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JB Commentary

Ectodomain shedding of HB-EGF: A potential target for cancer therapy

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Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is synthesized as a membrane-anchored protein, known as proHB-EGF. ProHB-EGF is cleaved by metalloproteases through a process referred to as 'ectodomain shedding', resulting in the formation of soluble HB-EGF. Both proHB-EGF and soluble HB-EGF are biologically active; the former acts on neighbouring cells through juxtacrine signalling, whereas the latter can move to distant locations. Elevated HB-EGF expression has been observed in ovarian and some other cancers. CRM197, a diphtheria toxin (DT) mutant, binds directly to the epidermal growth factor (EGF)-like domain and represses the mitogenic activity of HB-EGF. Recently, monoclonal antibodies (mAbs) specific for human HB-EGF were generated by immunizing HB-EGF-deficient mice with human HB-EGF (Hamaoka *et al.* (2010) *J. Biochem.* 148, 55–69). Most of the mAbs can bind to the EGF-like domain of HB-EGF, but fail to inhibit the mitogenic activity of soluble HB-EGF. However, some mAbs prevented the ectodomain shedding of proHB-EGF and inhibited the proliferation of EGF receptor-expressing cells stimulated by proHB-EGF-expressing cells. Hamaoka *et al.* showed that CRM197 prevents the ectodomain shedding of proHB-EGF. Thus, these mAbs function as specific inhibitors for the ectodomain shedding of HB-EGF and may be useful for treating cancers exhibiting elevated levels of HB-EGF.

Keywords: EGF/EGF receptor/ectodomain shedding/HB-EGF/diphtheria toxin.

Abbreviations: ADAM, a disintegrin and metalloprotease; DT, diphtheria toxin; EGF, epidermal growth factor; EGFR, EGF receptor; HB-EGF, heparin-binding EGF; HGF, hepatocyte growth factor; IL, interleukin; mAb, monoclonal antibody; TGF-β, transforming growth factor-β.

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a member of the epidermal growth factor (EGF) family of growth factors (*1*). The EGF family includes 13 members, all of which are produced as membrane-anchored proteins containing a functional EGF-like domain and a transmembrane domain (*2–4*). ProHB-EGF, the membrane-anchored form of HB-EGF, is cleaved by metalloproteases, including a disintegrin and metalloproteases (ADAMs), through a process known as ectodomain shedding (*2*, *3*), leading to the production of the mitogenically active, soluble form of HB-EGF. Of the four different EGF receptors (EGFRs or ErbB proteins), HB-EGF binds and activates the EGF receptor (EGFR, also known as ErbB1) and ErbB4 (*5*). Interestingly, proHB-EGF is also biologically active and binds to the EGFRs expressed on the surface of adjacent cells. Additionally, proHB-EGF serves as the receptor for diphtheria toxin (DT) and forms a complex with the membrane protein DRAP27/CD9 to increase the sensitivity to DT (*6*, *7*).

Hamaoka *et al.* (*8*) generated seven monoclonal antibodies (mAbs) specific for human HB-EGF by immunizing HB-EGF-deficient mice with human HB-EGF. One mAb recognized the heparin-binding domain, while the other 6 mAbs, as well as a commercially available mAb (MAB259), recognized the EGF-like domain (Fig. 1). Through epitope mapping, Hamaoka *et al.* subclassified the HB-EGF mAbs that bound to the EGF-like domain into three groups: the E141 group, the H135 group and the I133/H135 group. Although these human HB-EGF mAbs bound to the EGF-like domain of proHB-EGF, they failed to directly inhibit the growth-promoting activity of HB-EGF. However, mAbs in the E141 group efficiently repressed the ectodomain shedding of proHB-EGF, thereby preventing proHB-EGF-induced cell proliferation in a coculture system using proHB-EGF- and EGFR-expressing cells. It is unknown how these mAbs can specifically prevent ectodomain shedding without affecting the growth-promoting activity of soluble HB-EGF. The HB-EGF mAbs of the E141 and I133/H145 groups also suppressed the binding of DT to proHB-EGF. Thus, these mAbs may be useful for examining the function of HB-EGF and for establishing a novel method of treating diseases induced by HB-EGF.

Inflammatory cytokines are produced by specific cell types and often show specific expression profiles. For example, Th17 cells are induced under certain conditions through the action of transforming growth factor (TGF)-β and interleukin (IL)-6; thus, expression of IL-17 is observed only in specific tissues (*9*). In contrast, most growth factors are produced by many different cell types and are ubiquitously expressed. Platelet-derived growth factor was originally purified from human platelets, but is now known to be produced by a wide variety of cells. However, the functions of growth factors are regulated by various mechanisms, and activation (or regulation) steps are required for their biological activities. TGF-β is produced in latent high-molecular-weight forms, which are activated by proteases or mechanical forces (*10*). Hepatocyte growth factor (HGF) is also produced in a

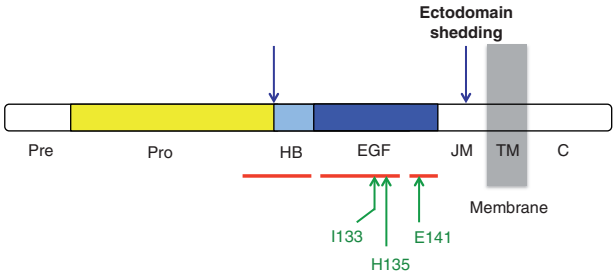

Fig. 1 Schematic representation of proHB-EGF structure and HB-EGF mAb binding sites. ProHB-EGF is composed of an amino-terminal signal sequence (Pre), followed by a pro-domain (Pro), heparin-binding domain (HB), EGF-like domain (EGF), juxtamembrane domain (JM), transmembrane domain (TM) and cytoplasmic domain (C). Proteolytic cleavage sites are indicated by arrows (blue). 'Ectodomain shedding', which is cleavage at the juxtamembrane domain, results in the production of soluble HB-EGF. mAbs generated by Hamaoka *et al*. (*8*) bind to either the heparin-binding domain, amino-terminal parts of the EGF-like domain or carboxy-terminal parts of the EGF-like domain (indicated by red bars). Amino acid residues critical for the binding of some mAbs are shown in green.

latent form; HGF activator, a blood coagulation factor XII-like serine protease, efficiently converts the latent form of HGF to the active form (*11*). ProHB-EGF is biologically active in its membrane-anchored form; however, it acts only on neighbouring cells in a juxtacrine fashion. HB-EGF can diffuse to distant locations only after proteolytic cleavage through ectodomain shedding (*2*, *3*). Additionally, the cytoplasmic remnant of proHB-EGF produced after ectodomain shedding plays a role in growth factor receptor signalling (*3*). Thus, ectodomain shedding is a critical step in regulating the biological action of HB-EGF and other members of the EGF family.

EGF ligand and receptor systems are perturbed in various types of cancer (*5*). Over-expression and mutations of EGFRs are frequently observed in human cancers, including breast and lung cancer, and mAbs to EGFRs and small molecule inhibitors for EGFR kinases have been developed as treatments for these cancers (*5*, *12*). Elevated HB-EGF expression has been observed in ovarian, gastric and breast cancer, melanoma and glioma cells. Ovarian cancer cells produce high levels of proHB-EGF, and the expression levels of HB-EGF are associated with clinical outcome in ovarian cancer (*13*). Therefore, HB-EGF is a potential molecular target for treating ovarian cancer (*14*). Although mAbs to EGFRs and small molecule inhibitors for EGFR kinases are used clinically or are under development for clinical use, they inhibit the action of many EGF family proteins. On the other hand, ectodomain shedding of proHB-EGF can be blocked by metalloprotease inhibitors; however, these inhibitors broadly act on various metalloproteases and prevent ectodomain shedding of many other proteins. Thus, specific HB-EGF inhibitors may be valuable for treating certain types of cancers exhibiting elevated HB-EGF expression.

CRM197 is a DT mutant with weak toxicity (*15*). CRM197 binds to proHB-EGF and inhibits the growth-promoting activity of HB-EGF by preventing its binding to EGFRs (*16*). Hamaoka *et al*. (*8*) showed that CRM197 also inhibits ectodomain shedding of proHB-EGF, which is consistent with the finding that E141 of HB-EGF is the most important amino acid for DT and CRM197 binding to HB-EGF (*17*). A preclinical trial of CRM197 is currently underway, but mAbs to proHB-EGF that prevent ectodomain shedding may also be useful for treating ovarian and other cancers whose pathogenesis involves overproduction of HB-EGF.

Conflict of interest
None declared.

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