

Deep Insight Section

Role of HB-EGF in cancer

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Published in Atlas Database: November 2010

Online updated version : <http://AtlasGeneticsOncology.org/Deep/HB-EGFInCancerID20090.html>
DOI: 10.4267/2042/45990

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Introduction

Peptide growth factors regulate diverse processes from cell survival and proliferation to migration and programmed cell death. Due to their central role in growth regulation, growth factors are major players in the development and progression of cancer. Among this broad class of molecules, those comprising the EGF-like family are amongst the best characterized. In this Deep Insight, I will elaborate on the evidence implicating one such protein, heparin-binding EGF-like growth factor (HB-EGF), in tumor biology and how its activity may be targeted for therapeutic gain.

HB-EGF: a member of the EGF-like growth factor family

Heparin-binding EGF-like growth factor (HB-EGF) is a member of the epidermal growth factor (EGF)-like growth factor family of proteins that bind to and activate the EGF receptor (EGFR) and its associated receptors ERBB2, ERBB3 and ERBB4. The extended family comprises 15 members, all of which conform broadly to common structural framework centered around 6 cysteine residues in the sequence: CX₇CX₄₋₅CX₁₀₋₁₃CXCX₈GXRC. Disulphide bond formation between 3 pairs of cysteines gives rise to the characteristic 3-looped EGF-like motif that mediates high-affinity binding to receptors (reviewed in Wilson et al., 2009).

The EGF-like growth factors achieve their effects through interaction with one or more ErbB receptor tyrosine kinases. The ERBB receptors are class I transmembrane proteins that homo- or heterodimerize following ligand binding and undergo auto-phosphorylation on defined tyrosine residues to generate recognition motifs for interactors that mediate

downstream signaling cascades. Among the ERBB proteins, ERBB2 is an orphan receptor with no known ligand, whereas ERBB3 lacks functional intrinsic tyrosine kinase activity. In addition, the EGF-like growth factors show specificity in ERBB binding, with some factors such as EGF, amphiregulin (AREG) and TGF α selective for ERBB1 but others such as HB-EGF and betacellulin (BTC) able to interact with ERBB1 and ERBB4. Consequently, as a result of differential intrinsic receptor activity, ligand selectivity and modulation of ligand availability by interactions with heparin, a wide range of downstream responses can be evoked following ligand-ERBB interaction (Citri and Yarden, 2006).

HB-EGF: structure, molecular interactions and function

HB-EGF was originally identified as a secreted product of macrophages that was purified on the basis of its high affinity for heparin (Higashiyama et al., 1991). The gene is encoded on the long arm of chromosome 5, at 5q23, and gives rise to a 208 amino acid protein of 20-22 kDa in size. Determination of the primary peptide sequence revealed the presence in HB-EGF of an extended N-terminal domain that was absent in the prototypical EGFR ligands EGF and transforming growth factor-alpha (TGF α). Notably, the N-terminal sequence in HB-EGF is enriched in basic amino acids that are positively charged at physiological pH and enable interaction with negatively charged heparin sulphate proteoglycans both on the cell surface and in the extracellular matrix.

HB-EGF is synthesized as a single pass membrane-anchored precursor with a short cytoplasmic tail (Figure 1).

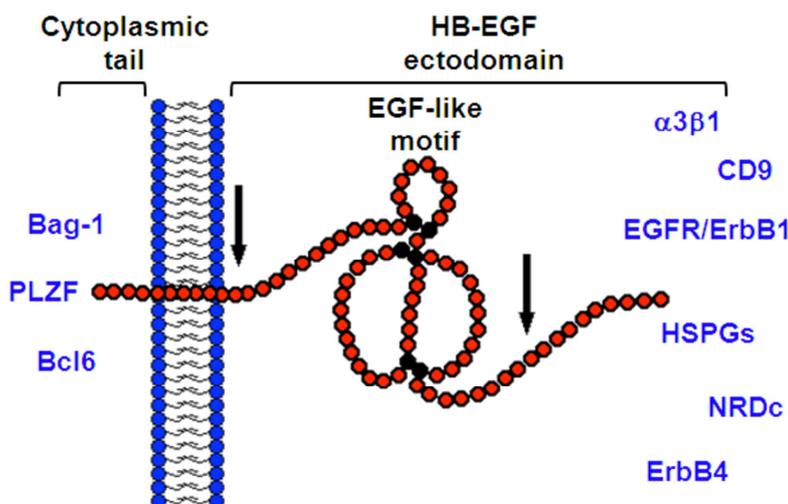


Figure 1. Molecular structure of HB-EGF. The figure illustrates in schematic form the secondary structure of HB-EGF. The protein possesses a short cytoplasmic region, a single transmembrane domain, and an ectodomain that harbors the 3-looped EGF-like motif characteristic of this growth factor family. Vertical arrows indicate primary sites of ectodomain cleavage. Molecules that interact with HB-EGF are indicated in blue.

Membrane-anchored proHB-EGF undergoes a number of post-translational modifications ranging from O-linked glycosylation of the N-terminal ectodomain, N-terminal truncations, phosphorylation of the cytoplasmic domain and regulated cleavage of the entire ectodomain. The significance of these modifications will be considered in subsequent sections.

Among the EGFR ligands, proHB-EGF is notable for the number of proteins and other molecules with which it interacts, including transmembrane receptors, adhesion molecules, and transcriptional regulators as described below.

(i) Transmembrane receptors: the best-studied functions of HB-EGF are as a ligand for the EGFR/ErbB1 and the related receptor ErbB4. High-affinity interactions with receptors are mediated via the 3-looped EGF-like motif and result in receptor autophosphorylation and initiation of downstream signaling cascades. Interestingly, the biological effects evoked by HB-EGF binding to ErbB1 and ErbB4 are distinct, with the former typically promoting proliferation but the latter stimulating chemotaxis and migration (Elenius et al., 1997). Interactions between the membrane-anchored form of HB-EGF and ErbB receptors expressed on adjacent cells also mediate both cell survival (Singh et al., 2007) and intercellular adhesion functions (Raab et al., 1996; Paria et al., 1999). More recently, radioligand binding assays with ^{125}I -labeled HB-EGF using the breast cancer cell line MDA-MB 453 revealed the existence of a novel receptor that was subsequently identified as the metalloendopeptidase N-arginine dibasic convertase (NRDC). In that study, NRDC was found to enhance HB-EGF stimulated migration of tumor cells in an EGFR/ErbB1-dependent manner (Nishi et al., 2001).

(ii) Heparan sulphate proteoglycans (HSPGs): HB-EGF was purified from the conditioned medium of a

macrophage-like cell line on the basis of its high affinity for heparin (Higashiyama et al., 1991). Interaction of HB-EGF with heparin, in the form of HSPGs present on cell surfaces and in the extracellular matrix is known to enhance ErbB receptor binding affinity as well as bioactivity (Paria et al., 1999; Higashiyama et al., 1993).

(iii) CD9 and integrins: CD9 is a member of the tetraspanin family of transmembrane proteins that interacts with membrane-anchored HB-EGF via its heparin-binding domain (Sakuma et al., 1997) and upregulates its ability to stimulate juxtacrine activation of the EGFR expressed on adjacent cells (Higashiyama et al., 1995). HB-EGF and CD9 were also found to be co-expressed in gastric cancers (Murayama et al., 2002), although the prognostic significance of these observations has not been determined. CD9 and HB-EGF were also demonstrated to exist in complex with integrin $\alpha3\beta1$ at sites of cell-cell junctions (Nakamura et al., 1995) where the multiprotein complex was predicted to participate in cell-cell adhesion.

(iv) Cytoplasmic tail interactors: several binding partners for the cytoplasmic domain of proHB-EGF were identified using either yeast 2-hybrid or co-immunoprecipitation strategies, including the cochaperone BAG-1 and the transcriptional repressors PLZF and Bcl6. Interaction between BAG-1 and proHB-EGF was found to augment the prosurvival function of proHB-EGF (Lin et al., 2001). Conversely, association between the C-terminal fragment of HB-EGF that is liberated following ectodomain shedding, with PLZF or Bcl6 leads to nuclear export or degradation, respectively of the transcriptional repressors and a resulting inhibition of repressive activity (Nanba et al., 2003; Kinugasa et al., 2007; Hirata et al., 2009). The relevance of these interactions to cancer will be considered in more detail below.

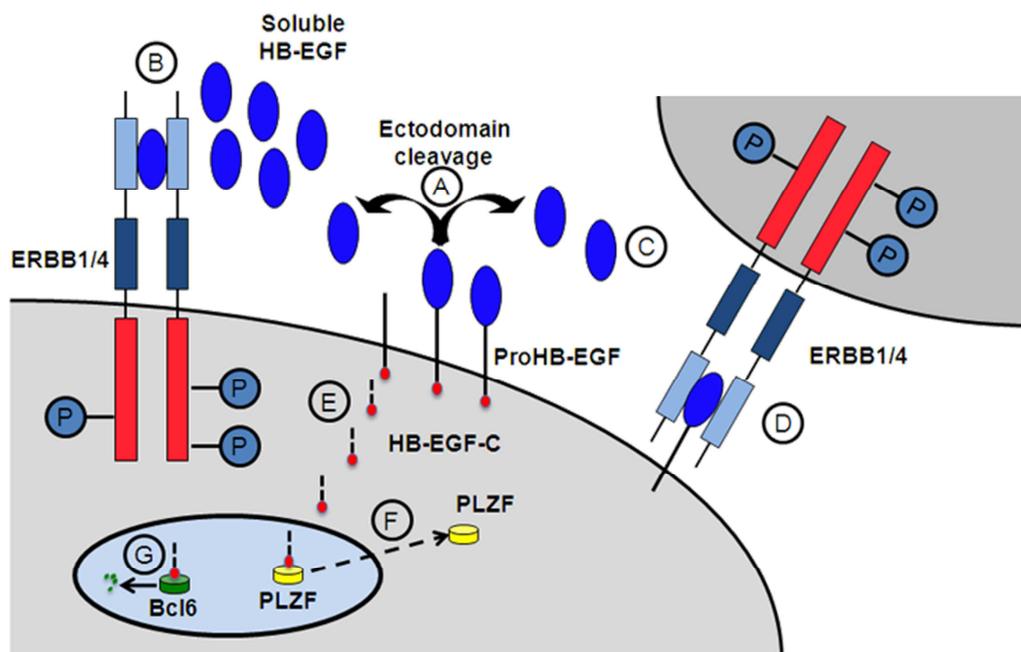


Figure 2. Regulated processing and activity of HB-EGF. ProHB-EGF expressed on the plasma membrane undergoes ectodomain cleavage mediated by enzymes of the matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase (ADAM) families (A). Release of the mature, soluble protein facilitates both autocrine (B) and paracrine (C) activation of ERBB receptors expressed on the same or adjacent cells, respectively. The membrane-anchored proHB-EGF can also activate ERBB receptors via juxtacrine signaling (D). In addition to release of the ectodomain, regulated cleavage releases the C-terminal cytoplasmic fragment, HB-EGF-C (E), which can translocate to the nucleus to effect either nuclear export (F) or degradation (G), respectively, of the transcriptional repressors PLZF or Bcl6.

Regulated processing of proHB-EGF

Like all ERBB ligands, HB-EGF is synthesized as a membrane-anchored precursor that is trafficked to the plasma membrane and subsequently processed to yield the mature, soluble growth factors. Regulated processing of the HB-EGF precursor represents a critical control point in ligand function since it represents the conversion from a membrane-anchored, non-diffusible state to a diffusible protein that has a greatly expanded sphere of influence on surrounding cells and tissues (Figure 2).

In addition to normal post-translational maturation of HB-EGF, regulation of precursor processing is highly relevant to cancer, since many of the enzymes that cleave HB-EGF and other EGFR ligands are themselves upregulated in cancer versus normal cells (Murphy, 2008). The signals and enzymes responsible for liberation of the HB-EGF ectodomain will be considered in the following sections.

Several metalloproteinases have been implicated in ectodomain shedding of proHB-EGF including matrix metalloproteinase-3 (MMP-3) (Suzuki et al., 1997), MMP-7 (Yu et al., 2002), ADAM10 (Yan et al., 2002), ADAM12 (Asakura et al., 2002) and TNF α -converting enzyme (TACE)/ADAM17 (Sahin et al., 2004). In forced expression experiments, ADAM9 was demonstrated to promote both basal and TPA-stimulated proHB-EGF processing (Izumi et al., 1998).

However, subsequent evaluation of ADAM9^{-/-} MEFs revealed no differences in processing with wild type MEFs (Weskamp et al., 2002) suggesting that there is some redundancy among ADAM factors that cleave proHB-EGF. Elegant studies using cells from mice deficient in specific ADAM family members identified ADAM-17/TACE as the primary mediator of HB-EGF cleavage (Sahin et al., 2004). Importantly, ADAM-17 itself is upregulated in a range of tumor types (Tanaka et al., 2005) and at least part of its association with tumor progression is likely to reflect increased processing of EGFR ligands including HB-EGF.

Ectodomain shedding of HB-EGF has also been implicated in EGFR transactivation in tumor cells downstream of multiple discrete agonists including G-protein coupled receptor activators (Filardo et al., 2000; Madarame et al., 2003; Schäfer et al., 2004a; Schäfer et al., 2004b; Yano et al., 2004; Itoh et al., 2005), Ser/Thr kinase activators (Ebi et al., 2010), ligands for gp130 cytokine receptors (Ogunwobi and Beales, 2008) and others. EGFR transactivation by GPCR-dependent HB-EGF cleavage has been discussed recently (reviewed in Higashiyama et al., 2008) and will not be considered further here.

Although much attention has focused on the fate of the soluble HB-EGF that is liberated following precursor cleavage, the C-terminal fragment of HB-EGF that remains following ectodomain shedding has also been shown to be functional, independently of proHB-EGF. HB-EGF-C, comprising both the transmembrane and

cytoplasmic domains, was demonstrated to undergo nuclear translocation following cleavage of the HB-EGF ectodomain (Nanba et al., 2003; Nanba et al., 2004; Toki et al., 2005). Detection of the C-terminal fragment of HB-EGF in nuclei was consistent with an earlier report from our group identifying nuclear localization of HB-EGF as a feature of aggressive disease in bladder cancer (Adam et al., 2003). Nuclear localization of HB-EGF-C was accompanied by nuclear export of the promyelocytic leukemia zinc finger (PLZF) protein, identified as an interactor for HB-EGF-C by yeast 2-hybrid analysis (Nanba et al., 2003). PLZF is a sequence-specific transcriptional repressor and inhibitor of cell cycle transit that achieves its effect by binding via its zinc finger domains to the promoters of target genes such as cyclin A (Yeyati et al., 1999). Export from the nucleus therefore prevents it from exerting its inhibitory function, resulting in enhanced movement through the cell cycle (Nanba et al., 2003). Interestingly, Bcl6, another transcriptional repressor, was also demonstrated to interact with HBEGF-C by a similar mechanism. In contrast to PLZF, however, binding to HB-EGF-C led to Bcl6 degradation and attenuation of its negative regulatory activity (Kinugasa et al., 2007; Hirata et al., 2009).

In light of the dual growth stimulatory effects of proHB-EGF cleavage, resulting in liberation of the HB-EGF ectodomain that can promote autocrine and paracrine stimulation of tumor cells, and the HB-EGF-C carboxyl terminal fragment, that induces cell cycle transit, attempts have been made to target both biological consequences pharmacologically to achieve tumor cell inhibition (Shimura et al., 2008).

HB-EGF in cancer

HB-EGF expression is altered in a number of cancer types including bladder (Adam et al., 2003; Kramer et al., 2007), breast (Ito et al., 2001c; Yotsumoto et al., 2010), colon (Ito et al., 2001a), hepatic (Inui et al., 1994), ovarian (Miyamoto et al., 2004; Tanaka et al., 2005), pancreatic (Kobrin et al., 1994; Ito et al., 2001b) and prostate cancers (Freeman et al., 1998) as well as gliomas (Mishima et al., 1998). In addition to quantitative increases in its expression in tumor versus non-tumor tissue, HB-EGF has also been found to undergo qualitative changes including altered subcellular localization, and cleavage to release N- and C-terminal fragments that mediate oncogenic behaviors. Notably, although HB-EGF in cancer is typically expressed in epithelial cells, we and others have reported robust HB-EGF expression in the stroma (Freeman et al., 1998; Adam et al., 1999) and endothelium (Nolan-Stevaux et al., 2010) in certain organs that exerts profound paracrine effects on tumor cells.

To understand the potential functions of HB-EGF in cancer, it is instructive to consider the defining characteristics of tumor cells. In their seminal article published 10 years ago Hanahan and Weinberg defined

six features or 'hallmarks' characteristic of tumor cells (Hanahan and Weinberg, 2000). These are: (i) self sufficiency in growth signals; (ii) limitless replicative potential; (iii) resistance to growth inhibitory signals; (iv) evasion of apoptosis; (v) ability to migrate, invade and metastasize; and (vi) ability to evoke sustained angiogenesis. Recently, it has been argued that the list should be updated to include inflammation as an additional hallmark of cancer (Colotta et al., 2009). In the following sections, we will consider how HB-EGF relates functionally these features.

Self-sufficiency in growth signals and limitless replicative potential

As a ligand for members of the ErbB family of receptor tyrosine kinases, it is well established that HB-EGF can promote proliferation of a wide range of cells, including tumor cells from diverse cancer types. HB-EGF gene expression is a target of several oncogenes including v-jun (Fu et al., 1999), Raf and Ras (McCarthy et al., 1995), and can therefore mediate growth-promoting effects subsequent to oncogenic transformation.

The growth promoting effects of HB-EGF are mediated largely, although not exclusively, through binding to ErbB receptors on the plasma membrane. HB-EGF binding to EGFR/ErbB1 activates downstream signaling that converges on the Raf/Ras/MEK/Erk and phosphoinositide-3-kinase (PI3K)/Akt pathways to promote survival and proliferation (reviewed in Yarden and Sliwkowski, 2001). However, recent studies have demonstrated receptor-independent activities for HB-EGF-C, the C-terminal fragment of HB-EGF that remains after ectodomain cleavage. In particular HB-EGF-C has been shown to inhibit the transcription-repressing capabilities of PLZF and Bcl6 through either nuclear export or degradation, respectively (Nanba et al., 2003; Kinugasa et al., 2007). This resulted in enhanced expression of cyclin A and cyclin D2, together with increased cell cycle transit.

Resistance to growth inhibitory signals evasion of apoptosis

HB-EGF has been implicated as a survival factor for multiple cell types exposed to growth inhibitory stimuli. One of the earliest demonstrations of HB-EGF-mediated cell survival revealed that whereas proHB-EGF could prevent TGF β -induced apoptosis in hepatoma cells in culture, this function could not be replicated with soluble HB-EGF (Miyoshi et al., 1997). That study provided the first demonstration of discrete functions for the soluble and cell-associated forms of HB-EGF, a concept that has been borne out in many subsequent studies both in non-malignant and tumor cells (Takemura et al., 1997; Singh et al., 2007; Ray et al., 2009).

It is important to appreciate that HB-EGF expressed by cells in the microenvironment has also been implicated in tumor cell survival. Circulating cells such as T lymphocytes and macrophages that infiltrate tumors

have been demonstrated to secrete HB-EGF that can act on tumor cells as well as other components critical for tumor expansion such as endothelial cells and pericytes (Blotnick et al., 1994; Peoples et al., 1995). The cytokine CXCL12 was shown to promote HB-EGF release from mononuclear phagocytes and subsequent activation of the EGFR/ErbB1 and initiation of pro-survival signaling in tumor cells (Rigo et al., 2010). This in turn stimulated release of the macrophage mitogen GM-CSF to further promote HB-EGF in a growth stimulatory loop.

Resistance to growth inhibition and evasion of apoptosis are relevant not only to cancer initiation, where cells lose responsiveness to normal cell death signals, but also in the setting of cancer treatment where tumor cells develop resistance to cytotoxic agents. Several recent reports have identified HB-EGF as a key mediator of treatment resistance and several tumor types. Exposure of cancer cells to either conventional chemotherapy or treatment with small molecule inhibitors was found to upregulate HB-EGF expression, release and activation of the EGFR, thereby enhancing survival signaling (Johnson et al., 2005; Yotsumoto et al., 2010). Both transcriptional and post-transcriptional mechanisms have been proposed to account for increased HB-EGF levels, including AP-1/NFκB-dependent transcription (Wang et al., 2007a; Sorensen et al., 2006) and enhanced mRNA stability contributing to upregulation of HB-EGF protein expression.

Ability to evoke sustained angiogenesis

Decades of work by Judah Folkman and colleagues led to the concept that tumor growth beyond a defined size is an angiogenesis-dependent process (reviewed in Bishop-Bailey, 2009) i.e. requiring the development of a tumor blood supply. Using bladder cancer cells stably expressing either soluble or membrane-anchored HB-EGF, Ongusaha and colleagues demonstrated that HB-EGF was a potent inducer of several oncogenic behaviors including growth and migration in vitro as well as xenograft growth and angiogenesis in vivo (Ongusaha et al., 2004). Consistent with distinct functions for soluble and membrane-anchored HBEGF, expression of non-cleavable proHB-EGF was unable to replicate the tumorigenic potential of either soluble or wild type proHB-EGF. The HB-EGF sheddase ADAM17 has also been implicated in pathological neovascularization. Studies in which ADAM17 was deleted conditionally in either endothelial cells or pericytes using tissue-specific promoters to drive Cre recombinase expression demonstrated that loss of ADAM17 expression specifically in endothelial cells attenuated growth of implanted tumor cells (Weskamp et al., 2002). Significantly, effects of ADAM17 ablation could be restored by administration of exogenous HB-EGF, consistent with a role for ADAM17-dependent release of HBEGF in regulation of angiogenesis. The proangiogenic function of HB-

EGF was verified in an independent study that employed the RIP1-Tag2 mouse model of pancreatic neuroendocrine tumorigenesis (Nolan-Stevaux et al., 2010). In that study the authors identified discrete roles for HB-EGF expressed by tumor endothelial and perivascular cells, and TGFα released by cancer cells both of which act through the EGFR to promote angiogenesis and tumor cell survival/growth, respectively.

Ability to migrate, invade and metastasize

To exit the primary tumor and disseminate to distant sites in the body, tumor cells must acquire the ability to migrate, intravasate, survive in the circulation, extravasate and establish in the secondary site. HB-EGF has been shown to promote prostate cancer cell migration and invasion both directly and as an intermediate in EGFR transactivation by G-protein coupled receptor agonists (Madarame et al., 2003; Schäfer et al., 2004a; Cáceres et al., 2008). HBEGF was also found to participate in the epithelial-mesenchymal transition (EMT) in gastric and ovarian cancer cells (Yagi et al., 2008). Gastric cancer cells exposed to the pathogen *Helicobacter pylori*, displayed increased MMP-7- and gastrin-dependent HB-EGF shedding and induction of EMT-associated genes. Inhibition of either gastrin or MMP-7 in vitro, or gastrin in vivo suppressed expression of HB-EGF and EMT-associated genes (Yin et al., 2010). Treatment of ovarian cancer cells with recombinant HB-EGF reduced E-cadherin levels and upregulated expression of Snail, a key regulator of the EMT. Conversely RNAi-mediated silencing of Snail attenuated HB-EGF expression and release of HB-EGF into the medium. Together these findings led the authors to conclude that HB-EGF could promote ovarian cancer metastasis through induction of the EMT (Yagi et al., 2008). Interestingly, Wang and colleagues demonstrated opposing effects on E-cadherin expression in pancreatic cells by retention of HB-EGF on the membrane. In cells either expressing non-cleavable proHB-EGF or treated with an inhibitor of HB-EGF ectodomain shedding, E-cadherin levels were up-regulated as a result of inhibition of ZEB1, a transcriptional repressor for E-cadherin (Wang et al., 2007b). Increased E-cadherin not only attenuated cell motility, but also sensitized cells to chemotherapy-induced apoptosis.

Regulation of neuroendocrine differentiation and inflammation

Although not strictly defined as 'hallmarks' of cancer, HB-EGF is known to participate in two additional processes linked to development and progression of cancer, namely inflammation and neuroendocrine differentiation. In intestinal cells exposed to cytokines (Mehta and Besner, 2003) or intestinal tissue exposed to ischemia/reperfusion injury (Rocourt et al., 2007) HB-EGF was found to exert anti-inflammatory activity in part through downregulation of NFκB and the ensuing reduction in expression of pro-inflammatory

cytokines. In contrast, however, HB-EGF expression by mesenchymal cells in the liver was upregulated in inflamed tissue and augmented by pro-inflammatory stimuli (Sagmeister et al., 2008). Moreover, increased HB-EGF expression contributed to enhanced DNA synthesis and mitogenesis in premalignant hepatocytes consistent with a facilitative role for HB-EGF in hepatocarcinogenesis.

In certain cancer types, such as prostate cancer the presence of neuroendocrine differentiation is associated with more aggressive tumors and worse patient outcome (Slovin, 2006). Our group showed that HB-EGF could drive the neuroendocrine phenotype in prostate cancer cells *in vitro* and *in vivo* (Kim et al., 2002; Adam et al., 2002). Notably, cells exposed to HB-EGF continued to traverse the cell cycle in contrast to previous reports showing inducers of NE differentiation promoting cell cycle arrest (Cox et al., 2000; Wang et al., 2004). Moreover, HB-EGF induced downregulation of androgen receptor (AR) expression in xenografts as well as AR expression and activity *in vitro* (Adam et al., 2002). Subsequent analysis revealed HB-EGF-mediated AR inhibition occurred through an mTOR-dependent mechanism involving cap-dependent mRNA translation (Cinar et al., 2005).

HB-EGF as a therapeutic target

In light of the involvement of HB-EGF in multiple aspects of tumor development, progression and metastasis, it is not surprising that attempts have been made to target it for therapeutic benefit. Promising targeting strategies include prevention of ligand binding to the EGFR, inhibition of proHBEGF cleavage and subsequent release of ectodomain and C-terminal fragments and exploitation of proHB-EGF as the receptor for diphtheria toxin. In this section, we will review the approaches used to inhibit HB-EGF and their potential for clinical use.

In situations where HB-EGF is overexpressed in tumors, some of its effects can obviously be blocked in the presence of either function blocking anti-EGFR antibodies or small molecule inhibitors of the intrinsic kinase domain. However this topic has been covered in many excellent reviews (Laskin and Sandler, 2004; Jimeno and Hidalgo, 2005) and will not be considered further here.

A number of studies have exploited the identity of proHB-EGF as the receptor for diphtheria toxin (DT) in human cells by treating cells, and in some cases patients, with a non-toxic DT mutant termed CRM197. CRM197 harbors a point mutation (G52E) in the DT A chain that diminishes its ability to perform the ADP-ribosylation of elongation factor 2 and inhibition of protein synthesis characteristic of wild type DT (Mekada and Uchida, 1985). Although CRM197 lacks the toxicity of DT, it nonetheless exerts potent growth inhibitory effects through binding to the EGF-like domain of cell surface and soluble HB-EGF (Mitamura et al., 1995; Kageyama et al., 2007). In experimental

evaluation, CRM197 alone has been found to induce apoptosis and inhibit oncogenic behaviors such as migration and invasion *in vitro* and to diminish growth, promote apoptosis and suppress angiogenesis in xenografts in nude mice (Sanui et al., 2010; Miyamoto et al., 2004; Martarelli et al., 2009). In addition, enhanced antitumor activity of CRM197 has been observed in combination with conventional chemotherapeutic agents such as paclitaxel (Yagi et al., 2009; Sanui et al., 2010). Although the results of CRM197 combination chemotherapy are provocative, it is important to note that exposure of cells to chemotherapeutic agents has been shown to upregulate HB-EGF levels that may in turn promote resistance to chemotherapy (Wang et al., 2007a). However, by careful attention to scheduling of drug administration, chemotherapy-induced upregulation of HB-EGF could be exploited to sensitize tumor cells to HB-EGF-targeted agents.

Based on its demonstrated bioactivity, CRM197 has been administered to patients with advanced, treatment-refractory malignant disease (Buzzi et al., 2004). One potential limitation of this strategy is that many in the general population are immunized against diphtheria and therefore may have innate resistance to CRM197 delivered systemically. Nevertheless, objective anti-tumor activity was observed in a small proportion of patients, with 3 responses and stable disease in a further 6 patients. Moreover, CRM197 demonstrated reasonable bioavailability, and toxicity associated with the treatment was deemed acceptable. Although the effect of CRM197 in that study was modest, more recent demonstrations of enhanced bioactivity in combination with conventional chemotherapeutic agents (Yagi et al., 2009; Sanui et al., 2010) suggests CRM197 may still have utility as an anti-cancer agent. As noted earlier, cleavage of membrane-anchored proHB-EGF represents a major control point for regulation of HB-EGF bioactivity. Consequently, several groups have focused on this event as a means to inhibit HB-EGF-dependent regulation of tumor cell behavior. Fridman and colleagues described the identification of selective inhibitors that could prevent shedding of ERBB ligands *in vitro* and went on to demonstrate potent anti-tumor effects in a range of assays, including survival pathway activation and growth and survival of xenografts (Fridman et al., 2007). Although such inhibitors are inhibiting shedding of multiple EGF-like ligands, in addition to HB-EGF, these results suggest the potential for combined inhibition of ligand shedding and ERBB receptor activation with small molecule inhibitors. In addition to preventing release of soluble HB-EGF, pharmacological inhibition of MMP/ADAM activity using KB-R7785 also suppressed generation and nuclear translocation of the HB-EGF C-terminal fragment (Shimura et al., 2008). This resulted in growth arrest, induction of apoptosis and decreased expression of proliferation-associated genes.

Summary

HB-EGF plays multiple important roles in cancer, and has been implicated in each of the hallmarks characteristic of neoplastic disease. Recent work has emphasized the potential for HB-EGF as a viable therapeutic target for certain cancer types, through a variety of strategies. Moreover, its unique identity among ERBB ligands as a receptor for DT/CRM197 may provide novel avenues for rational targeting in the context of cancer therapeutics. The future challenge will be to realize this potential and to translate our knowledge of this inimitable molecule into treatments that benefit humankind.

References

- Mekada E, Uchida T. Binding properties of diphtheria toxin to cells are altered by mutation in the fragment A domain. *J Biol Chem*. 1985 Oct 5;260(22):12148-53
- Higashiyama S, Abraham JA, Miller J, Fiddes JC, Klagsbrun M. A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science*. 1991 Feb 22;251(4996):936-9
- Higashiyama S, Abraham JA, Klagsbrun M. Heparin-binding EGF-like growth factor stimulation of smooth muscle cell migration: dependence on interactions with cell surface heparan sulfate. *J Cell Biol*. 1993 Aug;122(4):933-40
- Blotnick S, Peoples GE, Freeman MR, Eberlein TJ, Klagsbrun M. T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD4+ and CD8+ T cells. *Proc Natl Acad Sci U S A*. 1994 Apr 12;91(8):2890-94
- Inui Y, Higashiyama S, Kawata S, Tamura S, Miyagawa J, Taniguchi N, Matsuzawa Y. Expression of heparin-binding epidermal growth factor in human hepatocellular carcinoma. *Gastroenterology*. 1994 Dec;107(6):1799-804
- Kobrin MS, Funatomi H, Friess H, Buchler MW, Stathis P, Korc M. Induction and expression of heparin-binding EGF-like growth factor in human pancreatic cancer. *Biochem Biophys Res Commun*. 1994 Aug 15;202(3):1705-9
- Higashiyama S, Iwamoto R, Goishi K, Raab G, Taniguchi N, Klagsbrun M, Mekada E. The membrane protein CD9/DRAP 27 potentiates the juxtacrine growth factor activity of the membrane-anchored heparin-binding EGF-like growth factor. *J Cell Biol*. 1995 Mar;128(5):929-38
- McCarthy SA, Samuels ML, Pritchard CA, Abraham JA, McMahon M. Rapid induction of heparin-binding epidermal growth factor/diphtheria toxin receptor expression by Raf and Ras oncogenes. *Genes Dev*. 1995 Aug 15;9(16):1953-64
- Mitamura T, Higashiyama S, Taniguchi N, Klagsbrun M, Mekada E. Diphtheria toxin binds to the epidermal growth factor (EGF)-like domain of human heparin-binding EGF-like growth factor/diphtheria toxin receptor and inhibits specifically its mitogenic activity. *J Biol Chem*. 1995 Jan 20;270(3):1015-9
- Nakamura K, Iwamoto R, Mekada E. Membrane-anchored heparin-binding EGF-like growth factor (HB-EGF) and diphtheria toxin receptor-associated protein (DRAP27)/CD9 form a complex with integrin alpha 3 beta 1 at cell-cell contact sites. *J Cell Biol*. 1995 Jun;129(6):1691-705
- Peoples GE, Blotnick S, Takahashi K, Freeman MR, Klagsbrun M, Eberlein TJ. T lymphocytes that infiltrate tumors and atherosclerotic plaques produce heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor: a potential pathologic role. *Proc Natl Acad Sci U S A*. 1995 Jul 3;92(14):6547-51
- Raab G, Kover K, Paria BC, Dey SK, Ezzell RM, Klagsbrun M. Mouse preimplantation blastocysts adhere to cells expressing the transmembrane form of heparin-binding EGF-like growth factor. *Development*. 1996 Feb;122(2):637-45
- Elenius K, Paul S, Allison G, Sun J, Klagsbrun M. Activation of HER4 by heparin-binding EGF-like growth factor stimulates chemotaxis but not proliferation. *EMBO J*. 1997 Mar 17;16(6):1268-78
- Miyoshi E, Higashiyama S, Nakagawa T, Hayashi N, Taniguchi N. Membrane-anchored heparin-binding epidermal growth factor-like growth factor acts as a tumor survival factor in a hepatoma cell line. *J Biol Chem*. 1997 May 30;272(22):14349-55
- Sakuma T, Higashiyama S, Hosoe S, Hayashi S, Taniguchi N. CD9 antigen interacts with heparin-binding EGF-like growth factor through its heparin-binding domain. *J Biochem*. 1997 Aug;122(2):474-80
- Suzuki M, Raab G, Moses MA, Fernandez CA, Klagsbrun M. Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site. *J Biol Chem*. 1997 Dec 12;272(50):31730-7
- Takemura T, Kondo S, Homma T, Sakai M, Harris RC. The membrane-bound form of heparin-binding epidermal growth factor-like growth factor promotes survival of cultured renal epithelial cells. *J Biol Chem*. 1997 Dec 5;272(49):31036-42
- Freeman MR, Paul S, Kaefer M, Ishikawa M, Adam RM, Renshaw AA, Elenius K, Klagsbrun M. Heparin-binding EGF-like growth factor in the human prostate: synthesis predominantly by interstitial and vascular smooth muscle cells and action as a carcinoma cell mitogen. *J Cell Biochem*. 1998 Mar 1;68(3):328-38
- Izumi Y, Hirata M, Hasuwa H, Iwamoto R, Umata T, Miyado K, Tamai Y, Kurisaki T, Sehara-Fujisawa A, Ohno S, Mekada E. A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J*. 1998 Dec 15;17(24):7260-72
- Mishima K, Higashiyama S, Asai A, Yamaoka K, Nagashima Y, Taniguchi N, Kitanaka C, Kirino T, Kuchino Y. Heparin-binding epidermal growth factor-like growth factor stimulates mitogenic signaling and is highly expressed in human malignant gliomas. *Acta Neuropathol*. 1998 Oct;96(4):322-8
- Adam RM, Borer JG, Williams J, Eastham JA, Loughlin KR, Freeman MR. Amphiregulin is coordinately expressed with heparin-binding epidermal growth factor-like growth factor in the interstitial smooth muscle of the human prostate. *Endocrinology*. 1999 Dec;140(12):5866-75
- Fu S, Bottoli I, Goller M, Vogt PK. Heparin-binding epidermal growth factor-like growth factor, a v-Jun target gene, induces oncogenic transformation. *Proc Natl Acad Sci U S A*. 1999 May 11;96(10):5716-21
- Paria BC, Elenius K, Klagsbrun M, Dey SK. Heparin-binding EGF-like growth factor interacts with mouse blastocysts independently of ErbB1: a possible role for heparan sulfate proteoglycans and ErbB4 in blastocyst implantation. *Development*. 1999 May;126(9):1997-2005
- Yeyati PL, Shaknovich R, Boterashvili S, Li J, Ball HJ, Waxman S, Nason-Burchenal K, Dmitrovsky E, Zelent A, Licht JD. Leukemia translocation protein PLZF inhibits cell growth and expression of cyclin A. *Oncogene*. 1999 Jan 28;18(4):925-34

- Cox ME, Deeb PD, Bissonette EA, Parsons SJ. Activated 3',5'-cyclic AMP-dependent protein kinase is sufficient to induce neuroendocrine-like differentiation of the LNCaP prostate tumor cell line. *J Biol Chem.* 2000 May 5;275(18):13812-8
- Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via transactivation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol.* 2000 Oct;14(10):1649-60
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* 2000 Jan 7;100(1):57-70
- Ito Y, Higashiyama S, Takeda T, Okada M, Matsuura N. Bimodal expression of heparin-binding EGF-like growth factor in colonic neoplasms. *Anticancer Res.* 2001 Mar-Apr;21(2B):1391-4
- Ito Y, Higashiyama S, Takeda T, Yamamoto Y, Wakasa KI, Matsuura N. Expression of heparin-binding epidermal growth factor-like growth factor in pancreatic adenocarcinoma. *Int J Pancreatol.* 2001;29(1):47-52
- Ito Y, Takeda T, Higashiyama S, Noguchi S, Matsuura N. Expression of heparin-binding epidermal growth factor-like growth factor in breast carcinoma. *Breast Cancer Res Treat.* 2001 May;67(1):81-5
- Lin J, Hutchinson L, Gaston SM, Raab G, Freeman MR. BAG-1 is a novel cytoplasmic binding partner of the membrane form of heparin-binding EGF-like growth factor: a unique role for proHB-EGF in cell survival regulation. *J Biol Chem.* 2001 Aug 10;276(32):30127-32
- Nishi E, Prat A, Hospital V, Elenius K, Klagsbrun M. N-arginine dibasic convertase is a specific receptor for heparin-binding EGF-like growth factor that mediates cell migration. *EMBO J.* 2001 Jul 2;20(13):3342-50
- Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2001 Feb;2(2):127-37
- Adam RM, Kim J, Lin J, Orsola A, Zhuang L, Rice DC, Freeman MR. Heparin-binding epidermal growth factor-like growth factor stimulates androgen-independent prostate tumor growth and antagonizes androgen receptor function. *Endocrinology.* 2002 Dec;143(12):4599-608
- Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, Ohmoto H, Node K, Yoshino K, Ishiguro H, Asanuma H, Sanada S, Matsumura Y, Takeda H, Beppu S, Tada M, Hori M, Higashiyama S. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med.* 2002 Jan;8(1):35-40
- Kim J, Adam RM, Freeman MR. Activation of the Erk mitogen-activated protein kinase pathway stimulates neuroendocrine differentiation in LNCaP cells independently of cell cycle withdrawal and STAT3 phosphorylation. *Cancer Res.* 2002 Mar 1;62(5):1549-54
- Murayama Y, Miyagawa J, Shinomura Y, Kanayama S, Isozaki K, Yamamori K, Mizuno H, Ishiguro S, Kiyohara T, Miyazaki Y, Taniguchi N, Higashiyama S, Matsuzawa Y. Significance of the association between heparin-binding epidermal growth factor-like growth factor and CD9 in human gastric cancer. *Int J Cancer.* 2002 Apr 1;98(4):505-13
- Weskamp G, Cai H, Brodie TA, Higashiyama S, Manova K, Ludwig T, Blobel CP. Mice lacking the metalloprotease-disintegrin MDC9 (ADAM9) have no evident major abnormalities during development or adult life. *Mol Cell Biol.* 2002 Mar;22(5):1537-44
- Yan Y, Shirakabe K, Werb Z. The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. *J Cell Biol.* 2002 Jul 22;158(2):221-6
- Yu WH, Woessner JF Jr, McNeish JD, Stamenkovic I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev.* 2002 Feb 1;16(3):307-23
- Adam RM, Danciu T, McLellan DL, Borer JG, Lin J, Zurakowski D, Weinstein MH, Rajjayabun PH, Mellon JK, Freeman MR. A nuclear form of the heparin-binding epidermal growth factor-like growth factor precursor is a feature of aggressive transitional cell carcinoma. *Cancer Res.* 2003 Jan 15;63(2):484-90
- Madarame J, Higashiyama S, Kiyota H, Madachi A, Toki F, Shimomura T, Tani N, Oishi Y, Matsuura N. Transactivation of epidermal growth factor receptor after heparin-binding epidermal growth factor-like growth factor shedding in the migration of prostate cancer cells promoted by bombesin. *Prostate.* 2003 Nov 1;57(3):187-95
- Mehta VB, Besner GE. Inhibition of NF-kappa B activation and its target genes by heparin-binding epidermal growth factor-like growth factor. *J Immunol.* 2003 Dec 1;171(11):6014-22
- Nanba D, Mammoto A, Hashimoto K, Higashiyama S. Proteolytic release of the carboxy-terminal fragment of proHB-EGF causes nuclear export of PLZF. *J Cell Biol.* 2003 Nov 10;163(3):489-502
- Buzzi S, Rubboli D, Buzzi G, Buzzi AM, Morisi C, Pironi F. CRM197 (nontoxic diphtheria toxin): effects on advanced cancer patients. *Cancer Immunol Immunother.* 2004 Nov;53(11):1041-8
- Laskin JJ, Sandler AB. Epidermal growth factor receptor: a promising target in solid tumours. *Cancer Treat Rev.* 2004 Feb;30(1):1-17
- Miyamoto S, Hirata M, Yamazaki A, Kageyama T, Hasuwa H, Mizushima H, Tanaka Y, Yagi H, Sonoda K, Kai M, Kanoh H, Nakano H, Mekada E. Heparin-binding EGF-like growth factor is a promising target for ovarian cancer therapy. *Cancer Res.* 2004 Aug 15;64(16):5720-7
- Nanba D, Toki F, Higashiyama S. Roles of charged amino acid residues in the cytoplasmic domain of proHB-EGF. *Biochem Biophys Res Commun.* 2004 Jul 23;320(2):376-82
- Ongusaha PP, Kwak JC, Zwible AJ, Macip S, Higashiyama S, Taniguchi N, Fang L, Lee SW. HB-EGF is a potent inducer of tumor growth and angiogenesis. *Cancer Res.* 2004 Aug 1;64(15):5283-90
- Sahin U, Weskamp G, Kelly K, Zhou HM, Higashiyama S, Peschon J, Hartmann D, Saftig P, Blobel CP. Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J Cell Biol.* 2004 Mar 1;164(5):769-79
- Schäfer B, Gschwind A, Ullrich A. Multiple G-protein-coupled receptor signals converge on the epidermal growth factor receptor to promote migration and invasion. *Oncogene.* 2004 Jan 29;23(4):991-9
- Schäfer B, Marg B, Gschwind A, Ullrich A. Distinct ADAM metalloproteinases regulate G protein-coupled receptor-induced cell proliferation and survival. *J Biol Chem.* 2004 Nov 12;279(46):47929-38
- Wang Q, Horiatis D, Pinski J. Interleukin-6 inhibits the growth of prostate cancer xenografts in mice by the process of neuroendocrine differentiation. *Int J Cancer.* 2004 Sep 10;111(4):508-13

- Yano S, Macleod RJ, Chattopadhyay N, Tfelt-Hansen J, Kifor O, Butters RR, Brown EM. Calcium-sensing receptor activation stimulates parathyroid hormone-related protein secretion in prostate cancer cells: role of epidermal growth factor receptor transactivation. *Bone*. 2004 Sep;35(3):664-72
- Cinar B, De Benedetti A, Freeman MR. Post-transcriptional regulation of the androgen receptor by Mammalian target of rapamycin. *Cancer Res*. 2005 Apr 1;65(7):2547-53
- Itoh Y, Joh T, Tanida S, Sasaki M, Kataoka H, Itoh K, Oshima T, Ogasawara N, Togawa S, Wada T, Kubota H, Mori Y, Ohara H, Nomura T, Higashiyama S, Itoh M. IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells. *Cytokine*. 2005 Mar 21;29(6):275-82
- Jimeno A, Hidalgo M. Blockade of epidermal growth factor receptor (EGFR) activity. *Crit Rev Oncol Hematol*. 2005 Mar;53(3):179-92
- Johnson FM, Saigal B, Donato NJ. Induction of heparin-binding EGF-like growth factor and activation of EGF receptor in imatinib mesylate-treated squamous carcinoma cells. *J Cell Physiol*. 2005 Nov;205(2):218-27
- Tanaka Y, Miyamoto S, Suzuki SO, Oki E, Yagi H, Sonoda K, Yamazaki A, Mizushima H, Maehara Y, Mekada E, Nakano H. Clinical significance of heparin-binding epidermal growth factor-like growth factor and a disintegrin and metalloprotease 17 expression in human ovarian cancer. *Clin Cancer Res*. 2005 Jul 1;11(13):4783-92
- Toki F, Nanba D, Matsuura N, Higashiyama S. Ectodomain shedding of membrane-anchored heparin-binding EGF like growth factor and subcellular localization of the C-terminal fragment in the cell cycle. *J Cell Physiol*. 2005 Mar;202(3):839-48
- Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol*. 2006 Jul;7(7):505-16
- Slovin SF. Neuroendocrine differentiation in prostate cancer: a sheep in wolf's clothing? *Nat Clin Pract Urol*. 2006 Mar;3(3):138-44
- Sorensen BS, Ornskov D, Nexø E. The chemotherapeutic agent VP16 increases the stability of HB-EGF mRNA by a mechanism involving the 3'-UTR. *Exp Cell Res*. 2006 Nov 1;312(18):3651-8
- Fridman JS, Caulder E, Hansbury M, Liu X, Yang G, Wang Q, Lo Y, Zhou BB, Pan M, Thomas SM, Grandis JR, Zhuo J, Yao W, Newton RC, Friedman SM, Scherle PA, Vaddi K. Selective inhibition of ADAM metalloproteases as a novel approach for modulating ErbB pathways in cancer. *Clin Cancer Res*. 2007 Mar 15;13(6):1892-902
- Kageyama T, Ohishi M, Miyamoto S, Mizushima H, Iwamoto R, Mekada E. Diphtheria toxin mutant CRM197 possesses weak EF2-ADP-ribosyl activity that potentiates its anti-tumorigenic activity. *J Biochem*. 2007 Jul;142(1):95-104
- Kinugasa Y, Hieda M, Hori M, Higashiyama S. The carboxyl-terminal fragment of pro-HB-EGF reverses Bcl6-mediated gene repression. *J Biol Chem*. 2007 May 18;282(20):14797-806
- Kramer C, Klasmeyer K, Bojar H, Schulz WA, Ackermann R, Grimm MO. Heparin-binding epidermal growth factor-like growth factor isoforms and epidermal growth factor receptor/ErbB1 expression in bladder cancer and their relation to clinical outcome. *Cancer*. 2007 May 15;109(10):2016-24
- Rocourt DV, Mehta VB, Besner GE. Heparin-binding EGF-like growth factor decreases inflammatory cytokine expression after intestinal ischemia/reperfusion injury. *J Surg Res*. 2007 May 15;139(2):269-73
- Singh AB, Sugimoto K, Harris RC. Juxtacrine activation of epidermal growth factor (EGF) receptor by membrane-anchored heparin-binding EGF-like growth factor protects epithelial cells from anoikis while maintaining an epithelial phenotype. *J Biol Chem*. 2007 Nov 9;282(45):32890-901
- Wang F, Liu R, Lee SW, Sloss CM, Couget J, Cusack JC. Heparin-binding EGF-like growth factor is an early response gene to chemotherapy and contributes to chemotherapy resistance. *Oncogene*. 2007 Mar 29;26(14):2006-16
- Wang F, Sloss C, Zhang X, Lee SW, Cusack JC. Membrane-bound heparin-binding epidermal growth factor like growth factor regulates E-cadherin expression in pancreatic carcinoma cells. *Cancer Res*. 2007 Sep 15;67(18):8486-93
- Cáceres M, Tobar N, Guerrero J, Smith PC, Martínez J. c-jun-NH2JNK mediates invasive potential and EGFR activation by regulating the expression of HB-EGF in a urokinase-stimulated pathway. *J Cell Biochem*. 2008 Feb 15;103(3):986-93
- Higashiyama S, Iwabuki H, Morimoto C, Hieda M, Inoue H, Matsushita N. Membrane-anchored growth factors, the epidermal growth factor family: beyond receptor ligands. *Cancer Sci*. 2008 Feb;99(2):214-20
- Murphy G. The ADAMs: signalling scissors in the tumour microenvironment. *Nat Rev Cancer*. 2008 Dec;8(12):929-41
- Ogunwobi OO, Beales IL. Leptin stimulates the proliferation of human oesophageal adenocarcinoma cells via HB-EGF and Tgfalpha mediated transactivation of the epidermal growth factor receptor. *Br J Biomed Sci*. 2008;65(3):121-7
- Sagmeister S, Drucker C, Losert A, Grusch M, Daryabeigi A, Parzefall W, Rohr-Udilova N, Bichler C, Smedsrød B, Kandioler D, Grünberger T, Wrba F, Schulte-Hermann R, Grasl-Kraupp B. HB-EGF is a paracrine growth stimulator for early tumor prestages in inflammation-associated hepatocarcinogenesis. *J Hepatol*. 2008 Dec;49(6):955-64
- Shimura T, Kataoka H, Ogasawara N, Kubota E, Sasaki M, Tanida S, Joh T. Suppression of proHB-EGF carboxy-terminal fragment nuclear translocation: a new molecular target therapy for gastric cancer. *Clin Cancer Res*. 2008 Jun 15;14(12):3956-65
- Yagi H, Yotsumoto F, Miyamoto S. Heparin-binding epidermal growth factor-like growth factor promotes transcoelomic metastasis in ovarian cancer through epithelial-mesenchymal transition. *Mol Cancer Ther*. 2008 Oct;7(10):3441-51
- Bishop-Bailey D. Tumour vascularisation: a druggable target. *Curr Opin Pharmacol*. 2009 Apr;9(2):96-101
- Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009 Jul;30(7):1073-81
- Hirata Y, Ogasawara N, Sasaki M, Mizushima T, Shimura T, Mizoshita T, Mori Y, Kubota E, Wada T, Tanida S, Kataoka H, Kamiya T, Higashiyama S, Joh T. BCL6 degradation caused by the interaction with the C-terminus of pro-HB-EGF induces cyclin D2 expression in gastric cancers. *Br J Cancer*. 2009 Apr 21;100(8):1320-9
- Martarelli D, Pompei P, Mazzoni G. Inhibition of adrenocortical carcinoma by diphtheria toxin mutant CRM197. *Chemotherapy*. 2009;55(6):425-32
- Ray KC, Blaine SA, Washington MK, Braun AH, Singh AB, Harris RC, Harding PA, Coffey RJ, Means AL. Transmembrane and soluble isoforms of heparin-binding epidermal growth factor-like growth factor regulate distinct processes in the pancreas. *Gastroenterology*. 2009 Nov;137(5):1785-94

Wilson KJ, Gilmore JL, Foley J, Lemmon MA, Riese DJ 2nd. Functional selectivity of EGF family peptide growth factors: implications for cancer. *Pharmacol Ther.* 2009 Apr;122(1):1-8

Yagi H, Yotsumoto F, Sonoda K, Kuroki M, Mekada E, Miyamoto S. Synergistic anti-tumor effect of paclitaxel with CRM197, an inhibitor of HB-EGF, in ovarian cancer. *Int J Cancer.* 2009 Mar 15;124(6):1429-39

Ebi M, Kataoka H, Shimura T, Kubota E, Hirata Y, Mizushima T, Mizoshita T, Tanaka M, Mabuchi M, Tsukamoto H, Tanida S, Kamiya T, Higashiyama S, Joh T. TGF β induces proHB-EGF shedding and EGFR transactivation through ADAM activation in gastric cancer cells. *Biochem Biophys Res Commun.* 2010 Nov 19;402(3):449-54

Nolan-Stevaux O, Truitt MC, Pahler JC, Olson P, Guinto C, Lee DC, Hanahan D. Differential Contribution to Neuroendocrine Tumorigenesis of Parallel Egfr Signaling in Cancer Cells and Pericytes. *Genes Cancer.* 2010 Apr;1(2):125-141

Rigo A, Gottardi M, Zamò A, Mauri P, Bonifacio M, Krampera M, Damiani E, Pizzolo G, Vinante F. Macrophages may

promote cancer growth via a GM-CSF/HB-EGF paracrine loop that is enhanced by CXCL12. *Mol Cancer.* 2010 Oct 14;9:273

Sanui A, Yotsumoto F, Tsujioka H, Fukami T, Horiuchi S, Shirota K, Yoshizato T, Kawarabayashi T, Kuroki M, Miyamoto S. HB-EGF inhibition in combination with various anticancer agents enhances its antitumor effects in gastric cancer. *Anticancer Res.* 2010 Aug;30(8):3143-9

Yin Y, Grabowska AM, Clarke PA, Whelband E, Robinson K, Argent RH, Tobias A, Kumari R, Atherton JC, Watson SA. Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7. *Gut.* 2010 Aug;59(8):1037-45

Yotsumoto F, Oki E, Tokunaga E, Maehara Y, Kuroki M, Miyamoto S. HB-EGF orchestrates the complex signals involved in triple-negative and trastuzumab-resistant breast cancer. *Int J Cancer.* 2010 Dec 1;127(11):2707-17

This article should be referenced as such:

Adam RM. Role of HB-EGF in cancer. *Atlas Genet Cytogenet Oncol Haematol.* 2011; 15(7):610-619.
