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Protein kinase C mediated internalization of ErbB2 is independent of clathrin, ubiquitination and Hsp90 dissociation

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ABSTRACT

Overexpression of ErbB2 is frequent in cancer and understanding the mechanisms which regulate its expression is important. ErbB2 is considered endocytosis resistant. It has no identified ligand, but upon heterodimerization it is a potent mediator of proliferative signaling. A recent study established a role for protein kinase C (PKC) in internalization and recycling of ErbB2. We have now further investigated the molecular mechanisms involved in PKC-mediated downregulation of ErbB2. We confirm that PMA-induced PKC activation causes ErbB2 internalization, but while the Hsp90 inhibitor 17-AAG induced ErbB2 degradation, PMA had no such effect. When combined with 17-AAG, PMA had additive effect on ErbB2 internalization indicating that Hsp90 inhibition and PKC activation induce internalization by alternative mechanisms. We confirm that while 17-AAG-induced internalization was clathrin-mediated, PMA-induced internalization was clathrin independent. This difference may be explained by while both 17-AAG and PMA reduced the constitutive tyrosine phosphorylation of ErbB2, only 17-AAG induced Hsp90 dissociation, Hsp70 recruitment and ubiquitination of ErbB2. Importantly, since PMA induced internalization of ErbB2, but not dissociation of Hsp90, Hsp90 does not per se retain ErbB2 at the plasma membrane. The morphology of the compartment into which receptors are sorted upon PKC activation has not previously been identified. By immuno-electron microscopy, we show that PMA sorts ErbB2 into a complex tubulovesicular or cisternal organelle resembling a previously described endocytic recycling compartment.

Abbreviations