CPPC 2021

sciforum

Abstract

MALDI/MS as a Tool to Rapidly Screen Peptide Libraries for Novel Substrates of Farnesyltransferase †

Garrett L Schey [1], Emily R Hildebrandt [2], Sadie X Novak [3], Walter K Schmidt [2], James L Hougland [2] and Mark D Distefano [1]

[1] Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, USA; schey013@umn.edu (G.L.S.); diste001@umn.edu (M.D.D.)
[2] Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602, USA; erh@uga.edu (E.R.H.); wschmidt@uga.edu (W.K.S.); hougland@syr.edu (J.L.H.)
[3] Department of Chemistry, Syracuse University, Syracuse, New York 13244 USA; sxnovak@syr.edu (S.X.N.)

† Presented at the First Canadian Peptide and Protein Community Virtual Symposium, 27–28 May 2021; Available online: https://cppc2021.sciforum.net/.

Published: 27 May 2021

Abstract: Protein farnesylation is a post-translational modification where a 15 carbon farnesyl isoprenoid is appended to the C-terminal end of a protein by Farnesyltransferase (FTase). In the canonical understanding of FTase, the isoprenoids are attached to the Cysteine residue of a four amino acid CaaX box sequence. However, recent work has shown that five amino acid sequences can be recognized, such as the pentapeptide CMIIM. This new discovery greatly increases the number of potential FTase substrates, as the FTase is already known to tolerate a wide variety of amino acids in the canonical CaaX sequence. Due to the large number of potential substrates it is difficult to assay for novel CaaX sequences. With the goal of developing a more rapid and methodical method to evaluate potential substrates, we envisioned using MALDI to assay libraries of 10 peptides at a time, varying one amino acid in the CaaX box to all 20 canonical amino acids over two libraries. Through this method we observed 30 hits in the mass spectrum and chose eight for further evaluation. Seven of these sequences are novel substrates for FTase, with several meeting or surpassing the in vitro efficiency of the benchmark sequence CMIIM. Additionally, in vivo experiments in yeast demonstrate that proteins bearing these sequences can be efficiently prenylated in a biological context.