:335-406.
Levitan IB, Kaczmarek LK (2015) The neuron: cell and molecular biology, 4th ed. New York, USA: Oxford University Press.
Mondal A, Black B, Kim YT, Mohanty S (2014) Loop formation and self-fasciculation of cortical axon using photonic guidance at long working distance. Sci Rep 4:6902.
Morishita H, Miwa JM, Heintz N, Hensch TK (2010) Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. Science 330:1238-1240.
Muller HW, Gebicke-Harter PJ, Hangen DH, Shooter EM (1985) A specific 37,000-dalton protein that accumulates in regenerating but not in nonregenerating mammalian nerves. Science 228:499-501.
Stupp GS, Clendinen CS, Ajredini R, Szewc MA, Garrett T, Menger RF, Yost RA, Beecher C, Edison AS (2013) Isotopic ratio outlier analysis global metabolomics of Caenorhabditis elegans. Anal Chem 85:11858-11865.
Tedeschi A, Bradke F (2017) Spatial and temporal arrangement of neuronal intrinsic and extrinsic mechanisms controlling axon regeneration. Curr Opin Neurobiol 42:118-127.
Tomassy GS, Lodato S, Trayes-Gibson Z, Arlotta P (2010) Development and regeneration of projection neuron subtypes of the cerebral cortex. Sci Prog 93:151-169.

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