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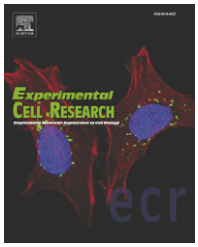

Review

The role of protease activity in ErbB biology

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ABSTRACT

Proteases are now recognized as having an active role in a variety of processes aside from their recognized metabolic role in protein degradation. Within the ErbB system of ligands and receptors, proteases are known to be necessary for the generation of soluble ligands from transmembrane precursors and for the processing of the ErbB4 receptor, such that its intracellular domain is translocated to the nucleus. There are two protease activities involved in the events: proteases that cleave within the ectodomain of ligand (or receptor) and proteases that cleave the substrate within the transmembrane domain. The former are the ADAM proteases and the latter are the γ-secretase complex and the rhomboid proteases. This review discusses the roles of each of these protease systems within the ErbB system.

Contents

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Adam proteases

All ligands of ErbB1 are synthesized with a membrane tether, which must be cut in order to release the soluble growth factors [1]. This process, which is referred to as protein ectodomain shedding (Fig. 1A), is therefore a critical regulator of the ability of ErbB-ligands to activate ErbB receptors in a paracrine manner (for previous reviews, see [1,2]). Moreover, shedding also can be important for juxtacrine signaling through ErbB1, at least under certain conditions, as signaling of TGFα to adjacent ErbB1-expressing cells can be blocked with a metalloproteinase inhibitor [2,3]. Since ErbB receptors have important functions in development and in diseases such as cancer [4,5], there is considerable interest in elucidating the identity of the enzymes responsible for processing and releasing ErbB-ligands, and understanding their regulation during development and adult homeostasis as well as their dysregulation in disease.

ADAMs 10 and 17 are the principal sheddases for ErbB1-ligands

In mammalian cells, two membrane-anchored enzymes belonging to the ADAM (a disintegrin and metalloprotease) family of membrane anchored metalloproteinases, ADAMs 10 and 17, have emerged as key molecules in shedding of the seven ErbB1-ligands (Fig. 1B shows a typical domain organization of an ADAM, for previous reviews on ADAMs, please see [2,6–8]). Based on studies with cells lacking various ADAMs and with selective inhibitors, ADAM17 is considered the principal sheddase for TGFα, HB-EGF, amphiregulin, epiregulin and epigen [9–16], whereas ADAM10 was identified as the major sheddase for EGF and betacellulin [14,17,18]. The relevance of ADAM17 in shedding of TGFα, HB-EGF and amphiregulin *in vivo* was corroborated by studies in mice lacking ADAM17, which resemble mice lacking TGFα in that they have open eyes at birth and wavy whiskers [15], or mice lacking amphiregulin with respect to defects in mammary ductal development [12], or HB-EGF with respect to defects in heart valve development [10,14]. Moreover, ADAM17 is responsible for the PMA-stimulated shedding of several isoforms of the ErbB ligand neuregulin [19,20]. The role of ADAM10 in shedding EGF has been further corroborated using ADAM10 selective inhibitors [17]. However, an evaluation of the contribution of ADAM10 to activating EGF and BTC *in vivo* is currently not possible, because mice lacking ADAM10 die very early during embryogenesis (E 9.5) [21], so conditional knockout mice will be necessary to attempt to address the relevance of ADAM10 in shedding EGF and BTC *in vivo*.

Phorbol esters are activators of ADAM17

In light of the well-established role of proteolysis in activating ErbB1 ligands, it is important to understand how the responsible sheddases are regulated. Tumor promoting phorbol esters (PMA/TPA) were among the first identified activators of protein ectodomain shedding [22–24]. Since then, ADAM17 has emerged as the principal ErbB1-ligand-sheddase that is stimulated by short-term treatment of cells with PMA (25 ng/ml for <1 h) [14,16,18,25]. Addition of PMA to cells leads to a burst of shedding of ADAM17-substrates that have accumulated in the late secretory pathway and on the cell surface, but once these substrates are consumed, shedding returns to constitutive levels even in the presence of PMA [16].

There are conflicting reports on the mechanism of activation of ADAM17 by PMA. Several studies have provided evidence that PMA stimulation increases relocalization and transport of ADAM17 to the cell surface and increases processing of ADAM17 by furin, which removes the inhibitory pro-domain [25,26]. On the other

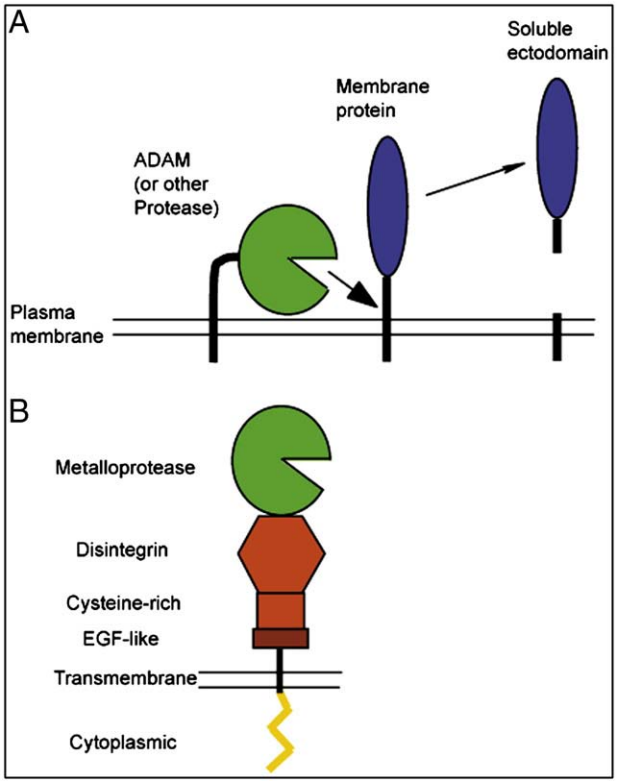

Fig. 1 – Protein ectodomain shedding refers to the proteolytic release of the extracellular domain or "ectodomain" of a membrane protein from its membrane anchor (A). ADAM (a disintegrin and metalloprotease) 10 and 17 as well as other enzymes, such as ADAMTS1 (a disintegrin and metalloprotease with thrombosponding motifs) have been implicated in the ectodomain shedding of ErbB-ligands and of ErbB3. A schematic representation of the domain organization of a typical ADAM (B) shows an amino-terminal metalloprotease domain, followed by a disintegrin domain, a cysteine-rich region, an EGF-like repeat, a transmembrane domain and a cytoplasmic domain. The cytoplasmic domain of ADAMs frequently contains signaling motifs such as phosphorylation sites or proline-rich SH3 ligand binding domains.