Development of a fluorogenic assay for high-throughput screening and identification of ABHD6 modulators in metabolic liver disease

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Résumé

Metabolic liver diseases, including Metabolic-Associated Steatotic Liver Disease (MASLD), have emerged as a major global health concern due to their rising prevalence and their strong association with metabolic disorders. In addition, MASLD progression can lead to advanced liver pathologies such as fibrosis, cirrhosis, and ultimately Hepatocellular Carcinoma (HCC), a malignancy characterized by poor clinical outcomes and limited therapeutic options. Identifying and addressing novel molecular targets involved in early lipid dysregulation is thus essential for potent therapeutic intervention in this domain.

In this context, the alpha/beta hydrolase domain-containing protein 6 (ABHD6) has gained a growing interest as a key regulator of lipid homeostasis. Indeed, this plasma membrane enzyme that was initially described for its role in the degradation of the endocannabinoid 2-Arachidonoylglycerol in the CNS, was recently highlighted for its key implication in hepatic lipid metabolism and metabolic disease progression. Moreover, recent studies reported its contribution to MASLD-associated hepatocarcinogenesis, positioning ABHD6 as a promising therapeutic target. Up to now, the discovery and fine characterization of ABHD6 modulators remains however limited by the lack of robust, scalable, and high-throughput-compatible screening methodologies.

To address this limitation, we established an original and global procedure, including a robust and tailored methodology for producing and purifying ABHD6 in membrane-mimicking environments, as well as a fluorogenic enzymatic assay relying on the hydrolysis of a methylumbelliferyl-based substrate enabling a direct monitoring of ABHD6 activity. The activity assay has been optimized for ABHD6 isolated in detergent micelles and is compatible with quantitative and kinetic analyses. It was further miniaturized, demonstrating its compatibility with high-throughput screening formats. In addition, preliminary validation using reference inhibitors confirmed its suitability for identifying ABHD6 modulators.

Altogether, this study aims to establish a robust platform for the systematic identification of ABHD6-targeting compounds and to provide a valuable tool for drug discovery efforts in metabolic liver diseases.

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Mots-Clés: ABHD6, ABHD6 modulators, fluorogenic enzymatic assay, high throughput screening, Metabolic Associated Steatotic Liver Disease (MASLD)