HB-EGF Is a Potent Inducer of Tumor Growth and Angiogenesis

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ABSTRACT

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) has been shown to stimulate the growth of a variety of cells in an autocrine or paracrine manner. Although HB-EGF is widely expressed in tumors compared with normal tissue, its contribution to tumorigenicity is unknown. HB-EGF can be produced as a membrane-anchored form (pro-HB-EGF) and later processed to a soluble form (s-HB-EGF), although a significant amount of pro-HB-EGF remains uncleaved on the cell surface. To understand the roles of two forms of HB-EGF in promoting tumor growth, we have studied the effects of HB-EGF expression in the process of tumorigenesis using in vitro and in vivo systems. We demonstrate that in EJ human bladder cancer cells containing a tetracycline-regulatable s-HB-EGF or pro-HB-EGF expression system, s-HB-EGF expression increased their transformed phenotypes, including growth rate, colony-forming ability, and activation of cyclin D1 promoter, as well as induction of vascular endothelial growth factor in vitro. Moreover, s-HB-EGF or wild-type HB-EGF induced the expression and activities of the metalloproteases, MMP-9 and MMP-3, leading to enhanced cell migration. In vivo studies also demonstrated that tumor cells expressing s-HB-EGF or wild-type HB-EGF significantly enhanced tumorigenic potential in athymic nude mice and exerted an angiogenic effect, increasing the density and size of tumor blood vessels. However, cells expressing solely pro-HB-EGF did not exhibit any significant tumorigenic potential. These findings establish s-HB-EGF as a potent inducer of tumor growth and angiogenesis and suggest that therapeutic intervention aimed at the inhibition of s-HB-EGF functions may be useful in cancer treatment.

INTRODUCTION

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a heparin-binding member of the EGF family (1), which was initially identified in the conditional medium of human macrophages (2). It is a potent mitogen and chemotactic factor for fibroblasts and smooth muscle cells (3–5). As with other EGF family members, HB-EGF binds and activates EGF receptors 1 and 4 (1, 6, 7). Moreover, HB-EGF has been shown to stimulate the growth of a variety of cells in an autocrine or paracrine manner and to be involved in stromal proliferation (8). HB-EGF is initially synthesized as a transmembrane protein of 208 amino acids (1). Although the membrane-anchored form of HB-EGF (pro-HB-EGF) is cleaved on the cell surface to yield a soluble growth factor of 75–86 amino acids, a considerable amount of pro-HB-EGF remains uncleaved on the cell surface (1). Pro-HB-EGF is not merely a precursor of the soluble form; it is biologically active such that it forms complexes with both CD9 (9) and integrin α5β1 (10) and transduces biological signals to neighboring cells in a nondiffusible manner (7). The transmembrane form of HB-EGF is a juxtacrine growth factor, which is immobilized on the surface of the cell and interacts with neighboring cells (1). It has also been shown that the transmembrane HB-EGF synthesized by one type of cell can stimulate tyrosine phosphorylation of the EGF receptor in another type of cell in coculture (11). A recent study demonstrated that HB-EGF can bind to a novel 140-kDa receptor identified as N-arginine dibasic convertase, a metalloendopeptidase of the M16 family, and that binding to N-arginine dibasic convertase is highly specific for HB-EGF among EGF family members (12). Its specific binding modulates HB-EGF-induced cell migration via EGF receptor (12).

Several laboratories have described HB-EGF as being up-regulated in response to oncogenes and in oncogene-transformed cells (13, 14). In chicken embryo fibroblasts transfected with regulatable v-Jun, the expression of HB-EGF is greatly induced as v-Jun levels are increased (15), suggesting that HB-EGF plays an important part in mediating Jun-induced cell transformation. Furthermore, HB-EGF has been identified as an immediate-early response gene that can be activated by the Ras/Raf signaling pathway that mediates the autocrine activation of the c-Jun kinase in NIH3T3 cells (15). Phosphorylation of the transcription factor Ets-2 by activation of the Raf1/MAPK cascade regulates the induction of HB-EGF transcription in mouse fibroblasts (16). Additionally, in nontransformed human mammary epithelial cells, HB-EGF expression is induced by EGF and Ha-Ras overexpression (17), strongly implying that HB-EGF is a direct target of mitogen-activated protein kinase (MAPK). Our previous observations demonstrated that HB-EGF is induced in response to tumor suppressor p53, as well as DNA damage, and HB-EGF induction antagonizes apoptosis mediated by genotoxic stress through the activation of the Ras/Raf/MAPK cascade and the AKT pathway (18, 19), strongly suggesting a survival factor function for HB-EGF.

HB-EGF expression has been implicated in tumor progression because of its overexpression in many tumors, including hepatocarcinoma, colon, melanoma, myeloma, breast, prostate, and bladder tumors. It also has been implicated in increased proliferation and metastasis (20–27). Many tumor cells with HB-EGF overexpression are diphtheria toxin sensitive, suggesting that these cells accumulate the transmembrane form of HB-EGF. There is growing evidence of increased HB-EGF expression in tumors compared with normal tissue, e.g., pancreatic (28), liver (29), esophageal (30), melanoma (20), bladder (31), and gastric tumors (32). Although HB-EGF is widely expressed in tumors and may be enhanced compared with normal tissue, its contribution to tumorigenicity is unknown. Moreover, it is not known whether the membrane-bound or the soluble form of HB-EGF contributes to tumorigenic processes.

In this study, we examined the biological effects of the two forms of HB-EGF on tumor growth and angiogenesis, using a tetracycline (tet)-regulated expression system in human EJ bladder carcinoma cells, which have a low basal level of HB-EGF. EJ cells expressing soluble form HB-EGF (s-HB-EGF) or wild-type HB-EGF (wt-HB-EGF) resulted in an increased growth rate, activation of the cyclin D promoter, colony-forming ability, and tumor growth in athymic nude mice. We also found that s-HB-EGF induced vascular endothelial growth factor (VEGF) expression, implicating an autocrine loop that may play a role in regulating these growth factors. Additionally, in response to s-HB-EGF, cells induced the expression and activities of the metalloproteases MMP-9 and MMP-3, leading to enhanced cell

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migration. In this study, we provide evidence of the biological activity of HB-EGF in tumorigenesis.

MATERIALS AND METHODS

Cell Culture and Transfection of Different Forms of HB-EGFs. Human bladder carcinoma cell line, EJ, expressing a tet activator, was transfected with wt-, s-, or pro-HB-EGF cDNA/pTet-puro and selected by continuous growth in puromycin (2 μg/ml) to isolate stable tet-regulated clones. Mutant constructs and expression of s-HB-EGF and pro-HB-EGF were described previously (33). Tet-regulatable EJ-HB-EGF cells were maintained in the presence of tet (1–2 μg/ml) in DMEM plus 10% FBS, 75 μg/ml hygromycin, and 2 μg/ml puromycin. Induction of each form of HB-EGF expression was achieved by washing the cells three times with PBS followed by addition of culture media without tet. For zymograms for MMP activity, cell culture media was concentrated 50-fold and loaded on to a 10% zymogram gel (Invitrogen, Carlsbad, CA). The MMP gel was renatured by incubating in renaturing buffer (Invitrogen) for 30 min at room temperature, and the MMP activity was examined by incubating in developing buffer (Invitrogen) for 24 h. MMP bands were visualized by simply blue dye reagent (Invitrogen). The concentrated culture media were also immunoblotted with the mouse anti-MMP-9 and anti-MMP-3 monoclonal antibodies (Neomarkers, Fremont, CA). For cell proliferation assay, three different EJ-HB-EGF cell lines expressing wt-HB-EGF, s-HB-EGF, or pro-HB-EGF, respectively, were seeded on 6-well plates with or without tet at a density of 2 × 10^4 cells/well. At days 2, 4, 6, and 8, cells were trypsinized, stained with trypan blue, and counted using a hemocytometer. Each data point was the average of three independent experiments.

Northern Blot and Western Blot Analyses. Total RNA was extracted, denatured, and electrophoresed through a 1% agarose-formaldehyde gel as described previously (19). Total protein extracts were immunoblotted with the respective antibodies. Tumors were dissected from the mice and snap frozen by liquid nitrogen. The tumors were then homogenized in lysis buffer containing 1% Triton X-100, 10 mM HEPES (pH 7.5), 130 mM NaCl, 5 mM EDTA, 10 mM Na_2PO_4, 100 mM NaF, 2 mM NaVO_4, 1 mM phenylmethylsulfonyl fluoride, 50 μg/ml leupeptin, and 50 μg/ml aprotinin. Twenty μg of proteins were loaded into each well and separated by a 4–12% NuPAGE gel (Invitrogen).

Transwell Migration Assay. The cells were plated at a cell density of 2 × 10^5 cells in a 100-mm diameter dish. Each form of HB-EGF was induced by the removal of tet for 24 h, followed by serum starvation for another 24 h. Control cells were plated in similar conditions as HB-EGF-expressing cells, except that tet was added to suppress HB-EGF expression. Migration assays were performed using a chemotaxis chamber (Becton Dickinson and Co., Franklin Lakes, NJ) and transwell tissue culture plates (6.5 mm and 8-μm pore size). The bottom of the chamber was coated with either 10 μg/ml fibronectin, collagen I, or Matrigel (Sigma, St. Louis, MO). The uncoated sites were blocked with 10% BSA. One-hundred μl of 1 × 10^5 cells/ml were introduced into each well and were allowed to migrate for 6 h. Cells were then fixed with methanol and stained with crystal violet. The migrated cells were quantified by counting the number of cells in five random ×200 fields. Each experiment was done in triplicates, and the experiment was repeated twice.

Wound-Healing Assay. HB-EGF was expressed for 24 h in the absence of tet, and control cells were seeded in the same fashion, except in the presence of tet. Cells were then seeded in a 6-well plate at a density of 1 × 10^6 cells in the presence or absence of tet for 24 h. A wound was made using the tip of a
HB-EGF as a potent inducer of tumor growth

RESULTS

Effects of HB-EGF on the Transformed Phenotype. To elucidate the role of HB-EGF overexpression in the process of tumorigenesis in human cancer cells, a tet-regulated HB-EGF-inducible system was generated in EJ cells that contain a low basal level of HB-EGF. Three different forms (wt-, s-, and pro-HB-EGF) of HB-EGF expression induced by the removal of tet resulted in an increase of each form of HB-EGF (Fig. 1A). Using this system, we first examined the role of HB-EGF in cell proliferation. The growth rate of EJ-HB-EGF cells was measured in the presence or absence of tet at 1 × 10^6 cells in 6-well plates for up to 8 days. Induction of wt- or s-HB-EGF by removal of tet resulted in a substantial growth rate increase, as seen by the 75–100% increase, when compared with HB-EGF-repressed cells (tet+; Fig. 1B). However, pro-HB-EGF induction in EJ cells resulted in a slight increase of growth rate by ~25%. To examine the effect of HB-EGF on cell colony formation ability, EJ-HB-EGF cells expressing three different types of HB-EGF were seeded at 200 cells/100-mm diameter dish. Cells expressing s- or wt-HB-EGF produced approximately two to three times more colonies than that of pro-HB-EGF-induced cells (Fig. 1C). We next evaluated the effect of each HB-EGF on colony formation in soft agarose as an additional measure of tumorigenicity in vitro using tet-regulated EJ-HB-EGF cells. The results of agar colony tests are shown in Fig. 1D. Soluble- or wt-HB-EGF (tet-) inducing cells significantly stimulated anchorage-independent growth, but anchorage-independent growth was not seen in pro-HB-EGF-induced cells. A colony formation assay in soft agarose was also used to examine whether HB-EGF could promote a transformed phenotype in a different cell line using HeLa cells. Transfection of HeLa cells with wt- or s-HB-EGF following a selection under G418 significantly increased sizes of colonies in soft agarose as compared with those in cells transfected with vector alone or a plasmid expressing pro-HB-EGF (Fig. 1E). Taken together, these results suggest that s-HB-EGF expression causes an enhanced transformed phenotype in vitro but pro-HB-EGF overexpression does not.

s-HB-EGF Elevates Cyclin D Expression via Cyclin D Promoter. It is well known that the signaling pathways of oncoproteins, including ErbB2 and Ras, can up-regulate cyclin D expression. Recent analysis of the cell lysates with antibodies against HB-EGF, cyclin D1, and β-actin (loading control). For activation of cyclin D1 promoter by HB-EGF, EJ cells were transiently cotransfected with the cyclin D1 promoter reporter construct (~1745C1) and three different forms of HB-EGF [wt-HB-EGF, s-HB-EGF, and membrane-anchored form (pro)-HB-EGF] in expression vector, respectively, or the empty vector then assayed for luciferase activity. pRL-TK Renilla luciferase reporter construct was cotransfected with each sample to normalize transfection efficiency. All results are expressed as x ± SD of three independent experiments with duplicates.
studies demonstrate that HB-EGF activates MAPK cascade through the activation of the Ras/Raf pathway (34). Therefore, we tested whether HB-EGF had any effect on cyclin D expression. To examine this possibility, we transiently transfected three different HB-EGF expression constructs, respectively, into EJ cells and then examined the effects on endogenous cyclin D1 levels. Forced expression of s- or wt-HB-EGF led to a 3-fold increase in cyclin D1 protein levels, whereas pro-HB-EGF did not result in an increase in cyclin D expression. (Fig. 2A). To determine whether HB-EGF regulates the transcription of cyclin D1, we also measured the effects of HB-EGFs on the cyclin D1 promoter using a cyclin D1-luciferase reporter construct (−1745CD1; Ref. 35). The reporter containing the cyclin D1 promoter was strongly activated in response to the expression of s- or wt-HB-EGF in EJ cells and 293T cells (Fig. 2B), whereas pro-HB-EGF did not show any significant effect on cyclin D promoter activity, regardless of serum presence. These results indicate that the activation of the cyclin D promoter and the −1745CD1 promoter fragment retains complete responsiveness to s-HB-EGF.

HB-EGF Overexpression Promotes Cancer Cell Growth in Vivo. To determine whether HB-EGF increases tumorigenicity in vivo, xenograft studies were conducted using EJ cells expressing s-HB-EGF, wt-HB-EGF, and pro-HB-EGF, respectively, and control cells expressing CAT (EJ-CAT) in the absence or presence of dox. Six mice per each HB-EGF form were injected with cells (2 × 10⁶ cells mixed with Matrigel). As shown in Fig. 3, A and B, EJ cells expressing s- or wt-HB-EGF showed an increased overall tumor size when injected into nude athymic mice (EJ-HB-EGF tumors in −tet condition), compared with EJ cells expressing a control CAT protein (EJ-CAT tumors in −tet and +tet) and EJ-HB-EGF cells in tet+ conditions. The mean size of EJ-HB-EGF tumors maintained in the absence of dox was 1.5 g, significantly larger than tumors from control mice, ∼0.1 g (Fig. 3C). However, tumors expressing pro-HB-EGF were similar to those of control mice. Data from the in vitro tumorigenicity assays and the xenograft assays support the conclusion that s-HB-EGF exerts a potent oncogenic potential but pro-HB-EGF does not.

HB-EGF Overexpression in Bladder Carcinomas Up-Regulates VEGF and Enhances Tumor Angiogenesis in Mice. The growth of tumors beyond a minimal size has been hypothesized to be dependent upon the induction of new blood vessel growth or angiogenesis, which in turn supplies needed nutrients to rapidly dividing tumor cells (36). Several studies indicated that HB-EGF can be up-regulated in some pathological states that may involve angiogenesis (37, 38). Some studies showed that VEGF, a critical factor in the development of new blood vessels, could induce HB-EGF in vascular endothelial cells and speculated that HB-EGF induction by VEGF may act in a paracrine fashion to promote angiogenesis (39). This prompted us to evaluate
the effect of HB-EGF on the existence of a growth factor-mediated autocrine response, possibly involving VEGF up-regulation. Northern blot analysis was performed with total RNA from EJ cells with a tet-regulated HB-EGF-inducible system. Soluble-HB-EGF or wt-HB-EGF expression by removal of tet resulted in an increase of VEGF mRNA (Fig. 4A), but no increase of VEGF mRNA by pro-HB-EGF induction in EJ-pro-HB-EGF cells was seen. These results implicate the up-regulation of HB-EGF as an angiogenesis-related change, occurring through an autocrine response, which is closely associated with tumorigenesis of epithelial cells.

Next, we examined the effect of HB-EGFs on blood vessel growth/angiogenesis in mice. Three weeks after injection, tumors harvested from control and three forms of HB-EGF-expressing mice in the xenograft studies (Fig. 3) were first homogenized and examined for HB-EGF expression by Western blotting. C, immunostaining of CD31. Immunostaining of anti-CD31 (PECAM-1) monoclonal antibody demonstrated rarefaction of tumor blood vessels in HB-EGF overexpressing tumors as compared with control tumors (+/-CAT and +/dox). Bar = 100 μm. CD31-stained blood vessels were evaluated in three different ×10 fields obtained from five different tumors for each condition. Right panel represents quantitative computer-assisted image analysis that revealed a significant increase of angiogenesis in soluble (s)- or wild-type (wt)-HB-EGF-overexpressing EJ tumors but not in membrane-anchored form (pro)-HB-EGF tumors, as measured by the number of blood vessels/mm² tumor area.

were determined by computer-assisted image analysis of representative digital images. The relative tumor area occupied by vessels increased ~2-3-folds in s- or wt-HB-EGF-expressing tumors (Fig. 4C, right panel).

Fig. 5. Up-regulation of metalloprotease (MMP)-9 and MMP-3 in response to heparin-binding epidermal growth factor-like growth factor (HB-EGF) induction. EJ-HB-EGF cells expressing soluble (s)-, wild-type (wt)-, and membrane-anchored form (pro)-HB-EGF were grown in the presence or absence of tetracycline (tet) for 48 h, respectively, then analyzed by zymography for MMP-9 and MMP-3 activities. Expression levels of MMP-9 and MMP-3 were also determined by Western blotting. Ponceau staining shows the loading levels of total proteins.
Up-Regulation of the MMP by HB-EGF. It is well documented that the up-regulation of MMP activities results in the proteolytic degradation of the extracellular matrix and the basement membrane, which promotes tumor growth and metastasis (41). To better evaluate the participation of HB-EGF in tumor migration and progression, we tested the effects of HB-EGF on MMP-9 activation. As shown in Fig. 5, HB-EGF induction after tet removal activated the 92-kDa pro-MMP-9 zymogen to the 67-kDa active form. The processing/activation was analyzed by zymography analysis. MMP-9 expression level was also determined by Northern and Western blotting, which showed that MMP-9 levels correlated with an increase of HB-EGF levels (Fig. 5). MMP-3, also known as a stromelysin-1, is the most efficient activator of MMP-9 and MMP-3 can be enhanced in response to HB-EGF induction. However, MMP-3 activation was not observed in response to pro-HB-EGF overexpression (data not shown). These results demonstrate that the expression level and activity of MMP-9 and MMP-3 can be enhanced in response to s-HB-EGF in EJ cells.

HB-EGF Effect on Migration and Wound Healing. We next investigated whether HB-EGF might be involved in extracellular matrix interactions in EJ cells and cause an increase in the migration of cells expressing HB-EGF because HB-EGF has been shown to be chemotactic (1, 12, 42) and to mediate migration in some cell types (12). Tet-regulated EJ-HB-EGF cells were used for migration assays in a transwell migration assay (haptotaxis). We tested for their ability to migrate through extracellular matrices, including fibronectin, collagen 1, and Matrigel. As shown in Fig. 6A, HB-EGF expression in EJ cells after the removal of tet showed a strong increase in migration on all three substrates but not in cells grown in the presence of tet. Although s-HB-EGF and wt-HB-EGF were more effective, pro-HB-EGF expression also increased cell migration on all three matrices.

It is well established that successful wound healing involves a number of processes, including cell proliferation, cell migration, vascular permeability, and angiogenesis (43). HB-EGF is known to be up-regulated in the wound-healing process of certain cell types, including keratinocytes (44, 45). Because HB-EGF expression triggers tumor angiogenesis and cell migration in EJ cells, we studied its effect on wound healing using the same cell system. One × 10^6 EJ-HB-EGF cells of each form (EJ-wt-HB-EGF, EJ-s-HB-EGF, and pro-HB-EGF) were seeded in a 6-well plate. Then, the cells were grown with or without tet for 24 h. A wound was introduced by the use of a pipette tip. After 32 h, the cells were fixed, and pictures were taken at different regions of the wound (Fig. 6C). Soluble- or wt-HB-EGF expression (−tet condition) increased the effectiveness of wound healing, but no significant effect was seen in pro-HB-EGF-expressing cells.

DISCUSSION

In this article, we provide evidence that HB-EGF exerts oncogenic potentials in vitro and in vivo. We found that overexpression of HB-EGF enhanced the transformed phenotype in vitro as determined by cell proliferation, anchorage-independent growth, and foci formation assays. We also showed that HB-EGF can activate the cyclin D promoter. We established a tet-regulated HB-EGF expression system using three different forms of HB-EGF in EJ human bladder cancer cells to test the tumorigenicity in vitro and in vivo using a xenograft assay. Inducible overexpression of s- or wt-HB-EGF caused increased...
tumor formation in mice, supporting the conclusion that s-HB-EGF does not function as a membrane-bound form of HB-EGF, indicating that HB-EGF does not exert oncogenic potential. This conclusion is supported by previous studies that pro-HB-EGF may act as a negative regulator of cell proliferation (46).

HB-EGF is known as a potent mitogen for keratinocytes, hepatocytes, smooth muscle cells, and fibroblasts (2, 29, 44). HB-EGF expression is elevated in human cancers, including hepatocellular and gastric carcinoma, breast carcinoma, melanoma, colon cancer, pancreatic cancer, glioma, and glioblastoma (23, 24). Furthermore, HB-EGF is induced in NIH3T3 cells transformed by v-Ras or v-Raf (34). HB-EGF was identified as a target of v-Jun, a potent oncogene, and HB-EGF plays a role in v-Jun-mediated oncogenic transformation (15). The significance of HB-EGF overexpression in tumorigenesis is also supported by our findings that HB-EGF activates the cyclin D promoter, implying that v-Jun could stimulate cyclin D transcription through HB-EGF induction. We have previously reported the identification of HB-EGF as a p53 downstream target gene, and p53 induction of HB-EGF could activate cell survival signaling, including AKT and MAPK cascades (19). A recent article has demonstrated that inducible expression of oncogenic Raf in normal epithelial cells strongly induced autocrine expression of HB-EGF, transforming growth factor α, and amphiregulin, which were directly implicated in the ability of sustained Raf/MAPK pathway stimulation to protect cells from apoptosis (47).

It is now well established that VEGF is an endothelial cell-specific, multifunctional growth factor that plays a major role in the initiation of angiogenesis by acting directly as a mitogenic and chemotactic factor (48). Although HB-EGF is known to have no effect on the proliferation of endothelial cells, some studies demonstrated that VEGF induces expression of angiogenic growth factors, including HB-EGF, in vascular endothelial cells and suggested that HB-EGF induction in response to VEGF provides a critical endothelial cell-derived signal, perhaps the activation of the MAPK and AKT cascade, for the process of new blood vessel formation and maturation (39). In addition, the reviews have proposed that HB-EGF acts as one of the recruiting signals for mesenchymal cells during the late phase of angiogenesis (49), implying that HB-EGF plays a critical role in angiogenesis as well as tumor development. In this study, we also demonstrate that s- or wt-HB-EGF induces VEGF expression, whereas it inhibits thrombospondin-1 expression (data not shown), implying the presence of an autocrine growth factor stimulation. Moreover, HB-EGF promotes tumor angiogenesis in vivo and exerts the migration-stimulating and wound-healing potential for cancer cells.

It is now clear that the up-regulation of MMP activities results in the proteolytic degradation of the extracellular matrix and the basement membrane, which promotes tumor growth, angiogenesis, and metastasis (41). It is possible that the effect of HB-EGF on the functional interaction between VEGF and MMP-9 is a key mechanism for initiation and maintenance of angiogenesis. In our cell system, we found a significant difference in the levels of active MMP-9 and MMP-3 in s-HB-EGF-induced cells. Moreover, recent studies clearly demonstrated that MMP-9 mediates the release and accumulation of VEGF from the cell matrix and that MMP-9 triggers the angiogenic switch (50). The generation of HB-EGF overexpressing mice or a targeted deletion of HB-EGF should provide additional understanding of mechanisms underlying HB-EGF-associated functions in angiogenesis.

In summary, our findings provide evidence that HB-EGF enhances transformed phenotypes and is associated with the stimulation of MMP-9, MMP-3, and cyclin D activation, which promotes tumorigenesis and angiogenesis. Given its elevated expression in human cancers along with our findings of HB-EGF contribution to enhanced transformed phenotypes, we hypothesize that HB-EGF behavior as an oncogene and, as such, could have importance as a therapeutic target.

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