Seminars in Cell & Developmental Biology 28 (2014) 22–30

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) and proteolytic processing by a disintegrin and metalloproteinases (ADAM): A regulator of several pathways

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ARTICLE INFO

Article history:
Available online 25 March 2014

Keywords:
HB-EGF
HB-EGF processing
ADAM
Wound healing
Reproductive biology
Transgenic mice
Angiogenesis
Tumor formation
Adipogenesis

ABSTRACT

HB-EGF is a member of the EGF family of ligands that is initially synthesized as a membrane-bound growth factor termed, proHB-EGF. The membrane bound proHB-EGF undergoes extensive proteolytic processing by several metalloproteinases capable of stimulating cellular proliferation. Soluble, mature HB-EGF binds to and activates EGF receptors. HB-EGF is a critical molecular component to a number of normal physiological processes including but not limited to tissue injury and wound healing, reproduction, angiogenesis and recently, adipogenesis. Misexpression of HB-EGF is linked to tumor formation and cancer including hepatocellular, pancreatic, gastric, breast, colon and melanoma, gliomas and glioblastomas. HB-EGF is a likely tool for therapeutic approaches to enhance treatment of injuries as well as a target for prevention of several cancers and obesity.

Contents

1. Introduction

Nearly a quarter of a century ago, a human macrophage derived heparin-binding growth factor was isolated, termed MD-HBGF, that exhibited mitogenic properties for Balb/c 3T3 cells and smooth muscle cells (SMC) and lacked mitogenicity for endothelial cells [1]. A year later, MD-HBGF was purified and characterized from the conditioned medium of a human macrophage-like cell line (U937) by heparin-affinity chromatography and reverse phase liquid chromatography, identified as a member of the epidermal growth factor (EGF) family and termed heparin-binding epidermal growth factor-like growth factor (HB-EGF) [2]. The human HB-EGF cDNA exhibits an open reading frame of 208 amino acids encoding a signal peptide, propeptide, soluble HB-EGF, transmembrane and cytoplasmic

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http://dx.doi.org/10.1016/j.semcdb.2014.03.004
1084-9521/

Table 1
Ligands of the EGF family and their receptors.

EGF ligand	Reference	EGF receptor	Reference
Amphiregulin (ARG)	[23,24]	HER1	[23,24]
Betacellulin (BTC)	[145]	HER1, HER4	[30,145]
Epidermal growth factor (EGF)	[142]	HER1	[146]
Epiregulin (EPR)	[147]	HER1	[147]
Heparin-binding EGF-like growth factor (HB-EGF)	[2]	HER1, HER4	[2,148,149]
Neuregulin-1 (NRG-1) (Type I-VI)	[21]	HER3, HER4	[21,25]
Neuregulin-2 (NRG-2)	[150,151]	HER1, HER3, HER4	[150,151]
Neuregulin-3 (NRG-3)	[32]	HER4	[32]
Neuregulin-4 (NRG-4)	[32]	HER4	[32]
Transforming growth factor-α (TGF-α)	[152]	HER1	[153]
Vaccinia growth factor (VGF)	[144]	HER1	[144]

domains. Proteolytic processing of proHB-EGF has been shown to occur resulting in several biological actions including adipogenesis, angiogenesis, blastocyst implantation, SMC hyperplasia, wound healing, atherosclerosis and tumor progression. The goal of this review is to describe the structural and biological properties of HB-EGF including relevant physiological and pathological roles.

2. HB-EGF structure and location

The HB-EGF gene is made up of six exons and five introns encoding an open reading frame of 208 amino acid corresponding to proHB-EGF and has been mapped to chromosome 5 (5q23) in human and chromosome 18 in mouse spanning approximately 13.8 kb of DNA [2–4]. proHB-EGF is initially synthesized as a type I single transmembrane precursor protein that undergoes extensive proteolytic processing. Initially, the extracellular domain of HB-EGF is processed by a furin-like enzyme, which cleaves the 208 amino acid proHB-EGF precursor at Arg_{62}-Asp_{63} [5] and ADAM cleaves between Pro_{148}-Val_{149} or Glu_{151}-Asn_{152}, resulting in the release of soluble, mature HB-EGF that is collectively referred to as ectodomain shedding [6–10]. Subsequent to and dependent upon ectodomain shedding, the HB-EGF intracellular domain is processed by an unidentified protease resulting in a carboxy-terminal HB-EGF domain, termed HB-EGF C [11,12]. Soluble, mature and carboxy-terminal domains of HB-EGF are potent stimulators of cellular proliferation whose action is mediated by EGF receptor (EGFR)-dependent and EGFR-independent mechanisms, respectively [1,11,12].

The soluble, mature HB-EGF extracellular motif contains six conserved cysteines referred to as the EGF-like domain and is thought to be required for EGF family members to bind and activate EGFRs [13,22]. The three dimensional structure of HB-EGF [14] and EGF [15,16] specify an amino-terminal domain containing the first two disulfide bonds forming a two-stranded anti-parallel β-sheet and a carboxy-terminal domain containing the third disulfide bond contributing to two short β-sheets for the EGF-like domain of HB-EGF or EGF itself. The EGF-like domain within HB-EGF contains a highly basic heparin-binding amino-terminal extension that is not present in EGF.

A novel property of the membrane bound form of HB-EGF is that it acts as the receptor for diphtheria toxin (DT) [143,18]. DT binds to the EGF-like domain of proHB-EGF and upon binding internalizes the DT. Interestingly, mouse and rat HB-EGF are insensitive to DT most likely because DT lacks affinity to rodent proHB-EGF [17].

3. EGF family and receptors

HB-EGF is a member of the EGF family, secreted peptide signaling molecules that induce proliferation and differentiation in all cells [19]. Initially synthesized as transmembrane protein, EGF family members are cleaved from the membrane by metalloproteinases to form the soluble, mature growth factors. Therefore, EGF family ligands can induce juxtacrine, autocrine, paracrine, or endocrine signaling, depending on the peptide's cellular environment [20].

Members of the EGF ligand family include Epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin (ARG), heparin-binding EGF-like growth factor (HB-EGF), betacellulin (BTC), epiregulin (EPR), neuregulin-1 (NRG-1), neuregulin-2 (NRG-2), and more recently, neuregulin-3 (NRG-3) and neuregulin-4 (NRG-4) (Table 1). NRG-1 has several different isoforms created by alternative splicing, including Type I/Heregulin/NEU differentiation factor (NDF)/aceytlcholine receptor inducing activity (ARIA), Type II/glial growth factor (GGF2), Type III/sensory and motor neuron-derived factor (SMDF), Type IV, Type V, and Type VI [21]. All ligands have a common amino acid motif, the EGF domain, which is made up of 6 cytosine residues that form the consensus sequence $CX_7CX_4CX_{10}$-$CXCX_8C$ [22]. It is the three intramolecular disulfide bonds between the cysteine residues (C_1-C_3, C_2-C_4, C_5-C_6) that are necessary for HB-EGF mitogenic activity [2].

Four members of the EGF receptor family have been identified and labeled using the HER (human EGF-receptor) and erbB nomenclature – EGF receptor (EGFR)/HER 1/erbB1, HER2/erbB2/p185/neu, HER3/erbB3, and HER4/erbB4 [23–26]. These receptors are structurally related tyrosine kinases with a single membrane spanning domain and an intrinsic kinase domain in the cytoplasm. Ligand binding induces homo or heterodimerization between HER receptors, which leads to the activation of their intrinsic tyrosine kinase activity, trans phosphorylation of intracellular tyrosine residues and activation of multiple signal transduction cascades [27]. HER2 lacks ligand-binding capacity, whereas HER3 is intrinsically inactive or at least, weakly active as a kinase [28]. Both HER2 and HER3 heterodimerize with other HER family members to activate intracellular signaling [29].

The EGF ligands are specific when binding to receptors (Table 1). EGF, TGF-α, and ARG bind to EGFR; HB-EGF, BTC, and EPR bind to both EGFR and HER4; NRG-1 and NRG-2 bind EGFR, HER3 and HER4 [30,31] and NRG-3 and NRG-4 bind to HER4 [32].

4. HB-EGF protein processing and signaling

G-protein coupled receptors (GPCR) agonists including endothelin-1, lysophosphotidic acid [33,34], angiotensin-II [7,35,36], or stromal cell-derived factor-1, also known as C-X-C motif chemokine 12 (CXCL12) [37] induce extracellular activity of ADAM 9, 10, 12, 17 and matrix metalloproteinase (MMP) 3 and 7 that have been reported to stimulate ectodomain shedding of proHB-EGF in specific cellular environments resulting in binding and activation of EGFRs [6,7,9,10,38,39]. Transactivation of EGFR by GPCR signaling is a common biological role in a number of cell