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Article

Bioinformatic Prediction of ERK1/2 Downstream Phosphorylation Targets in Oligodendrocytes

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ERK1/2 (MAPK3/1) signaling plays a critical role in oligodendrocyte development, while the downstream phosphorylation targets that mediate its effects on myelination remain unclear. Here, we applied computational motif discovery and protein functional analysis to identify candidate phosphorylation targets of ERK1/2 in mouse oligodendrocytes. Using protein sequences derived from genes expressed in one-month wild-type mouse oligodendrocytes, we performed iGPS-based phosphorylation site prediction followed by motif matching with programming pipelines. Gene Ontology (GO) enrichment analysis for function classifications reveals that these phosphorylation candidates are significantly associated with important biological processes and molecular functions relevant to myelination. Together, our findings suggest that ERK1/2 modulates oligodendrocyte lineage progression through phosphorylation of downstream effectors, including transcription factors in terms of a series of processes and functions. These results provide a foundation for future experimental validation and mechanistic studies of ERK-mediated phosphorylation in oligodendrocyte lineage progression.

Myelination in the central nervous system (CNS) is essential for rapid signal conduction and neuron survival. Oligodendrocytes, the myelinating glia of the CNS, arise from oligodendrocyte precursor cells (OPCs) and

undergo tightly regulated stages of proliferation, differentiation, and maturation (Gonsalvez et al., 2016). A key regulator/signal protein of these processes is the extracellular signal-regulated kinase 1/2 (ERK1/2, also known as MAPK1/3) that belongs to the mitogen-activated protein kinases (MAPK) signaling pathway (Guo et al., 2020), which has been shown to influence OPC proliferation and myelin thickness through phosphorylation via energy transfer of ATP (Bonora et al., 2012). However, the downstream mechanisms by which ERK1/2-mediated phosphorylation of candidate targets promote myelination remain incompletely understood.

While previous studies have demonstrated that ERK1/2 activity affects other neuron cells like astrocytes development by phosphorylation, a switch to activate target proteins (Li et al., 2021), the landscape of these specific targets that mediate these effects have not been fully elucidated. Understanding this regulatory network is critical, as dysregulation of myelination contributes to demyelinating diseases like multiple sclerosis (MS) (Dobson& Giovannoni, 2019).

Here, we apply a computational approach to predict the transcriptional targets of ERK1/2 phosphorylation in mouse oligodendrocytes. By integrating phosphorylation prediction, motif discovery, and GO enrichment analysis, we aim to identify candidate targets that link ERK1/2 activation to myelin gene expression.

Results

Overview of the Dataset

The dataset comprised oligodendrocyte cells isolated from the central nervous system of one-month-old mice, representing an active developmental stage. After quality control and imputation, expression data from over 10,000 genes were retained for analysis. Matched protein sequences are downloaded from Uniprot (https://www.uniprot.org/).

iGPS Prediction

Phosphorylation site prediction was performed by using iGPS, which effectively identifies kinase-specific phosphorylation targets based on a similarity scoring system. In total, 1107 unique protein targets were identified as potential substrates.

Motif Matches

To further refine specificity, motif-based scanning was conducted using two predefined ERK recognition patterns/motifs (Fig.1 a). Motif analysis revealed that 1857 unique proteins contained at least one instance of Pattern 1 or Pattern 2. Pattern 1 appeared 1317 times, while Pattern 2 was detected 923 times, indicating a broader representation of Pattern 1 across oligodendrocyte-expressed proteins (Fig.1.b).

A co-occurrence heat-map revealed that 918 proteins contained only Pattern 1, 631 only Pattern 2, and 224 proteins harbored both, suggesting distinct regulatory roles and possible combinatorial signaling mechanisms (Fig.1.c).

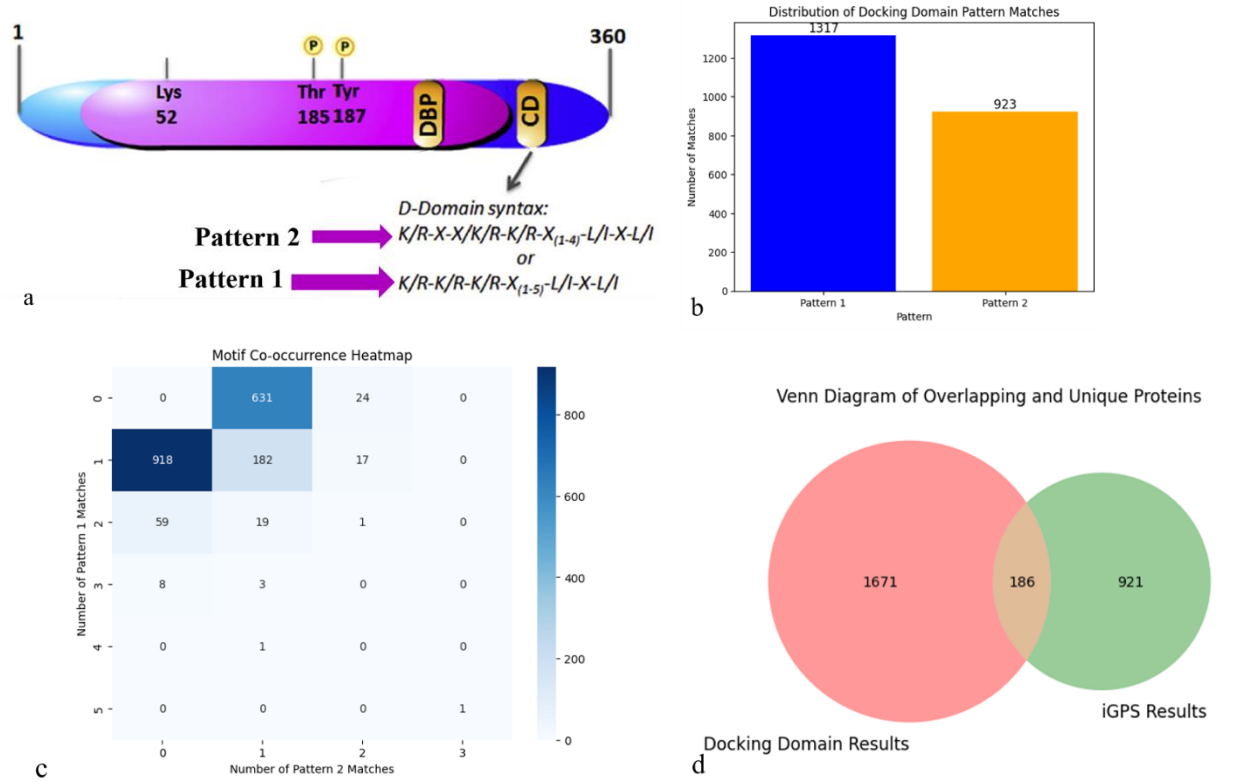

Fig. 1| Prediction of ERK1/2 phosphorylation targets in oligodendrocytes of mice. a, diagram of chemical structure of ERK2 from Gonsalvez et al., 2016, showing there are 2 kinds of sequences of D(docking) domain (pointed by purple arrows). b, pattern match frequency of pattern 1 and pattern 2. These are match-level counts, meaning if a single protein has 3 Pattern 1s and 2 Pattern 2s, it contributes 3 to pattern 1 and 2 to pattern 2. So these counts represent all individual pattern occurrences. c, the heat-map of motif co-occurrence reveals the distribution of Pattern 1 and Pattern 2 across proteins based on their respective matching counts. The x-axis represents the number of Pattern 2 matches, while the y-axis represents the number of Pattern 1 matches. d, overlap (186 proteins) of iGPS prediction and docking domain matching for further ORA analysis.

Overrepresentation Analysis

ORA is prepared for these 186 proteins carrying both predicted phosphorylation sites and D-domain motifs (Fig.1.d). The results revealed key enriched biological processes, including positive regulation of transcription, MAPK cascade, and RNA polymerase II-mediated transcriptional control. Molecular function terms highlighted MAP kinase activity, GTPase regulation, and transcription factor binding, aligning with the known role of ERK signaling in transcriptional modulation during oligodendrocyte development (Fig.2).

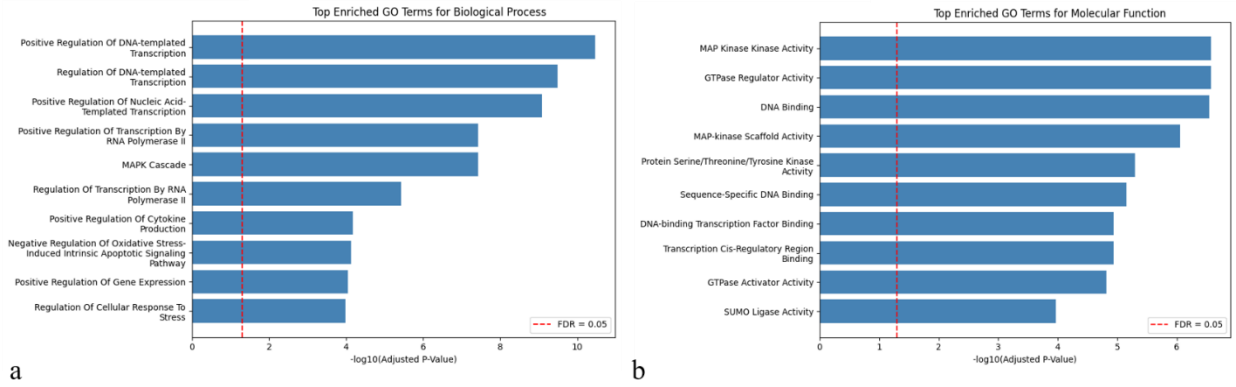

Fig. 2| Overrepresentation Analysis of Gene Ontology for proteins which contain positive results in iGPS prediction as well as in docking patterns match. a, top 10 enriched GO terms for biological process, showing these functions are highly significantly enriched in ERK1/2 phosphorylation targets. This means that these targets are more likely involved with these functions. b, top 10 enriched GO terms for molecular function, showing these functions are highly significantly enriched in ERK1/2 phosphorylation targets. This means that these targets are more likely involved with these functions

Discussion

This study provides a systematic prediction of ERK1/2 phosphorylation targets in oligodendrocytes, integrating motif scanning and machine learning-based approaches to improve confidence. By identifying over a thousand potential substrates and narrowing to 186 high-confidence targets, the results offer insight into how ERK1/2 signaling may regulate transcription and other essential processes in myelination.

Phosphorylation of these targets likely contributes to key functions such as oligodendrocyte maturation, survival, and myelin formation. Enrichment of transcription-related GO terms suggests that ERK1/2 activity may modulate gene expression programs essential for myelination.

Future analyses should include subtype- and spatial-resolved approaches as oligodendrocytes are a heterogeneous population with multiple distinct subgroups performing different functions (Park et al., 2023).

Without subtype stratification, it remains unclear which phosphorylation events are relevant to specific developmental transitions. Techniques like single-cell spatial transcriptomics or pseudotime-based trajectory alignment may help clarify the timing and localization of ERK-mediated phosphorylation.

Moreover, the functional interpretation of predicted targets could be improved by using more specific term categories. Standard or unbiased ORA may underrepresent terms like “myelination” due to limited annotation coverage or low gene counts. Enhanced annotation tools or ontology refinement could better capture specialized biological processes.

Together, this study lays a foundational framework for identifying ERK1/2 targets in oligodendrocytes and suggests multiple avenues for deeper functional and spatial resolution in future work.

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Methods

Data Selection

This study utilized a publicly available single-cell RNA sequencing (scRNA-seq) dataset of oligodendrocyte-lineage cells isolated from mouse brains at one month of age (a developing stage) (Park, H., et al. 2023). The dataset was originally processed by using CellRanger v3.0.2 and aligned to the mm10 genome with STAR, underwent standard quality control, including the removal of cells with >10% mitochondrial content or fewer than 200 and more than 6000 detected genes. Dimensionality reduction, batch correction (via Seurat's IntegrateData), and clustering were performed based on highly variable genes, followed by UMAP visualization. Oligodendrocyte clusters were annotated by reference and extracted using

Seurat's SubsetData. To mitigate dropout noise, imputation was performed using SAVER (v1.1.1), and trajectory inference was conducted using multidimensional scaling (MDS) and Slingshot. The resulting high-quality imputed expression matrix was used for downstream analysis.

ERK1/2 Target Prediction Using iGPS

Prediction of ERK1/2 phosphorylation targets was performed using iGPS 2.0 (Song et al., 2012), a kinase-substrate prediction tool that integrates sequence motif data with protein-protein interaction (PPI) network context. Protein sequences from oligodendrocyte-expressed genes were input. High-confidence targets were identified based on a confidence score to representative phosphor-peptide clusters, computed via BLOSUM62 matrix. Only proteins marked as high-confidence by iGPS were retained for further analysis.

Docking Motif Identification via Pattern Matching

To identify proteins with canonical ERK1/2 docking motifs, we implemented a custom Python script using the pandas and Biopython libraries (Cock et al., 2009). Motif detection was conducted for exact matches of known docking motifs with 2 patterns which facilitate ERK-substrate binding but are not themselves phosphorylated.

Gene Ontology Overrepresentation Analysis

To explore the functional significance of the identified candidate substrates, Gene Ontology (GO) enrichment analysis was conducted using Overrepresentation Analysis (ORA) via the gseapy Python package (Fang et al., 2023). The analysis is performed across 2 GO categories: Biological Process (BP) and Molecular Function (MF). Results were filtered using the False Discovery Rate (FDR) to correct for multiple hypothesis testing.