Seminars in Cell & Developmental Biology 28 (2014) 31–41

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

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

Review

Amphiregulin

Carmen Berasain[a,b], Matías A. Avila[a,b,*]

[a] Division of Hepatology and Gene Therapy, CIMA, University of Navarra, Avda. Pio XII, n55, 31008 Pamplona, Spain
[b] CIBERehd, Clinica Universidad de Navarra, Avda. Pio XII, n55, 31008 Pamplona, Spain

ARTICLE INFO

Article history:
Available online 23 January 2014

Keywords:
Amphiregulin
Epidermal growth factor receptor (EGFR)
Cell signaling
Proliferation
Survival
Cancer
Chemoresistance

ABSTRACT

Amphiregulin (AREG) is a ligand of the epidermal growth factor receptor (EGFR), a widely expressed transmembrane tyrosine kinase. AREG is synthesized as a membrane-anchored precursor protein that can engage in juxtacrine signaling on adjacent cells. Alternatively, after proteolytic processing by cell membrane proteases, mainly TACE/ADAM17, AREG is secreted and behaves as an autocrine or paracrine factor. AREG gene expression and release is induced by a plethora of stimuli including inflammatory lipids, cytokines, hormones, growth factors and xenobiotics. Through EGFR binding AREG activates major intracellular signaling cascades governing cell survival, proliferation and motility. Physiologically, AREG plays an important role in the development and maturation of mammary glands, bone tissue and oocytes. Chronic elevation of AREG expression is increasingly associated with different pathological conditions, mostly of inflammatory and/or neoplastic nature. Here we review the essential aspects of AREG structure, function and regulation, discuss the basis for its differential role within the EGFR family of ligands, and identify emerging aspects in AREG research with translational potential.

Contents

Abbreviations: ADAM17, a desintegrin and metalloproteinase 17; AREG, amphiregulin; BTC, betacellulin; COPD, chronic obstructive pulmonary disease; CRE, cAMP response element; CREB1, CRE binding protein 1; CTGF, connective tissue growth factor; DEP, diesel exhaust particles; EBS, ETS/ATF binding site (EBS); EGF, epidermal growth factor; EGFR, EGF receptor; EPGN, epigen; ERE, estrogen receptor binding element; EREG, epiregulin; HB-EGF, heparin-binding EGF; HBS, hypoxia-inducible transcription factor binding sites (HBS); HCV, hepatitis C virus; PGE2, prostaglandin E2; PM, particulate matter; PMA, phorbol 12-myristate 13-acetate; SRE, serum response element; TACE, tumor-necrosis factor-alpha converting enzyme; TBE, beta-catenin/TCF/LEF-binding sites; TLR, Toll-like receptor; WRE, Wilms' tumor suppressor WT1-responsive element; YAP, Yes associated protein.

* Corresponding author at: Division of Hepatology and Gene Therapy, CIMA, University of Navarra, Avda. Pio XII, n55, 31008 Pamplona, Spain. Tel.: +34 948 194700x4003.
E-mail addresses: cberasain@unav.es (C. Berasain), maavila@unav.es (M.A. Avila).

1084-9521/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcdb.2014.01.005

1. Introduction

The epidermal growth factor (EGF) family member amphiregulin (AREG) was identified 25 years ago in the supernatant of MCF-7 human breast carcinoma cells treated with phorbol 12-myristate 13-acetate (PMA). It was initially defined as a bifunctional growth factor, capable of inhibiting the proliferation of certain carcinoma cell lines while able to induce that of normal cells such as fibroblasts and keratinocytes [1]. Currently AREG is known to stimulate the proliferation of most cell types analyzed. This effect is mainly mediated through its binding and activation of the epidermal growth factor (EGF) receptor (EGFR, also known as ErbB1), a widely expressed transmembrane tyrosine kinase [2]. AREG promotes EGFR homodimerization or heterodimerization with ErbB2, ErbB3 and ErbB4, other receptors of the EGFR family, triggering the generation of intracellular signals [3–6]. Here we will summarize the essential structural, regulatory and functional features of AREG along with its increasingly recognized role in disease.

2. AREG gene and protein structure

In humans two copies of the *AREG* gene (*AREG* and *AREGB*) spanning about 10 kb of genomic DNA and located 160 kb apart are found at the EGF family gene cluster on chromosome band 4q13.3. These two *AREG* genes are flanked by the betacellulin (BTC) gene at the 3′ region and by the epiregulin (EREG) and epigen (EPGN) genes at the 5′ region, respectively (Fig. 1). The relative contribution of each *AREG* gene copy to the overall levels of cellular AREG expression is currently unknown. Several single-nucleotide polymorphisms have been identified in the intergenic region between *AREG* and *AREGB* [7]. *AREG* is transcribed as a 1.4-kb mRNA composed of six exons that code for a transmembrane polarized glycoprotein precursor (Pro-AREG) of 252 aminoacids (aa) [8].

Pro-AREG contains multiple domains: a hydrophobic signal peptide (aa 1–20), a N-terminal hydrophilic pro-region with a glycosylation site (aa 20–101), a heparin-binding (HB) domain (aa 102–140) containing another glycosylation site and a nuclear localization signal, the EGF-like domain (aa 141–181) with six spatially conserved cysteines that form disulfide bridges and the three-looped structure that define the EGF family, followed by a juxtamembrane stalk (aa 182–198) containing the cleavage site (Lys187) for “ectodomain shedding” [9]. A hydrophobic domain (aa 199–221) transverses the cell membrane and a cytoplasmic domain (aa 222–252) with another nuclear localization signal, a novel mono-Leu basolateral sorting motif [10] and a ubiquitination site at Lys240 implicated in AREG endocytosis [11], is found in the intracellular portion of the Pro-AREG molecule (Fig. 1).

Mature soluble AREG containing the EGF motif is produced upon proteolytic cleavage or “ectodomain shedding” of Pro-AREG at Lys187 [9]. This is carried out by the membrane anchored tumor-necrosis factor-alpha converting enzyme (TACE), a member of the desintegrin and metalloproteinase family also known as ADAM-17 [12]. AREG shedding induces the autocrine or paracrine activation of EGFR. As we will review latter, multiple stimuli acting through very different receptors can activate ADAM-17 and induce AREG shedding and EGFR transactivation [9,13]. Sequential proteolysis at other alternative cleavage sites generates several active soluble forms of AREG containing the HB and/or the EGF domains [14,15]. Transmembrane Pro-AREG can also activate EGFR in a juxtacrine manner, alone [16] or in combination with tetraspanin proteins [17].

3. Regulation of AREG availability

AREG availability can be regulated at three different levels: the transcriptional level through the binding of activators and repressors to different regions on the *AREG* promoter, the post-transcriptional level through modulation of mRNA stability and the post-translational level through covalent modifications and the regulation of AREG shedding (Fig. 1).

3.1. Transcriptional regulation

Several functional elements implicated in the induction of AREG expression have been identified in the human *AREG* promoter including a cAMP response element (CRE), a Wilms' tumor suppressor WT1-responsive element (WRE), SP1 binding consensus elements, an ETS/ATF binding site (EBS), three beta-catenin/TCF/LEF-binding sites (TBE), a serum response element (SRE), a Yes-associated protein (YAP)/TEAD binding site, an estrogen receptor binding element (ERE), an AP1 site, two hypoxia-inducible transcription factor 2α (HIF-2α) binding sites (HBS) and a p53 responsive element (Fig. 1) [18–26].

The recruitment of transcription factors to those sites can be triggered by a variety of signals depending on the cellular context. *AREG* expression is induced through the activation of the cAMP/PKA pathway by prostaglandin E2 (PGE2) [27], the protein kinase C (PKC) pathway [28], parathyroid hormone [29], polycystin-1 [25], tobacco smoke [30] or hypoxia [31]. Other pathways responsible for the induction of *AREG* expression are triggered by growth factors and cytokines. Interestingly *AREG* transcription is induced upon activation of the EGFR by AREG itself in an autostimulatory feedback mechanism, or by other members of the EGF family in several epithelial cells [16,32,33] and in liver fibrogenic cells [34]. Cytokines such as interleukin 1 and tumor necrosis factor alpha up-regulate AREG expression in cervical epithelial cells [35], hepatocytes [36], T cells [37], macrophages [38] and salivary gland epithelial cells [39]. Microbial products and infectious agents also promote AREG expression. Bacterial lipopolysaccharide (LPS) triggers *AREG* transcription in macrophages [38] and in intestinal epithelial cells [40]. Viral infection by the hepatitis C virus (HCV) in hepatoma cells [41], bacterial infections by *Helicobacter pylori* [42] in gastric epithelial cells or *Neisseria gonorrhoeae* in cervical epithelial cells [15], as well as nematode infections in TH2 cells [43] also promote *AREG* expression. Several hormones including androgens [44], parathyroid hormone [29], insulin [45] or estrogens, which induce ERalpha recruitment to the *AREG* promoter [23,28], in some cases depending on cyclin D1 expression [46], are also *AREG* inducers. Other stimuli triggering AREG expression in the respiratory tract include the activation of the protease-activated receptor 2 (PAR2) by trypsin-like protease (HAT) in airway epithelial cells [47], and by xenobiotics present in diesel exhaust particles (DEP) and particulate matter (PM) in bronchial epithelial cells [48]. Also in mouse lung tissue the transcription factor DMP1 has been shown to bind the *Areg* promoter and to induce AREG expression [49].

Regarding the repression of AREG promoter it has been recently shown that the tumor suppressor gene BRCA1 binds and represses AREG transcription in breast cells [50], suggesting that the loss of BRCA1 expression may contribute to the tumorigenic phenotype of breast cancer cells through the induction of *AREG* gene expression. Interestingly, it has been also shown that the maintenance of skin homeostasis and skin cancer prevention in mice depends on the inhibition of *EGF*, *heparin-binding EGF* (*HB-EGF*) and *Areg* promoter in keratinocytes by IKKalpha [51]. The protein coded by the coiled-coil domain containing 6 gene, frequently rearranged in papillary thyroid carcinomas, inhibits CRE binding protein 1 (CREB1)-dependent *AREG* gene expression in these cells inducing histone H3 deacetylation [52]. In periovulatory rat granulosa cells