Next generation drug connectivity mapping for acquiring therapeutic agents to differentially regulate myelination

Kasum Azim, Andrea Domenico Rivera*, Arthur Morgan Butt

The need for new therapeutic approaches: Conventional drug discovery is a lengthy and expensive process, taking decades and billions of dollars to get a drug from bench to bedside. Much of the costs incurred are at the pre-clinical stages, between drug design and synthesis to delineating the cellular “Mechanisms of Action” (MoA). Notably, there is a very high risk of failure, and only a very small proportion of therapeutic agents reach later-phase clinical trials. Accordingly, drug repositioning has become a valuable strategy aimed at fast-tracking treatments into clinical use and improving the chances of therapeutic success. A novel addition to this approach is connectivity mapping, which defines cell-specific transcriptional responses to small molecules in disease-dependent contexts. This commentary outlines how some of the latest innovations in connectivity mapping can be exploited for drug repurposing.

Using connectivity mapping to repurpose small molecules: The small molecule connectivity map database is a publicly available resource (https://cclu.io/) that utilizes gene expression profiling to connect biology and drug discovery (Figure 1), and has been used successfully to repurpose small molecules in diverse disease contexts (Azim et al., 2017; Gns et al., 2019). A major advance in the field is the Library of Integrated Cellular Signatures (LINCS), which contains the genomic fingerprint for over 20K small molecules systematically assayed in over 90 depleted pluripotent stem cell/human cell lines. We have developed the use of connectivity mapping and LINCS to first identify small molecules that drive discrete transcriptional changes associated with the regulation of neural cell fate, and secondly use successive network modeling to identify cell-specific downstream targets and MoA of these small molecules (Rivera et al., 2021). Importantly, LINCS integrates the dose-dependent actions of each small molecule, which enabled us to identify individual small molecules that have divergent cellular effects depending on their concentration (Rivera et al., 2022a). This comprehensive knowledge enables experiments in disease models to be refined and focused on the predicted MoA of small molecules with the greatest therapeutic potential, thereby reducing the overall number of costly and time-consuming pre-clinical laboratory experiments.

Next-generation drug connectivity mapping to promote central nervous system (CNS) myelination and repair: Oligodendrocytes are the myelinating cells of the CNS whereby their loss together with myelin has devastating effects on CNS function, as manifested in the demyelinating disease multiple sclerosis, as well as in neurodegenerative diseases such as stroke and Alzheimer’s disease. Notably, the adult CNS has an intrinsic capacity for remyelination through a significant population of oligodendrocyte precursor cells (OPCs) and this is critical for maintaining CNS function and integrity. However, these repair mechanisms ultimately fail and a major factor is age-related OPC senescence (Rivera et al., 2022b). In our recent study, therefore, we analyzed transcriptional changes that define oligodendrocyte differentiation in development compared to adults and used the LINCS database to identify small molecules that regulate OPC differentiation and have the potential to rejuvenate their regenerative capacity, which is a key therapeutic objective in multiple sclerosis (Rivera et al., 2022a). A key finding was that LY294002 was the most highly ranked small molecule for both promoting and inhibiting OPC differentiation, dependent on the dose. Initially, this bipartite effect of LY294002 appeared counter-intuitive because it is a potent inhibitor of PI3K/Akt/mTOR/PTEN signaling, which is essential for OPC survival, proliferation, and differentiation, but is consistent with a recent study showing that short-term genetic knock out of PTEN in OPCs surprisingly enhanced myelination and remyelination in contrast with long-term knock out of PTEN which had a negative effect on myelination (Gonzalez-Fernandez et al., 2018). The potential beneficial action of low LY294002 highlights the value of using the LINCS database to reassess small molecules that have failed to reach clinical use and this is being continuously refined by the addition of new resources, e.g. https://appters.mayanlab.cloud/# [Clarke et al., 2021].

Validation of LINCS-derived small molecules in regulating myelination: In our recent study (Rivera et al., 2022a), the predicted bipartite effect of LY294002 on oligodendrogensis and myelination was verified in both in vivo and ex vivo mouse models. For the in vivo studies, we used an optimized intrathecal route of administration in mice aged postnatal day 8 to target the subventricular zone (SVZ) and periventricular myelination and avoid side effects of LY290042 on systemic PI3K/Akt signaling. In this regard, delivering LY290042 to sites adjacent to the corpus callosum limits neuronal toxicities which could otherwise mask any beneficial effects on axonal myelination. Our analyses demonstrated that low LY290042 dramatically increased the production of “pre-OPCs” from NSCs in the SVZ; pre-OPCs express Ascl1 and Olig2 and are lineage-restricted pro-oligodendrogial transiently amplifying progenitors. Notably, low LY290042 also depleted the density of NSC in the dorsal SVZ and further investigations are required to determine whether this reflects NSC exhaustion, which would be critical when attempting to stimulate long-term oligodendrogenesis/regeneration from the SVZ (Cayre et al., 2021). Nonetheless, low LY290042 promoted differentiation of pre-OPCs along the subsequent oligodendrocyte lineage stages, increasing the generation of OPCs and myelinating oligodendrocytes in the corpus callosum and cerebral cortex. Furthermore, the positive effects of low LY294002 on OPCs and oligodendrocytes were completely mirrored in the ex vivo models of the intact optic nerve and cerebellar slice from both postnatal and adult mice. In contrast, high LY294002 severely inhibited oligodendrogenesis and myelination in both in vivo and ex vivo models. Additionally, using STITCH drug/protein-protein network analysis we determined common mechanisms of action of low LY294002 with drugs previously recognized as therapies in multiple sclerosis, including metformin, prednisolone, clemastine, benzoprine, siponimod, and bezartetone. Overall, our drug discovery approach identifies novel drugs that target OPCs and we have demonstrated some of these can promote remyelination in older mouse models of injury (Rivera et al., 2021).

Transcriptomic and protein network reconstruction to determine the mode of action of LY294002 on predicted oligodendrocyte lineage cells: Gene ontology and pathway analysis of the target genes of LY294002 from the LINCS database cross-referenced with oligodendrocyte lineage gene databases identified PI3K/Akt/mTOR/PTEN signaling as the highest-ranked mechanism for the effects of both low and high LY294002 in oligodendrocytes, and the predicted MoA were determined by subsequent STRING and Cytoscape Cluego analyses of the protein-protein interactions and signaling networks (see Figure 1 and also Supplementary Figure 1A and B in Rivera et al. (2022a)). Finally, we demonstrated that LY294002 caused a downregulation of PI3K/Akt/mTOR/PTEN signaling in a dose-dependent manner, but that low and high LY294002 induced differential transcriptional changes, consistent with that observed following OPC-specific knockout of PTEN (Gonzalez-Fernandez et al., 2018). We further anticipate that the two doses of LY294002 tested may have differential binding affinities and specificities and are aspects that we have discussed recently (Rivera et al., 2022c). Importantly, there are connections between the predicted and experimentally determined effects of LY294002, which validates and highlights the power of the in silico strategy to identify potential new therapies.

Future directions: In the present commentary we have highlighted the use of LINCS for predicting the dose-dependent actions of small molecules in promoting myelination in the context of myelinopathies. This strategy can be adapted for other systems and diseases, across basic and translational research. However, it can be difficult for non-experts to navigate around the databases used to derive target genes and pathways, which is where the main interest in therapeutics lies (Nassiri and McCull, 2018). To address these issues, our future efforts aim to devise bioinformatic workflows that can be done from any web browser (via Rshiny) or via R/Studio and Python with the following implemented features:

1. From the input of genes/proteins used to drive a particular transcriptional shift, to the output of small molecules;
2. Generation of plots (dot plots and violin plots) for the most significantly correlated small molecules (or for select small molecules) demonstrating the predicted changes in biological processes, signaling pathways, and the most...
common gene ontology databases; (3) Network visualizations using STRING db (https://string-db.org) and TETRAMER (https://ngsqc.org/tetramer/) which can show the main genes/co-expressed genes differentially altered. Including the possibility of downloading the data for use on the open-source Cytoscape software for improved network constructions are planned; (4) Visualisation of the most connected small molecule target-genes/proteins in a chord plot together with the pathways/genes ontologies they are associated with that are altered in expression both negatively and positively. This final set of visualization tools would be the most informative for hands-on investigators where the key target genes observed in this analysis can be measured via qPCR, western blot, histology, etc.

We have already implemented many of these systems biology readouts in our studies (Azim et al., 2017; Rivera et al., 2021, 2022a), and future implementations will enable meaningful data to be readily extracted by both experts and non-experts.

Conclusions: A major advantage of the LINCS in silico drug discovery approach is that not only can it be used to identify therapeutically relevant small molecules but also their predicted target genes and MoA. This feature enables the careful design of laboratory investigations to validate the transcriptomic findings in disease models, both using cell biology approaches as in our studies and physiological validation using neurophysiological recording and animal behavior (Kropiwicki et al., 2021). These techniques offer unprecedented opportunities to gain insights into hard-to-predict context-specific mechanisms of action of small molecules for stimulating endogenous regeneration and repair not only in neuropathological contexts but across translational medicine.

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Date of submission: February 28, 2022

Date of decision: May 13, 2022
Date of acceptance: July 30, 2022
Date of web publication: September 16, 2022

https://doi.org/10.4103/1673-5374.353486

How to cite this article: Azim K, Rivera AD, Butt AM (2023) Next generation drug connectivity mapping for acquiring therapeutic agents to differentially regulate myelination. Neural Regen Res 18(4):797-798.

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C-Editors: Zhao M, Liu WJ, Wang Lu; T-Editor: Jia Y

Figure 1 | Overview of the in silico pharmacogenomics strategy. (A) Gene expression profiles are curated from a system of interest or disease versus control states. (B) These are then fed into the LINCS database, which contains 20,000 small molecule-induced expression profiles which have been systematically tested in multiple human cell lines/induced pluripotent stem cells. Matches are performed in yielding small molecules with correlation scores in driving the desired cell state (or reverted disease to healthy states). (C) Each small molecule generated is provided with a reasonable number of genes, i.e. drug target genes that are prospectively altered for stimulating the desired phenotypes. Some small molecules as output can show bipartite effects on the experimental system according to their dosages, i.e. hormesis. (D) Drug target genes can be used for identifying pathways altered, their mechanisms, and any potential adverse effects (i.e. excessive proliferation, cell death) and allows selection of the most promising small molecules for further and in vivo analyses. (E) Novel small molecules can be further studied by constructing gene/protein networks for visualizing their target genes. LINCS: Library of Integrated Cellular Signatures; MoA: Mechanisms of Action.