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Abi1/Hssh3bp1 pY213 links Abl kinase signaling to p85 regulatory subunit of PI-3 kinase in regulation of macropinocytosis in LNCaP cells

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ABSTRACT

Macropinocytosis is regulated by Abl kinase via an unknown mechanism. We previously demonstrated that Abl kinase activity is, itself, regulated by Abi1 subsequent to Abl kinase phosphorylation of Abi1 tyrosine 213 (pY213) [1]. Here we show that blocking phosphorylation of Y213 abrogated the ability of Abl to regulate macropinocytosis, implicating Abi1 pY213 as a key regulator of macropinocytosis. Results from screening the human SH2 domain library and mapping the interaction site between Abi1 and the p85 regulatory domain of PI-3 kinase, coupled with data from cells transfected with loss-of-function p85 mutants, support the hypothesis that macropinocytosis is regulated by interactions between Abi1 pY213 and the C-terminal SH2 domain of p85—thereby linking Abl kinase signaling to p85-dependent regulation of macropinocytosis.

Structured summary:
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1. Introduction

Macropinocytosis is an actin polymerization-dependent cellular process responsible for extracellular fluid and macromolecule uptake; however, the details of the mechanisms regulating macropinocytosis remain unclear [2,3]. In mammalian cells macropinocytosis is thought to be regulated by multiple pathways, which include several actin polymerization regulatory complexes, and involves receptor and non-receptor tyrosine kinases [4,5]. Abl kinases (Abl and Arg) are unique among cytoplasmic tyrosine kinases in their capacity to directly bind actin and to regulate actin polymerization [6,7]. Abl kinase activity is regulated by Abi1 [1,8]. The fact that overexpression of Abi1 inhibits macropinocytic uptake of fluorescent, water-soluble markers in NIH 3T3 [9] together with recent reports indicating that the anti-Abl drug Gleevec (STI-571) inhibits macropinocytic uptake of pathogenic bacteria [10,11], suggests the possibility that Abl tyrosine kinase activity is a key regulatory component of macropinocytosis. Moreover, expression of isoform 2 of Abi1, which inhibits Abl tyrosine activity [1], also reduces macropinocytic uptake in LNCaP cells [12].

Abl tyrosine kinase is implicated in regulating phosphotyrosine-mediated Abi1 interactions with several actin polymerization regulatory complexes [13] including PI-3 kinase [14]. PI-3 kinase involvement in regulating actin dynamics is well established [15], but is mostly attributed to the regulatory subunits of PI-3 kinase [16–18]. Studies have most often pointed to a critical role of the p85 subunit in formation of actin-rich membrane ruffles, which precedes fluid phase uptake into macropinocytic vesicles. PI-3 kinase activity was proposed to play a role in the closure of immature membrane ruffles to produce a macropinosome [19,20]. Wortmannin, an irreversible, PI-3 kinase