

P003 Identification of Vimentin as a Novel PI3K γ -Dependent Tyrosine-phosphorylated Protein.
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PI3K γ is a kinase expressed in leukocytes and activated by GPCRs. We previously showed that neutrophils and macrophages lacking PI3K γ display impaired responses to chemokines and reduced recruitment to the inflammation site. Nonetheless, the signaling events causing a PI3K γ -dependent chemotactic response are still obscure. To clarify the role of PI3K γ in leukocyte movement, we compared the patterns of tyrosine phosphorylated proteins following chemokine stimulation, using bone marrow derived macrophages (BMDM) obtained from wild-type and PI3K γ -null mice. By developing a novel proteomic approach which combined an anion exchange chromatography and a phosphotyrosine-immunoprecipitation, we consistently identified 7 bands that displayed modified phosphorylation levels between wild-type and PI3K γ -null BMDM. Three of these bands were chosen for sequence analysis using a MS/MS approach, and one of those was identified as the class III intermediate filament vimentin. To confirm this finding, vimentin was immunoprecipitated from wild-type and mutant BMDM and analysed for its tyrosine phosphorylation levels. Consistently with the results of the proteomic screening, tyrosine phosphorylation of vimentin was reduced in the PI3K γ -null BMDM extracts. Moreover vimentin tyrosine phosphorylation was reduced in wild-type BMDM treated with the PI3K γ inhibitor LY294002. The finding that, upon chemokine stimulation, vimentin is tyrosine phosphorylated in a PI3K γ dependent manner is intriguing: being vimentin involved in the cytoskeletal dynamics, it is possible that the lack of vimentin phosphorylation contribute to the migration defect of PI3K γ -null macrophages.