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Detection of Ubiquitination on Syk and Documenting Syk Stability

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ABSTRACT

Post-translational modifications regulate the activities of proteins important to numerous diseases. Spleen Tyrosine Kinase (Syk) is particularly interesting to researchers because it modifies many targets and plays multiple roles in regulating cells in our bodies and its abnormal modifications may contribute to cancer, Alzheimer's disease and allergies. In an attempt to study these modifications of Syk, we first looked at detecting ubiquitination on Syk protein. Ubiquitin, a small 8 kDa molecule, attaches to lysine residues on protein. The attachment of ubiquitin to Syk may cause Syk to either propagate signals onwards to activate other proteins or signal it to undergo proteasomal degradation. To detect ubiquitination of Syk, B cell lymphoma DG75 with endogenous Syk expression was electroporated with HA-tagged Ubiquitin expression vector to introduce the ubiquitin molecule into the cells. Immunoprecipitation of Syk was performed to isolate the total Syk and to visualize the ubiquitination by Western Blot with anti-HA antibody. When cells were treated with Cyclohexamide (CHX), a protein translation inhibitor, we did not observe significant decrease of Syk in protein level, indicating that Syk is an exceptionally stable protein with a half-life longer than 72 hours. Upon treatment of cells with both CHX and MG132, a proteasome inhibitor, we reproducibly observed a detectable accumulation of Syk protein in 24 hours. The established technique will not only facilitate the study of the impact of ubiquitination on Syk in signal transduction, it also will lead us to identify the potential significance of ubiquinated lysine residues on Syk in cellular function.

KEYWORDS

Syk, Spleen tyrosine Kinase, Ubiquitination, Ubiquitin, post-translational mutation, cancer, allergies, electroporation, MG132, CHX, stability,