

Trans-subunit regulation of ectodomain shedding of glycoprotein Ib α

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Treatment of calmodulin inhibitors, such as W7, can induce ectodomain shedding of many membrane receptors from the cell surface. It is thought that W7 induces dissociation of calmodulin from the cytoplasmic domain of receptors, which in turn alters their susceptibility to shedding. Shedding of glycoprotein (GP) Ib α , an effective marker for platelet lesion and aging, has been suggested to play a critical role in platelet clearance. Like in platelets, GPIb α expressed in transfected Chinese hamster ovary cells, as a subunit of GPIb-IX complex, is shed constitutively by ADAM17. Its shedding can be further up-regulated by W7 in a dose-dependent manner. To define the element in GPIb-IX complex that responds to W7 treatment and mediates GPIb α shedding, we systematically deleted the cytoplasmic domains of each subunit in GPIb-IX complex or replaced them with unrelated sequences, and measured the effect on GPIb α shedding. As expected, removing either cytoplasmic domain of GPIb α or GPIIX, or the membrane-distal region of GPIb β cytoplasmic domain, all of which are devoid of a calmodulin-binding site, did not affect the W7-induced shedding of GPIb α . The membrane-proximal region of GPIb β cytoplasmic domain, which contains a calmodulin-binding site, is essential to surface expression of GPIb-IX complex and therefore could not be removed for our study. Instead, site-specific mutations in this region, presumably to abolish calmodulin association to GPIb β , have been identified to elevate constitutive shedding level of GPIb α . Moreover, the mutational effect was amplified in a mutant GPIb-IX complex in which both GPIb α /GPIb β disulfide bonds were removed and their binding interface perturbed. Overall, our results have provided evidence for the regulation of GPIb α shedding by the GPIb β cytoplasmic domain. This is, to the best of our knowledge, the first reported case of trans-subunit regulation of ectodomain shedding of a membrane receptor.