

简介:

Christopher M. Overall
Centre for Blood Research, University of British Columbia, Canada

照片:

见附件

题目:

N-terminomics. How TAILS Positional Proteomics Mechanistically Deciphers Pathology and led to an Allosteric Molecular Corrector Rescuing Function in an Immunodeficient Patient

摘要:

To specifically enrich for mature protein N-termini and neo-N-termini of proteins we developed 6 and 10-plex TMT TAILS (Terminal Amine Isotopic Labeling of Substrates) (Nature Biotech 28, 281-288 (2010); Nature Protocols 6, 1578-1611 (2011)). In analyzing the N-terminome of normal human tissues we find that the N-termini of protein chains *in vivo* can commence at many points C terminal to the predicted start site and result from proteolytic processing to generate stable protein chains: Proteolytic processing generates new protein species with characteristic neo-N termini that are frequently accompanied by altered half-lives, function, interactions and location. We used TAILS to mechanistically dissect a severe human immunodeficiency disease.

The paracaspase MALT1 proteolytic activity and its molecular scaffolding are central for transducing lymphocyte antigen receptor activation of NF- κ B with unregulated MALT1 activity leading to B cell lymphomas and autoimmune disease. We developed nanomolar, selective allosteric inhibitors of MALT1 paracaspase activity that bind by replacing the side chain of Trp580 and locking the protease in an inactive conformation. Interestingly, we had previously identified a patient homozygous for a hypomorphic *MALT1* mutation suffering from combined immunodeficiency who carries a serine mutation at Trp580. We used TAILS, the N-terminal *positional proteomics* approach to compare lymphocytes from the *MALT1^{mut/mut}* patient with healthy *MALT1^{+/mut}* family members and normal individuals using 10-plex Tandem Mass Tag in the N-terminomics TAILS approach with MS3 synchronous peak selection quantification, with and without B Cell receptor stimulation. From the MALT1 cleaved neo-N terminal peptide (prime side) and the natural N terminus of HOIL1 identified by TAILS, and the nonprime side of the HOIL1 cleavage site identified by preTAILS shotgun proteomics, we identified HOIL1 of the linear ubiquitin chain assembly complex (LUBAC) as a novel MALT1 substrate. Upon B and T cell receptor stimulation HOIL1 cleavage resulted in disassembly of LUBAC and loss of linear ubiquitination in T and B cells that prevented reactivation of NF- κ B signaling. However, in this immunodeficiency, the Trp580Ser mutation weakened the interaction between the paracaspase and C-terminal immunoglobulin domains resulting in protein instability (T_m 46.1°C *versus* 52.3°C) and consequently reduced MALT1 function and protein levels. The new allosteric inhibitors also bind MALT1-Trp580Ser, stabilizing the mutant protein and increasing the T_m to that of wild-type MALT1. Allosteric, but not MALT1 active site inhibition of patient *MALT1^{mut/mut}* lymphocytes restored MALT1 protein levels *in vivo* and rescued NF- κ B and JNK signaling. Following compound washout, MALT1 substrate cleavage was also rescued. Thus, a low molecular weight compound can rescue an enzyme deficiency by substituting for the mutated residue, inspiring potential novel precision therapies to increase mutant enzyme activity.