Effect of Amyloid-beta Exposure on Phospholipid Dynamics: A Computational and Cellular Perspective

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DOI: 10.46793/ICCBIKG25.331P

Abstract: Advancements in computational analysis of lipid markers have significantly improved our understanding of lipid-based regulation in cellular physiology and disease mechanisms. In this study, we investigated phospholipid alterations in human SK-N-SH neuroblastoma cells following with amyloid-beta (Aβ), a peptide associated with the onset and progression of Alzheimer's disease. Our objective was to uncover lipid signatures indicative of early-stage neurodegenerative changes by analyzing complex datasets requiring computational processing. Data analysis was conducted in the R programming language, utilizing a reproducible and scalable workflow. The lipidr package was employed for data normalization, lipid species annotation, and statistical evaluation of differential abundance, while ggplot2 was used to generate detailed visualizations of lipid expression patterns. The computational approach enabled us to pinpoint statistically significant alterations in specific phospholipids, notably PE 16:0-18:1, PC 18:2-16:1, and PC 20:2-18:0, under Aβ exposure. These notable findings highlight the critical role of computational approaches in lipidomic data processing and demonstrates their application in neurological disease models through integrative analysis.

Keywords: lipidomics, lipidr library, Alzheimer's disease, phospholipids, amyloid-beta

1. Introduction

Neurodegenerative diseases pose a critical challenge in biomedical research, with Alzheimer's disease (AD) being the most common and devastating form. AD is characterized by progressive memory impairment and cognitive decline, with early- onset cases often linked to genetic mutations such as in the APP gene, while late-onset cases are associated with multifactorial mechanisms. Early identification of molecular biomarkers remains essential to delay disease progression and guide therapeutic strategies. Phospholipids are fundamental for neuronal membrane stability and signaling. Dysregulation of lipid levels has been associated with amyloid-beta accumulation and neurodegeneration. These innovations provide deeper insight into lipid metabolism and its alterations under pathological conditions. Within the central nervous system, phospholipids such as phosphatidylinositol, phosphatidylethanolamine, and phosphatidylcholine are essential for maintaining

membrane organization and regulating signaling pathways [Raghu et al., 2019]. Their disruption has been linked to amyloid-beta accumulation and neuronal impairment in Alzheimer's disease [Witt et al., 2015]. Beyond their structural role, lipid profiles are emerging as valuable markers, with computational approaches such as Biocmanager and lipidr facilitating the analysis of large datasets and uncovering therapeutic targets [Datki et al., 2018; Kaneko et al., 2000]. The goal of the study is to identify phospholipid signatures associated with early-stage neurodegenerative changes in human SK-N-SH neuroblastoma cells following exposure to amyloid-beta (Aβ), using computational analysis of complex lipid datasets.

2. Methodology

The workflow combined data extraction, statistical evaluation, and visualization, implemented through R programming, with specific libraries chosen to optimize performance and reproducibility. Visualization was performed using ggplot2, following the principles of the Grammar of Graphics. The library translated mathematical measures into clear graphical representations such as scatter plots with error bars, enabling intuitive interpretation of lipid distributions. Lipidr functions such as tidy_lipid() and summarise_lipid() were employed for aggregation, quantification, and visualization of lipid classes, providing insight into metabolic shifts induced by Aβ. This approach ensured a robust, reproducible, and interpretable workflow, tailored specifically for analyzing phospholipid alterations in cellular models exposed to amyloid-beta.

3. Results and Discussion

Data analysis indicated that the exposure of cells to different concentrations of Aβ had a direct and statistically significant effect on the levels of specific phospholipids. t- tests were performed for all pairwise comparisons (Control vs Treated_5nM, Control vs Treated_50nM, Treated_5nM vs Treated_50nM; 5 nM and 50 nM is the concentration of added amyloid beta in the cellular culture). The results are summarized in Table 1, where lower p-values are observed in the comparisons involving the Treated_50nM condition, indicating significant differences compared to the Control.

Table 4: Summary of t-test results for pairwise comparisons between conditions (Control, Treated_5nM, Treated_50nM). Lower p-values in comparisons involving Treated_50nM indicate significant lipid alterations relative to control. Treated_5nM, Treated_50nM).

Phospholipid	LogFC (Control vs Treated5)	p-value	Significance
PI 18:0-16:1	0.95	0.019	Significant
PI 18:0-18:1	-0.45	0.161	Not Significant
PI 18:0-18:2	0.89	0.006	Significant
PE 16:0-18:1	0.95	0.019	Significant
PC 18:2-16:1	-0.45	0.161	Not Significant
PC 20:2-18:0	0.89	0.006	Significant

Figure 1 presents the mean levels of phospholipids per condition. It is evident that the increase in Aβ concentration is associated with a gradual decrease in phosphatidylcholines (PC) and phosphatidylethanolamines (PE). These reductions are more pronounced in the Treated_50nM group, suggesting a dose-dependent effect of Aβ.

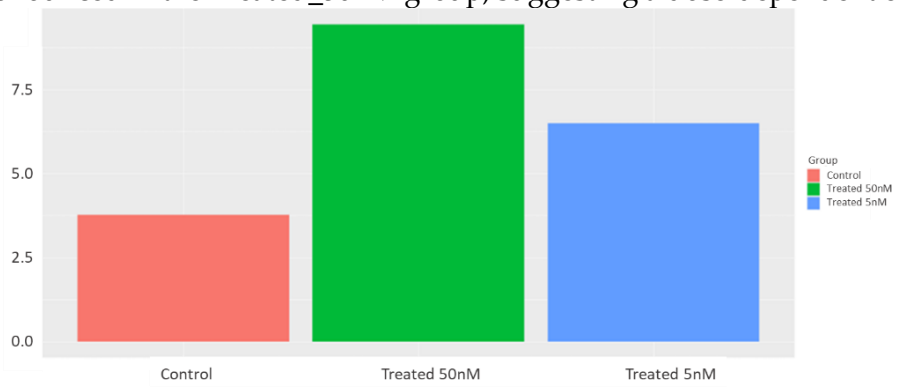

Figure 32: Mean levels of phospholipids across experimental conditions (Control, Treated_50nM, Treated_5nM).

Differential expression analysis revealed significant changes in specific lipids, which are summarized in Table 2. Certain PCs and PIs showed high negative LogFC values, confirming the decreasing trend under Aβ exposure. At the same time, some PEs exhibited greater variability, which may reflect more complex regulatory mechanisms.

Table 5: Differential expression analysis. LogFC values reflect the direction and magnitude of change, with pronounced decreases observed in specific PC and PI species under Aβ.

Phospholipid	Group Comparison	LogFC	p-value	Significance
PI 18:0-16:1	Control vs Treated5	0.95	0.019	Significant
PI 18:0-18:2	Control vs Treated5	0.89	0.006	Significant
PE 16:0-18:1	Control vs Treated5	0.95	0.019	Significant
PC 20:2-18:0	Control vs Treated5	0.89	0.006	Significant
PI 18:0-18:2	Control vs Treated50	0.92	0.004	Significant
PI 18:1-18:2	Control vs Treated50	0.84	0.007	Significant
PE 16:0-18:2	Control vs Treated50	0.91	0.002	Significant

Furthermire, the analysis showed that the reduction in PCs is consistent and evident, while PEs show higher heterogeneity, particularly in the Treated_5nM condition. In contrast, PIs display smaller changes, which nevertheless become statistically significant in the comparison with Treated_50nM.Furthermore, control samples formed a distinct cluster from the two Treated groups, while Treated_5nM and Treated_50nM showed intermediate similarity, with Treated_50nM being more distant from the Control. This finding suggests that the extent of lipid alterations is directly related to the concentration

of Aβ. Overall, the results demonstrate that exposure to Aβ leads to dose-dependent changes in the levels of specific phospholipids. PCs consistently decrease, PEs show more heterogeneous responses, and PIs are mainly altered at higher concentrations. The combination of statistical tests, boxplots, volcano plot, and heatmap strengthens the reliability of the observations, providing a comprehensive picture of the lipid remodeling induced by Aβ.

4. Conclusions

The present study indicates that amyloid-beta exposure induces dose-dependent alterations in the phospholipid profile of human neuroblastoma cells. The most pronounced differences were observed at 50 nM, where phospholipids such as PE 16:0- 18:1, PC 18:2-16:1, and PC 20:2-18:0 exhibited statistically significant changes compared to controls. Phosphatidylcholines showed consistent reductions, while phosphatidylethanolamines and phosphatidylinositols displayed more variable responses, particularly at higher amyloid-beta concentrations. These findings underline the role of amyloid-beta in disrupting lipid metabolism and highlight the complexity of lipid signaling in neuronal cells. By providing a comprehensive overview of lipidomic alterations, this work offers valuable insights for future research and supports the development of lipid-based therapeutic approaches targeting neurodegenerative processes.

Acknowledgments

This work was carried out within the framework of the Action 'Flagship Research Projects in challenging interdisciplinary sectors with practical applications in Greek industry', implemented through the National Recovery and Resilience Plan Greece 2.0 and funded by the European Union—NextGenerationEU (project code: TAEDR- 0535850).

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