REVIEW

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HEPARIN-BINDING EGF-LIKE GROWTH FACTOR: MECHANISMS OF BIOLOGICAL ACTIVITY AND POTENTIAL THERAPEUTIC APPLICATIONS

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The diphtheria toxin receptor on sensitive mammalian cells is known as the membrane anchored precursor of heparin-binding EGF-like growth factor (HB-EGF). When the precursor is cleaved by metalloproteinases, a soluble form (sHB-EGF) is formed that can bind to the EGF receptors, resulting in activation of signaling pathways that regulate cell proliferation, differentiation, migration, and inhibition of apoptosis. The ability of HB-EGF to cause both positive and negative consequences for organism underscores the complexity of its biological functions and the need for a nuanced understanding of its role in health and disease. In this review the data on the HB-EGF structure, biological activity, involvement in the mechanism of diphtheria toxin action, wound healing, tumor progression as well as the methods of HB-EGF delivery are summarized.

K e y w o r d s: heparin-binding EGF-like growth factor, diphtheria toxin, EGF receptor, signal transduction, cell proliferation, wound healing.

Epidermal growth factor (EGF) is a protein that plays a key role in the regulation of cell growth, proliferation, and differentiation. It is a crucial component in various physiological processes, including tissue development, wound healing, and the maintenance of epithelial tissues [1].

The EGF family includes the following protein compounds in addition to EGF itself: heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor-α (TGF-α), amphiregulin (AR), epiregulin (EPR), epigen, betacellulin (BTC), neuregulin-1 (NRG1), neuregulin-2 (NRG2), neuregulin-3 (NRG3), and neuregulin-4 (NRG4). Members of this protein family have very similar structural and functional characteristics [2].

Heparin-binding epidermal growth factor (HB-EGF) was first identified in cultures of human monocytes and macrophages. In 1991/92, it was described by Higashiyama as a highly glycosylated member of the EGF family and was purified from the conditioned medium of the human myeloid leukemia cell line U937 using heparin-affinity chromatography. Since HB-EGF is structurally a member of the EGF family, it is expected to have biological properties characteristic of EGF [3].

The biological functions of membrane-bound and soluble HB-EGF are multifaceted and significant in various physiological and pathological processes. Foremost, HB-EGF is involved in the development and functioning of heart, skin and liver regeneration, blastocyst implantation, smooth muscle cell hyperplasia, tumor progression, pulmonary hypertension and neurodegenerative diseases [4]. This wide spectrum of biological activities makes HB-EGF and its receptors promising targets for the creation of new drugs for different therapeutic purposes.

HB-EGF is synthesized in the form of a membrane-anchored proHB-EGF precursor capable of forming a soluble form of the factor (sHB-EGF) when it is cleaved by metalloproteinases of the

ADAMs (short for a disintegrin and metalloproteinases) and MMPs types. Membrane-bound HB-EGF, or proHB-EGF, is known to play a crucial role in cell-cell and cell-matrix interactions, influencing cellular responses such as migration, growth, and structural organization of tissues. Furthermore, the ability of proHB-EGF to bind diphtheria toxin suggests a potential role in cellular susceptibility to this bacterial pathogenic factor. Upon proteolytic cleavage, proHB-EGF releases soluble HB-EGF (sHB-EGF), which can activate the EGF family receptors, leading to promitogenic signaling and gene expression alterations in target cells. This activation is critical for processes like wound healing, where rapid cellular proliferation and migration are necessary. However, the same mechanisms can contribute to tumor progression and metastasis, as they may enhance the growth and spread of cancer cells. Receptors for sHB-EGF are homodimers of receptors of the family of epidermal growth factors of the first and fourth types – HER-1 and HER-4 (Human EGF Receptors 1 and 4), as well as their heterodimers with HER-2 and HER-3. The binding of sHB-EGF to the receptor leads to the formation of the sHB-EGF/EGFR ligand-receptor complex and the activation of signaling pathways that play a crucial role in regulating cell growth, proliferation, differentiation, migration, and also in the inhibition of apoptosis, which is important in wound healing and malignization treatment.

The activation mechanisms of HB-EGF are intricate and involve several steps that are crucial for its biological functions. Initially, HB-EGF exists as a membrane-bound precursor, proHB-EGF, which can interact with cell surface receptors and the extracellular matrix. The transition from proHB-EGF to its active form involves proteolytic cleavage by specific enzymes known as sheddases, which release the soluble form, sHB-EGF. This soluble ligand can then bind to EGF receptors, such as EGFR or HER4, leading to the formation of receptor homo- or heterodimers. These dimers initiate a cascade of intracellular signaling by activating the phosphorylation of tyrosine residues within the cytoplasmic parts of these receptors. Such phosphorylation triggers multiple downstream pathways, including the STAT3 and ERK signaling pathways, which regulates gene expression and promotes cellular processes like proliferation, migration, and differentiation. Additionally, HB-EGF can bind to heparan sulfate proteoglycans (HSPGs), which also modulate its activity and availability to receptors. The regulation of HB-EGF activity is further influenced by transcription factors such as AP-1, SP1, and NF-κB, and by microRNAs, which can form an autocrine loop amplifying its effects. These mechanisms collectively ensure that HB-EGF fulfills its role in physiological processes such as wound healing and developmental biology, as well as in pathological conditions like cancer progression. Understanding these mechanisms is vital for developing targeted therapies that can modulate HB-EGF activity in disease treatment. In addition, the results of our research showed that proHB-EGF plays an important role in the regulation of the immune system cell activity (at least macrophage-like and B cells), providing (along with other factors) various responses to activating factors and supporting immune system cells functioning to create full protection of the organism against pathogenic agents [5].

It is known that the formed ligand-receptor complex sHB-EGF/EGFR is subjected to lysosomal degradation or return to the surface of the plasma membrane (receptor recycling). In addition, research in recent years has revealed that the binding of other ligands (EGF, TGFβ) to EGFR leads to nuclear translocation of the ligand-receptor complex, where the latter, due to the presence of kinase activity in the cytoplasmic part of EGFR, performs the functions of a transcription regulator by phosphorylation of a number of nuclear proteins. This leads to an increase in the proliferative potential of the cell.

Characteristics of human HB-EGF

Heparin-binding EGF-like growth factor was first discovered in the culture medium of human monocytes and macrophages, as well as human histiocytic lymphoma cells U937 [3]. HB-EGF belongs to the family of epidermal growth factors (EGFs), which combines proteins, the primary structure of which is characterized by the presence of a unique sequence – an EGF-like domain. The EGF-like domain contains 6 cysteine residues that form three intramolecular disulfide bonds in the following sequence: C1 – C3, C2 – C4, C5 – C6. The EGF-like domain that is responsible for the binding of HB-EGF to receptors of the HER family (short for Human epidermal growth factor receptors) on the surface of cells. HB-EGF, like all other members of the family of epidermal growth factors, belongs to type 1 transmembrane proteins and is synthesized in the form of a membrane-anchored precursor –

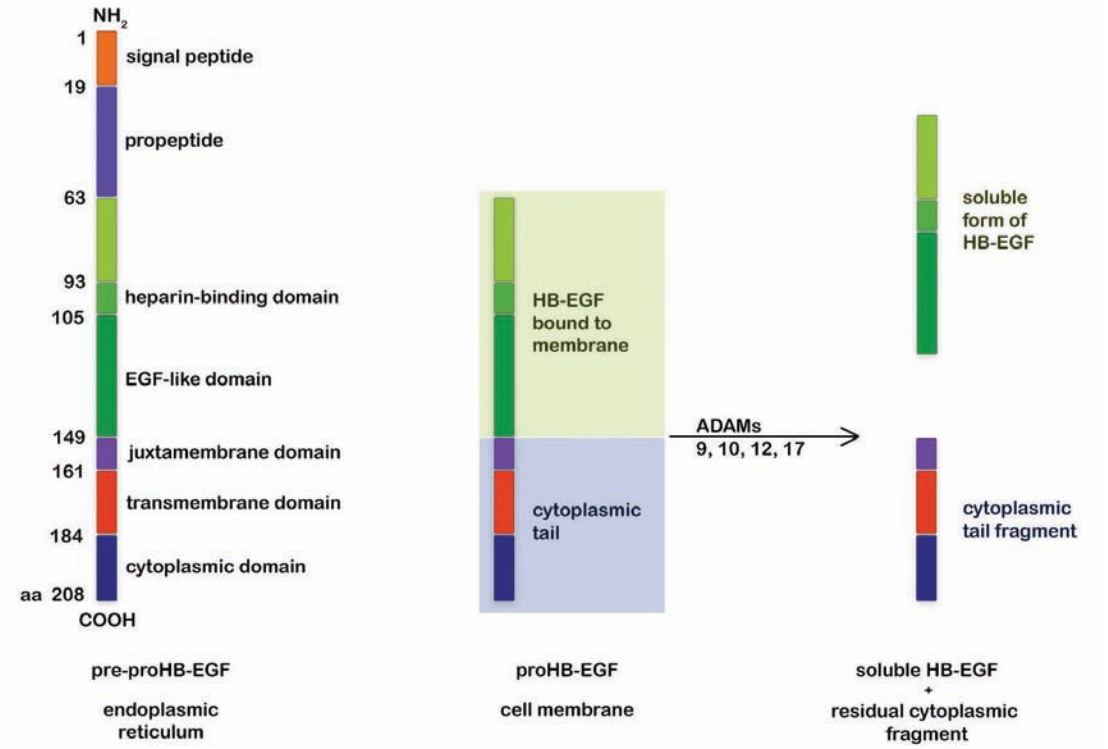

Fig. 1. Schematic representation of the structure of pro-HB-EGF. Pro – pro-peptide, HB – heparin-binding site, EGF-like – epidermal growth factor-like domain, JM – juxtamembrane region, TM – transmembrane region, Cytoplasmic – cytoplasmic domain [7]

pro-HB-EGF with a mass of 20-30 kDa [3, 6]. In the structure of pro-HB-EGF there are six regions: propeptide sequence, heparin-binding (HB) site, EGF-like domain, juxta-membrane region, transmembrane and cytoplasmic domains are distinguished [3] (Fig. 1).

As a result of processing under the influence of metalloproteinases, pro-HB-EGF can turn into a soluble form of sHB-EGF, which has a weight from 14 to 19 kDa [3, 8, 9]. The main role in the formation of the soluble form of HB-EGF is played by metalloproteinases of the MMPs (matrix metalloproteinases) and ADAMs (a disintegrin and metalloproteases) families, namely ADAM 9, 10, 12, 17 [10-13]. Processing of pro-HB-EGF with the subsequent formation of a soluble form can be induced by the activation of protein kinase C (PKC), as well as by an increase in the intracellular concentration of Ca^{2+} ions [14]. In particular, it was established that ADAMs are activated by phosphorylation under the influence of PKCδ. In addition, the formation of a soluble form of HB-EGF can be induced by pharmacological stimuli, for example, 12-O-tetradecanoylphorbol-13-acetate [15], lysophosphatidic acids [16], insulin-like growth factor-1 [17], basic fibroblast growth factor [18], interleukin-8 [19], estrogen [20], angiotensin-2 [21] etc. Cellular stress caused by inflammatory cytokines, oxygen radicals or osmotic shock also leads to the formation of sHB-EGF [16] (Fig. 2).

Role in diphtheria toxin reception

ProHB-EGF also serves as a receptor for diphtheria toxin (DT), the main factor in the pathogenesis of *Corynebacterium diphtheriae* [23], allowing the toxin to enter cells. This interaction is crucial for the pathogenesis of diphtheria. Once inside, the toxin inhibits protein synthesis, leading to cell death. Two subunits are distinguished in the structure of DT: receptor-binding – B (from the English binding) and enzymatically active – A (from the English active). The B-subunit consists of two domains: R (receptor), which is directly responsible for binding to the receptor, and T (transmembrane), due to which the A-subunit is translocated into the cytosol of the cell, where its enzymatic activity is realized through ADP-ribosylation of the eukaryotic translation elongation factor-2 (eEF-2), which leads to the termina-

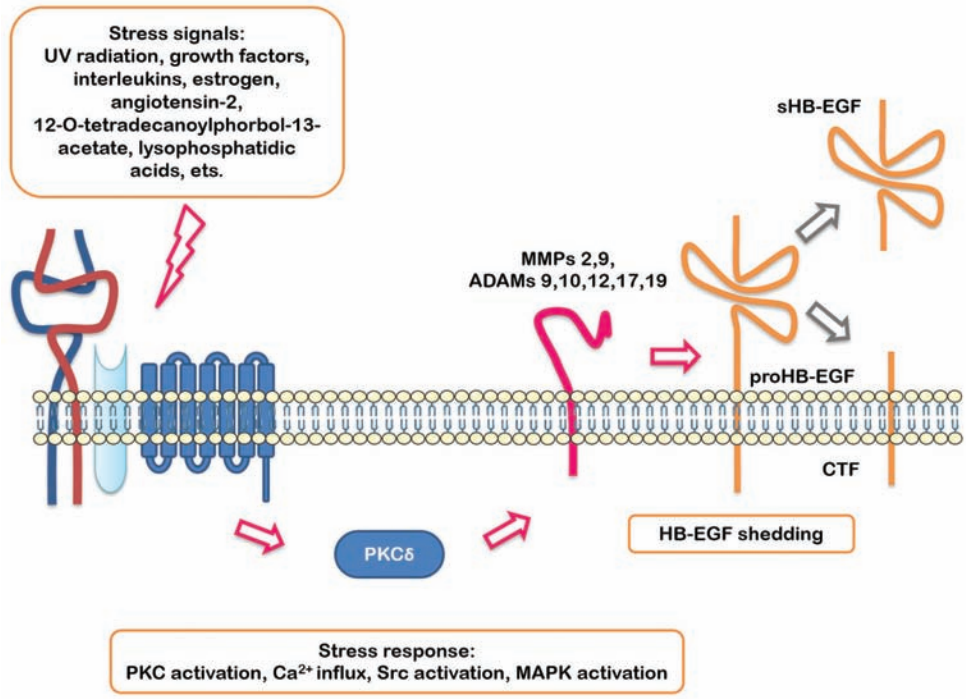

Fig. 2. Scheme of the formation of a soluble form of heparin-binding, EGF-like growth factor (sHB-EGF) under the action of exogenous and endogenous signals (according to [22])

tion of protein synthesis and cell death [24, 25]. The interaction between DT and proHB-EGF is a critical aspect of the toxin's mechanism of action (Fig. 3). After binding, the DT-proHB-EGF complex is internalized into the cell via receptor-mediated endocytosis. The internalization process involves the formation of endosomes, where the acidic environment triggers a conformational change in DT, allowing it to translocate into the cytoplasm. The T-domain of DT facilitates the movement of the C-domain across the endosomal membrane into the cytoplasm.

The sensitivity of cells to DT depends on the expression levels of proHB-EGF and the efficiency of the internalization and translocation processes. Some species exhibit resistance to DT due to variations in the primary structure of proHB-EGF, affecting the binding and internalization efficiency. Understanding the interaction between DT and proHB-EGF has led to the development of targeted therapies, such as the use of DT mutants (e.g., CRM197) to inhibit proHB-EGF shedding in cancer treatment.

Diphtheria toxin can penetrate via an alternative pathway only into immune system cells (macrophages and toxin-specific B cells), owing to phagocytosis and DT-specific immunoglobulin receptors [27].

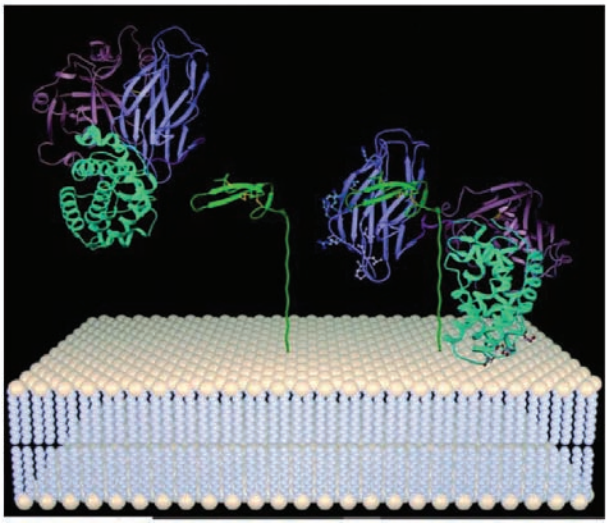

Fig. 3. A model for the interaction of DT molecule with proHB-EGF receptor (green) with respect to the membrane surface (by Louie G. V. [26])

Cell receptors for HB-EGF

The receptors for the membrane-bound and soluble forms of HB-EGF are human epidermal growth factor receptor 1 (HER-1 (EGFR)) and type 4 (HER-4). HER-2 and HER-3 receptors can also participate in the interaction with HB-EGF if

they heterodimerize with HER-1 or HER-4 receptors [28]. Another receptor for HB-EGF was also identified – N-arginine dibasic convertase (NRDC), a metalloendopeptidase that specifically interacts with pro-HB-EGF and increases its metalloproteinase-dependent processing by interacting with other metalloproteinases [29, 30].

It is likely that HER-4-mediated signaling activates complementary pathways of the MAPK cascade and may assist proliferation stimulation induced by EGFR autophosphorylation. It is also shown that the rate of proliferation is determined not only by the number of sHB-EGF receptors, but also by the ratio of their types. This can be explained by the formation of EGFR/HER-4 dimers, the activation of which leads to more effective stimulation of growth and division processes [31].

There are three main mechanisms of biological activity of HB-EGF – autocrine, paracrine and juxtacrine (Fig. 4).

The autocrine mechanism is carried out by the interaction of HB-EGF with the receptor on the surface of the same cell that secretes the soluble form. The juxtacrine mechanism is implemented with the participation of the membrane-bound form of pro-HB-EGF, which interacts with the receptor on the surface of the neighboring cell when both cells are in close contact. This regulation mechanism is implemented, for example, in epithelial cells. If the soluble form of HB-EGF (sHB-EGF) interacts with the receptor on the surface of distant cells, and not the cell that secreted it, the paracrine mechanism of the biological activity of this growth factor is realized.

In addition to the fact that EGF receptors can form dimers in various combinations, they can also bind 11 ligands: HB-EGF, epidermal growth factor (EGF), transforming growth factor-α (TGFA), betacellulin (BTC), amphiregulin (AREG), epiregulin (EREG) and 4 types of neuregulins (NRG1–4) [4]. Each growth factor does not act independently, but influences the functioning of the others, creating a wide range of possible biological effects that are realized through EGF receptor signaling.

Each type of EGF receptor can have several isoforms that differ in functional specialization, affinity for binding to one or another ligand, and the level of expression in different tissues. The existence of at least 4 such isoforms of the HER-4 receptor, all of which are differentially associated with different signaling pathways and can support proliferation or

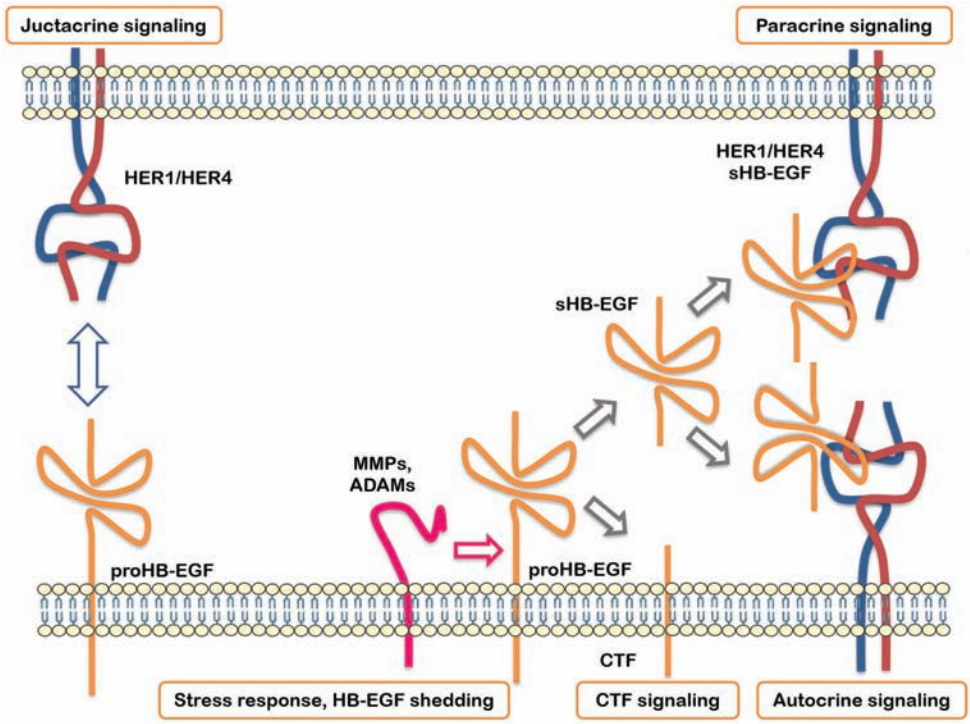

Fig. 4. The mechanisms of biological activity of HB-EGF. HER1/HER4 – human epidermal growth factor receptors for HB-EGF; ADAMs – disintegrin and metalloproteinases; CTF – C-terminal fragment of proHB-EGF [31]

migration [32]. Specific antagonists, for example, can influence the interaction of the EGF receptor with the ligand and its activation of the recently described glycoprotein MIF, which is able to block the activation of EGFR [33], or methylation processes of the receptor, which contribute to the high affinity of ligand binding [34].

Thus, the regulation of important cellular functions such as growth, proliferation, migration, differentiation, and survival, which can be mediated by EGF receptors and their ligands, including HB-EGF, is a very complex multilevel signaling system that uses many delicate safety mechanisms, and therefore, it is very difficult to study its individual components.

Mechanisms of biological activity of HB-EGF

As a result of pro-HB-EGF processing under the action of metalloproteinases, two molecules are formed: a soluble form of the factor – sHB-EGF, consisting of heparin-binding and EGF-like domains, as well as a membrane-anchored cytoplasmic fragment of CTF (C-terminal fragment) [10, 13, 14]. In the process of membrane retrograde transport, CTF is transported to the nucleus through the membrane of the endoplasmic reticulum. CTF interacts with transcription repressors PLZF (promyelocytic leukemia zinc finger protein) and Bcl6 (B cell leukemia 6) [35, 36], which downregulate the cell division by suppressing the expression of cyclin A2 [37], c-myc [38, 39] and others factors necessary for cell progression through the cell cycle. When CTF interacts with PLZF it can inactivate this factor, which leads to the activation of repressed genes. In addition, Wang et al. reported the effect of the whole HB-EGF molecule (proHB-EGF) on the expression of the E-cadherin gene [40].

At the same time, the soluble form of the factor – sHB-EGF interacts with receptors of the family of epidermal growth factors, which are involved in the activation of MAP-kinase (Ras-Raf mitogen-activated protein kinase pathway) and phosphatidyl inositol 3′-kinase pathways 3K [41, 42]. These signaling pathways play a crucial role in the activation of cell proliferation and suppression of apoptosis,

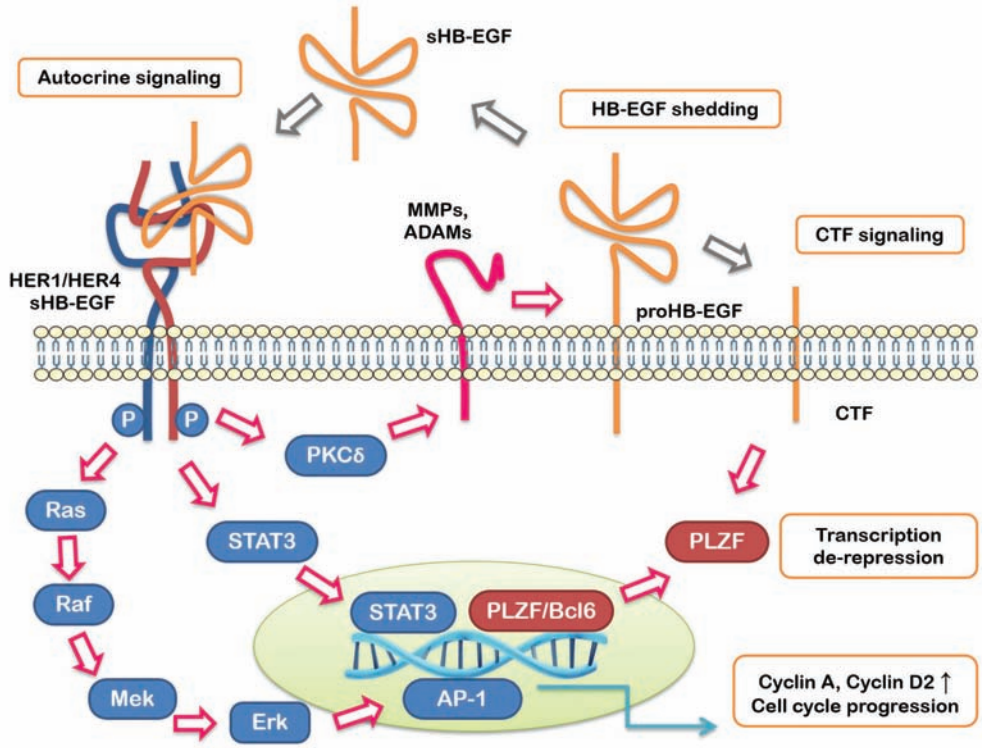

*Fig. 5. HB-EGF signaling. MARK – Ras-Raf mitogen-activated protein kinase pathway, ADAMs – disintegrin and metalloproteinases, PLZF – promyelocytic leukemia zinc finger protein, Bcl-6 – B cell leukemia 6, CTF – C-terminal fragment of proHB-EGF (based on [22]). *This activation pathway was proposed by Gechtman et al. [44]*

which is particularly important in the malignant transformation of cells [43]. Activation of the specified signaling pathways after sHB-EGF binding leads to increased expression of cyclins A and D2, as well as the c-myc factor, which, in turn, leads to the transition of the cell from G0 to the G1 phase of the cell cycle. Thus, metalloproteinase-dependent processing of proHB-EGF leads to the formation of two effector molecules – sHB-EGF and CTF, which are responsible for the generation of activating and derepressing signals, respectively. The simultaneous and complementary effect of two signals is a necessary condition for the normal life of the cell [28, 35] (Fig. 5) and its passage through the cell cycle.

Ryo Iwamoto and others reported different biological activities of membrane-anchored and soluble forms of HB-EGF. Thus, proHB-EGF acts as an antagonist of the mitogenic effect of sHB-EGF (as well as EGF) and is able to induce contact-dependent growth inhibition and apoptosis of neighboring cells. Moreover, this effect is not related to the competition of proHB-EGF and sHB-EGF for the same receptors. According to the authors, a possible explanation for this effect can be oligomerization of proHB-EGF, which, in turn, can cause oligomerization of HER 1 (or HER 4) receptors upon binding to oligomeric proHB-EGF. Such a complex of oligomerized receptors may also include other signaling molecules that can participate in growth inhibition and apoptosis induction [43].

Coreceptor molecules for proHB-EGF and their roles in realizing of its biological activity

The membrane-associated form of HB-EGF (proHB-EGF) forms a complex with CD9, CD44, α3β1 integrins, anti-apoptotic protein BAG-1, metalloproteinases of the ADAM type, as well as with membrane-associated heparan sulfate proteoglycans (HSPG). CD9 (DRAP 27) belongs to the family of tetraspanin membrane proteins (Fig. 6). CD9 interacts with the extracellular heparin-binding domain of proHB-EGF [45]. Tetraspanin membrane proteins are auxiliary molecules that promote or prevent interactions between proteins [46]. In addition to CD9, other tetraspanins are associated with proHB-EGF: CD63, CD81, CD82, however, only CD9 enhances the mitogenic activity of HB-EGF and the ability to interact with diphtheria toxin [47, 48]. In turn, CD9 is associated with α3β1 integrins, which play an important role in the processes of intercellular interactions [49, 50].

The mitogenic activity of HB-EGF is also significantly enhanced by membrane-associated heparan sulfate proteoglycans (HSPG), since the interaction of the N-terminal heparin-binding region of HB-EGF with HSPG leads to stabilization of the EGF-like domain of the molecule [52-54].

Surface glycoprotein CD44 is a receptor for many components of the extracellular matrix, including hyaluronic acid, fibronectin, osteoponin, collagen, and is involved in adhesion and migration processes [55].

Cytoplasmic domain of proHB-EGF interacts with cochaperone BAG-1. As part of this complex, BAG-1 can contribute to the HB-EGF-mediated cytoprotective effect [56, 57], increasing the secretion of its soluble form [58]. BAG-1 is a multifunctional protein and is able to interact with a large number of signaling molecules, including Bcl-2, which is involved in the regulation of apoptosis, 70 kDa heat shock proteins (Hsp70), nuclear hormone receptors, Raf -kinase, DNA [59]. One of the isoforms of the BAG-1 protein (BAG-1L) was found in the nuclei of some cell types [60, 61], which indicates the existence of a complex of BAG-1 and the cytoplasmic domain of HB-EGF, which is transported into the nucleus after its soluble form is separated from proHB-EGF. As part of such a complex, the BAG-1 protein can be involved in the activation of signaling mechanisms induced by the cytoplasmic domain of HB-EGF.

Two more proteins were also identified that form a complex with the extracellular domain of proHB-EGF: TGFβ-binding protein and fibulin-1, a calcium-binding extracellular glycoprotein. However, the biological significance of these interactions has yet to be determined [62].

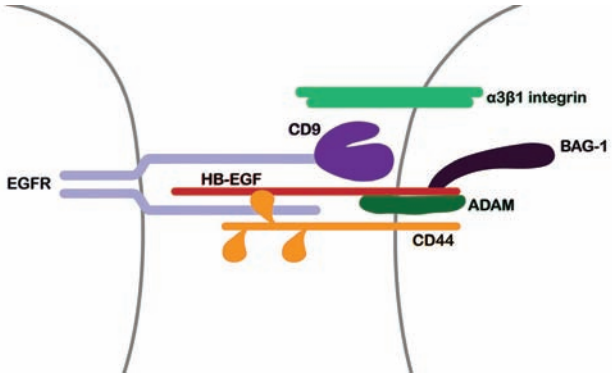

Fig. 6. The membrane complex is associated with proHB-EGF molecules (by Harris R. C. et al. [51])

Expression of HB-EGF on normal and tumor cells

Expression of the HB-EGF gene was detected in a number of tissues (Fig. 7), especially in lung, heart, brain, and skeletal muscle tissues [56]. Also, a high level of HB-EGF expression was found in the cells of a number of tumors: hepatocarcinoma, colon, melanoma, myeloma, glioma, glioblastoma, mammary gland, prostate, urinary bladder, which may indicate the important role of this growth factor in the processes of malignant transformation. In addition, the soluble form of HB-EGF is a potential mitogen and chemoattractant for keratinocytes, hepatocytes, smooth muscle cells, and fibroblasts [3, 52, 64].

The relationship between the increased level of HB-EGF expression and the processes of malignant transformation and tumor growth was investigated in human ovarian tumors. A high level of HB-EGF expression was observed in a significant number of tumors isolated from the ovaries of cancer patients, and ascitic fluid isolated from such patients contained a significant proportion of HB-EGF in relation to total protein [65, 66]. However, the level of expression of other EGFR ligands in ovarian tumors and in the total protein content of ascitic fluid was two to three times lower than that of HB-EGF.

A correlation was also found between the level of expression of HB-EGF in cancer patients and the clinical course of the disease, which was expressed in a higher level of survival of patients with a low level of expression of this growth factor in ovarian tumors [66]. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) plays intriguing roles in cancer. For instance, HB-EGF stimulates cell growth and angiogenesis (formation of new blood vessels) in tumors. sHB-EGF also induced vascular endothelial growth factor (VEGF) expression, promoting angiogenesis. In bladder cancer cells, soluble HB-EGF (sHB-EGF) increased transformed phenotypes, including growth rate, colony-forming ability, and activation of cyclin D1 promoter. Exposure of cancer cells to chemotherapy or radiotherapy often upregulates HB-EGF expression. This leads to EGFRs activation, enhancing the survival signaling of cancer cells. So, HB-EGF inhibition tolls could be valuable for cancer treatment.

The role of HB-EGF in wound healing and the response to injury

Due to the detection of HB-EGF in the wound fluid during the first day, it was assumed that this factor plays an important role in wound healing [7]. Peak levels of HB-EGF are observed 2-3 days after injury and return to background levels within a week of wound healing. HB-EGF levels were significantly higher than FGF-2 or PDGF. The origin of HB-EGF in wound fluid is not known for sure, but it is quite possible that it is synthesized by monocytic macrophages [53], T-cells [67], and keratinocytes [7, 68].

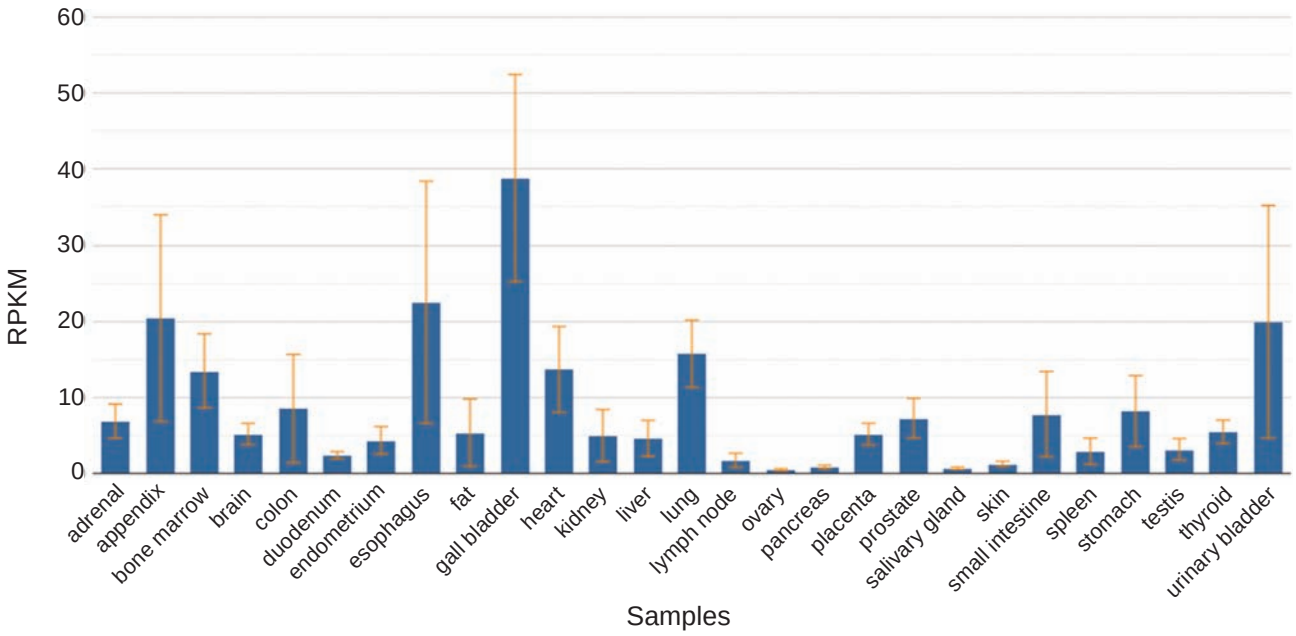

Fig. 7. HB-EGF mRNA expression levels in normal human tissue samples (by Fagerberg L. et al. [63])

Wound fluid also contains IGF-1, which is synergistic with HB-EGF to stimulate keratinocyte growth [69]. Due to the ability of HB-EGF to stimulate the mitogenesis of fibroblasts and keratinocytes, and to induce cell migration, it may be involved in the induction of granulation tissue and re-epithelialization [7, 69]. Also, HB-EGF was detected in the wound fluid of pediatric burn patients on the first day [68]. Immunohistochemical staining of burn wound specimens during healing revealed HB-EGF in islets or regenerating epithelium in the burn wound and in ducts and proximal tubules of eccrine sweat glands. In intact skin, HB-EGF was detected only in the basal epithelium [69, 70].

HB-EGF is upregulated in renal cells both in culture and *in vivo* models of diabetes and renal injury. In cultures, HB-EGF gene expression in kidney mesangial cells is stimulated by oxidized low-density lipoprotein, thrombin, endothelin-1, and HB-EGF itself. Since HB-EGF is a mitogen for mesangial cells, it is likely to be involved in their hyperproliferation and glomerulosclerosis, which may cause glomerulonephritis and renal damage. In rats with induced diabetes for three months, there is a constant regulation of HB-EGF mRNA in the kidneys [71]. Renal hypertrophy occurs in this model, and because HB-EGF is an autocrine growth factor for mesangial cells, abnormal production of HB-EGF may contribute to diabetic nephropathy. Renal ischemic reperfusion in rats also increases HB-EGF mRNA levels within 45 min of reperfusion. *In situ* analysis 6 h after ischemia showed increased HB-EGF mRNA in the inner cortex and outer medulla [64]. Induction of HB-EGF gene expression was also detected in the renal tubules of rats after repeated oxygenation. After 70% partial hepatectomy in rats, the level of HB-EGF mRNA increased 5-fold compared to control animals, which is associated with non-parenchymal cells. Hepatotoxins such as carbon tetrachloride also upregulate HB-EGF expression [72].

HB-EGF in regenerative medicine

HB-EGF is promising in the field of regenerative medicine due to its multifaceted role in tissue repair, cell proliferation, and wound healing. Its ability to enhance cell growth is especially valuable in regenerative medicine, where the goal is to replace or repair damaged tissue [73]. Research on the use of HB-EGF in regenerative medicine includes the following promising areas.

Promotion of cell proliferation and migration. HB-EGF has been shown to stimulate the proliferation and migration of various cell types that are critical for tissue regeneration. *In vivo* studies show that HB-EGF accelerates wound healing and hair growth by promoting keratinocyte proliferation and migration [74]. Administration of HB-EGF in animal models stimulates hepatocyte proliferation and liver regeneration after damage [75, 76]. HB-EGF induces chondrocyte proliferation and cartilage matrix production, offering potential for the treatment of cartilage defects and osteoarthritis [77], and enhances osteoblast activity and bone formation in bone defect models, promoting bone healing [78].

Angiogenic effects of HB-EGF. HB-EGF exhibits an angiogenic effect, promoting the formation of new blood vessels (angiogenesis). This is crucial for the supply of nutrients and oxygen to regenerating tissues, increasing their viability and functionality. HB-EGF directly affects endothelial cells, the building blocks of blood vessels, inducing their proliferation and migration, key stages of angiogenesis [79].

Modulation of inflammatory responses. Inflammation is a natural part of the regeneration process, but excessive or prolonged inflammation can impede healing. By influencing the immune response, HB-EGF helps maintain a balanced and controlled inflammatory environment. Excessive inflammation can disrupt epithelial homeostasis, and HB-EGF helps regulate this process [80].

Participation in tissue remodeling. Tissue remodeling is a complex process that includes the breakdown and synthesis of extracellular matrix components. HB-EGF is involved in tissue remodeling by affecting the activity of matrix metalloproteinases (MMPs) and other remodeling enzymes, contributing to the restructuring of damaged tissues [81]. Corneal wound healing studies demonstrate the efficacy of HB-EGF in promoting re-epithelialization and reducing scarring in corneal abrasion models [82-84].

Potential for stem cell differentiation. HB-EGF may play a role in promoting the differentiation of stem cells into specialized cell types. This property is of particular interest in regenerative medicine approaches that involve the use of stem cells for tissue repair and regeneration [85].

Regulation of epithelial homeostasis. HB-EGF contributes to the maintenance of epithelial tissues by influencing cellular homeostasis. HB-EGF binds

T a b l e. Overview of potential delivery methods, their advantages and limitations

Methods of HB-EGF delivery	Advantages of the method	Limitations of the method
Scaffolds, hydrogel, cream, or ointment [72]	Localized and controlled delivery to the affected skin or wound site; easily distributed all over the body; the possibility of creating a three-dimensional matrix for long-term release.	The variability of the distribution can lead to an uneven therapeutic effect; the need to ensure sufficient penetration through the skin barrier; maintenance of appropriate parameters such as pH, temperature, and interaction with other components of the cream can affect the stability of the growth factor; may not reach deep tissues, which prevents its therapeutic effect; the need for repeated dosing – multiple applications will be required, which increases the complexity and duration of treatment.
Internal administration (intramuscular, subcutaneous, local injection) [69]	Injection at the site of injury ensures targeted delivery; suitable for controlled release and can be used in various therapeutic contexts; use in the form of solutions for direct infusion into the bloodstream will allow to reach target tissues throughout the body.	May not reach all affected areas, especially in large tissues (subcutaneous and local injection); easily distributed throughout the body through direct introduction into the bloodstream (intravenous administration), reaching non-target tissues and potentially causing unwanted side effects; repeated dosing – local injections may require frequent administration, which increases the complexity of treatment and potential problems; rapid degradation (protein sensitive to degradation by enzymes in the blood, which limits its effectiveness at the target site) and elimination from the body.
Nanoparticle delivery systems [76, 78]	Improved stability – encapsulation protects HB-EGF from degradation, extending its service life and increasing its therapeutic potential; controlled release – nanoparticles can be designed to gradually release HB-EGF over time, minimizing systemic exposure and maximizing target site exposure; targeted delivery – nanoparticles can be equipped with specific ligands to bind to the receptors of target cells, increasing delivery efficiency and minimizing off-target effects.	Efficiency and control of HB-EGF release need further study; biocompatibility of nanoparticles to avoid adverse reactions when administered into the body; loading efficiency – inadequate loading can lead to suboptimal therapeutic effects, which requires careful optimization of formulation parameters; nanoparticles or their components can trigger an immune response, affecting the overall safety and effectiveness of the delivery system.
Gene therapy [80, 85]	Delivery of the HB-EGF gene directly into cells ensures continuous production at the site of damage, potentially reducing the need for re-injection; gene therapy vectors can be engineered to target specific cell types, further increasing the specificity of treatment.	Raises safety concerns regarding potential insertional mutagenesis; effective and safe gene delivery vectors are needed; raises ethical issues and requires complex manipulations.

to members of the ErbB receptor family, mainly ErbB1 and ErbB4, triggering downstream signaling cascades such as MAPK, PI3K/Akt, and STAT3. These pathways regulate various cellular processes important for epithelial homeostasis. HB-EGF stimulates cell proliferation by activating cyclin-dependent kinases, ensuring constant renewal of the epithelial layer [86].

Therapeutic implications in ischemic conditions. The angiogenic properties of HB-EGF make it a potential candidate for therapeutic interventions in ischemic conditions, where restoration of blood

flow and tissue perfusion is crucial for successful regeneration [1].

Methods of HB-EGF delivery

HB-EGF shows great potential for therapeutic use, so the question of possible delivery methods of HB-EGF is important, because this is a decisive factor in its use. Based on the literature review, different strategies for efficient delivery of HB-EGF to target tissues were analyzed. The results are presented in the Table.

The choice of delivery method depends on the specific therapeutic goals, the nature of the condition being treated, and the desired kinetics of HB-EGF release. Each delivery method has its advantages and limitations, and ongoing research is aimed at optimizing these approaches for increased efficacy in clinical applications.

When choosing a method, the following should be taken into account:

1) target tissues – different methods may be suitable for different tissues depending on availability and specific needs;

2) the desired duration of action – depending on the therapeutic conditions, short-term or long-term release strategies may be needed;

3) safety and efficacy – it is imperative to balance potential benefits with potential risks;

4) cost and feasibility – it is necessary to consider the costs of production and clinical application.

Effective delivery of HB-EGF is essential to unlock its therapeutic potential. Continued research and development are promising to identify safe and effective delivery strategies that can bring this promising molecule closer to reality.

Conclusions. While HB-EGF (Heparin-binding epidermal growth factor-like growth factor) is widely known for its role as a receptor for diphtheria toxin, it has broader functions beyond that. It acts as a growth factor, stimulating cell proliferation, migration, and tissue repair. So, HB-EGF is involved in wound healing, tissue regeneration, and development. Therefore, it has potential therapeutic applications. For example, HB-EGF promotes cardiac repair after injury. It plays a role in skin wound healing and tissue remodeling. Also, HB-EGF may protect neurons from damage. Different ways of HB-EGF inhibition are associated with cancer treatment. In cosmetology, EGFs (including HB-EGF) are used for skin rejuvenation. EGF-containing products claim to enhance collagen production, reduce wrinkles, and improve skin texture. However, scientific evidence supporting these claims is still evolving.

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ГЕПАРИН-ЗВ'ЯЗУВАЛЬНИЙ EGF-ПОДІБНИЙ ФАКТОР РОСТУ: МЕХАНІЗМИ БІОЛОГІЧНОЇ АКТИВНОСТІ ТА ПОТЕНЦІЙНІ ТЕРАПЕВТИЧНІ ЗАСТОСУВАННЯ

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Рецептор дифтерійного токсину на чутливих клітинах ссавців відомий як закріплений на мембрані попередник гепарин-зв'язувального EGF-подібного фактора росту (HB-EGF). Коли попередник розщеплюється металопротеїназами, утворюється розчинна форма (sHB-EGF), яка може зв'язуватися з рецепторами EGF, що призводить до активації сигнальних шляхів, які регулюють клітинну проліферацію, диференціювання, міграцію та інгібування апоптозу. Здатність HB-EGF спричинювати як позитивні, так і негативні наслідки для організму підкреслює складність його біологічних функцій і потреби в тонкому розумінні його ролі в здоровому організмі та за різних захворювань. В огляді узагальнено дані про структуру HB-EGF, біологічну активність, участь у механізмі дії дифтерійного токсину, загоєння ран, пухлинну прогресію, а також способи доставки HB-EGF.

Ключові слова: гепарин-зв'язувальний EGF-подібний фактор росту, дифтерійний токсин, рецептор EGF, трансдукція сигналу, клітинна проліферація, загоєння ран.

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