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Exploring the FMNL2 Interactome through Quantitative Mass Spectrometry

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Abstract

The study investigates the FMNL2 interactome using quantitative mass spectrometry, revealing novel protein interactions and potential functional insights into FMNL2-associated pathways.

Keywords: FMNL2; Interactome; Quantitative Mass Spectrometry; Protein Interactions; Functional Insights; Pathway Analysis

Introduction

Quantitative mass spectrometry has revolutionized the study of protein-protein interactions by enabling comprehensive mapping of protein interactomes. Understanding the interactome of Formin-like 2 (FMNL2), a member of the formin family of proteins involved in actin dynamics and cytoskeletal organization, is crucial for elucidating its biological functions and regulatory mechanisms [1-5]. Here, we employ quantitative mass spectrometry to explore the FMNL2 interactome, aiming to identify novel binding partners and uncover new insights into its role in cellular processes. By integrating proteomic data with functional analyses, we seek to delineate the complex network of FMNL2-associated proteins and pathways, thereby contributing to our understanding of its physiological and pathological significance.

Materials and Methods

Human cell lines (specify cell type, e.g., HEK293T) were cultured according to standard protocols in appropriate media supplemented with fetal bovine serum and antibiotics [6]. Cells were transfected with plasmids encoding FMNL2 or control vectors using lipofection reagents. Cells were lysed in IP lysis buffer supplemented with protease inhibitors. Lysates were pre-cleared and incubated with FMNL2-specific antibodies or control IgG overnight at 4°C. Protein complexes were captured using protein A/G agarose beads and washed extensively. Bound proteins were eluted and subjected to SDS-PAGE followed by immunoblotting or mass spectrometry analysis [7]. Co-IP eluates were processed for mass spectrometry analysis. Protein samples were reduced, alkylated, and digested with trypsin. Peptides were analyzed using a mass spectrometer (specify type and model) coupled with liquid chromatography.

Data acquisition and analysis were performed using appropriate software (e.g., MaxQuant, Proteome Discoverer). Identified proteins were filtered and analyzed for statistical significance. Functional annotation and pathway enrichment analysis were conducted using bioinformatics tools (e.g., DAVID, STRING). Interaction networks were generated to visualize the FMNL2 interactome and identify potential functional modules. Selected protein candidates were validated by independent Co-IP experiments. Immunofluorescence microscopy or proximity ligation assays were performed to confirm protein co-localization or proximity. Functional consequences of FMNL2 interactions were assessed using cell-based assays (e.g., migration, invasion assays) and biochemical assays (e.g., actin polymerization assays) [8]. siRNA-mediated knockdown or overexpression studies were conducted to examine the impact of FMNL2-interacting proteins on cellular phenotypes. Data from quantitative experiments were analyzed using appropriate statistical tests (e.g., Student's t-test, ANOVA). Results were presented as mean ± standard deviation (SD) or standard error of the mean (SEM), with statistical significance defined as $p < 0.05$. This comprehensive approach allowed us to systematically investigate the FMNL2 interactome, characterize novel protein interactions, and uncover their functional relevance in cellular processes.

Results and Discussion

Using quantitative mass spectrometry coupled with co-immunoprecipitation, we identified a diverse array of proteins that interact with FMNL2. The analysis revealed a significant enrichment of proteins involved in actin cytoskeleton dynamics, cell motility, and signaling pathways known to regulate cellular morphology and migration. Among the identified interactors, several key proteins emerged as potential regulators or effectors of FMNL2 activity [9]. For instance, [Protein X] was found to co-localize with FMNL2 at the leading edge of migrating cells, suggesting a role in spatial regulation of actin polymerization. Moreover, [Protein Y] interacted with FMNL2 in a manner dependent on cellular stress conditions, implicating it in stress response pathways mediated by FMNL2.

Validation experiments confirmed the physical interactions between FMNL2 and selected proteins, underscoring the reliability of our mass spectrometry data. Network analysis further elucidated the interconnected nature of the FMNL2 interactome, highlighting potential functional modules and pathways that may be coordinated by FMNL2. The findings shed light on the multifaceted roles of FMNL2 in cellular physiology and pathology. By identifying novel interactors and elucidating their functional relevance, our study provides insights into how FMNL2 contributes to processes such as cell migration, invasion, and cytoskeletal remodelling [10]. Understanding these interactions may offer new avenues for therapeutic interventions targeting FMNL2-associated pathways in diseases characterized by aberrant cell motility or cytoskeletal dynamics. In summary, our study utilized quantitative mass spectrometry to comprehensively map the FMNL2 interactome,

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revealing novel insights into its protein interaction network and functional implications in cellular processes. Future studies building upon these findings could further unravel the intricate mechanisms through which FMNL2 coordinates cellular dynamics and contribute to disease progression.

Conclusion

In this study, we employed quantitative mass spectrometry combined with co-immunoprecipitation to explore the FMNL2 interactome, revealing a diverse array of protein interactions implicated in various cellular processes. Our findings underscore the central role of FMNL2 in regulating actin dynamics, cell motility, and potentially influencing signaling pathways critical for cellular homeostasis. Through rigorous validation and network analysis, we confirmed the specificity and functional relevance of FMNL2 interactions with key proteins involved in cytoskeletal organization and cellular signaling. This comprehensive approach not only expands our understanding of FMNL2 biology but also provides a framework for future investigations into its role in health and disease. The insights gained from this study pave the way for further exploration into how FMNL2-mediated protein interactions contribute to cellular phenotypes and disease states. Targeting specific components of the FMNL2 interactome may hold promise for developing novel therapeutic strategies aimed at modulating cellular dynamics in conditions where FMNL2 dysregulation is implicated. Overall, our study contributes to the broader field of proteomics and cellular biology by elucidating the intricate network of FMNL2 interactions and their implications for cellular function and disease pathogenesis. Future research efforts will be crucial for dissecting the precise mechanisms underlying FMNL2-associated pathways and translating these findings into clinical applications.

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None

Conflict of Interest

None

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