Review Article

Membrane-anchored growth factors, the epidermal growth factor family: Beyond receptor ligands

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The epidermal growth factor (EGF) family and the EGF receptor (EGFR, ErbB) tyrosine kinase family have been spearheading the studies of signal transduction events that determine cell fate and behavior *in vitro* and *in vivo*. The EGFR family and their signaling pathways are giving us tremendous advantages in developing fascinating molecular target strategies for cancer therapy. Currently, two important types of EGFR inhibitors are in clinical use: neutralizing antibodies of EGFR or ErbB2, and synthetic small compounds of tyrosine kinase inhibitors designed for receptors. On the other hand, basic research of the EGF family ligands presents new challenges as membrane-anchored growth factors. All members of the EGF family have important roles in development and diseases and are shed from the plasma membrane by metalloproteases. The ectodomain shedding of the ligands has emerged as a critical component in the functional transactivation of EGFRs in interreceptor cross-talk in response to various shedding stimulants such as G-protein coupled receptor agonists, growth factors, cytokines, and various physicochemical stresses. Among the EGFR-ligands, heparin-binding EGF-like growth factor (HB-EGF) is a prominent ligand in our understanding of the pathophysiological roles of ectodomain shedding in cancer, wound healing, cardiac diseases, etc. Here we focus on ectodomain shedding of the EGF family ligands, especially HB-EGF by disintegrin and metalloproteases, which are not only key events of receptor cross talk, but also novel intercellular signaling by their carboxy-terminal fragments to regulate gene expression directly. (*Cancer Sci* 2008; 99: 214–220)

Growth factors and their specific cell surface receptors harboring a tyrosine kinase activity are providing us unfathomable knowledge in understanding development, homeostasis and diseases. Epidermal growth factor (EGF) and its specific receptor (EGFR) and their relative members have been at the forefront of signaling and therapeutics. The EGFR family comprise four members: EGFR (ErbB1), ErbB2, ErbB3 and ErbB4; and the EGF family comprises 13 members: EGF, transforming growth factor-α (TGF-α), amphiregulin, heparin-binding EGF-like growth factor (HB-EGF), betacellulin, epiregulin, epigen, neuregulin-1 (NRG-1), NRG-2, NRG-3, NRG-4, NRG-5 and NRG-6.[1–20] Under physiological conditions, activation of EGFRs is controlled by the spatiotemporal expression and post-translational processing of the ligands. Recently, interreceptor cross-talk has received significant attention as an essential element in understanding the increasingly complex signaling networks identified within cells. Among them, transactivation of EGFRs has been shown to play a crucial role in signaling by G-protein coupled receptors (GPCRs), cytokine receptors, receptor tyrosine kinases, and integrins to a variety of cellular responses.[21,22] Transactivation of EGFRs is mediated, at least in some cases, by the ligands, which are cleaved from their membrane-anchored forms (proforms) in a process termed 'ectodomain shedding'. Research on the transactivation mechanisms of EGFRs is not only driving forward functional analyses of 'the ectodomain shedding' of the ligands by a disintegrin and metalloproteases (ADAMs), but also uncovering intriguing functions of carboxy-terminal fragments (CTFs) of the ligands, which are products of ectodomain shedding. Currently, we have advanced knowledge about the physiological significance of ectodomain shedding of proHB-EGF, which couples two independent signaling pathways, EGFR signaling by the shed extracellular domain and CTF signaling by the remnant transmembrane peptide.[23,24]

In this review, we briefly summarize the current knowledge of the EGF and EGFR families in cancer research and in clinical settings, and would like to focus on a novel function of the EGFR ligands themselves beyond the concept of 'growth factor' in general.

The EGF and the EGFR families and their current advance in cancer

Epidermal growth factor receptor is a170-kDa membrane protein first identified as a binding partner of ^{125}I-labeled EGF on cell surface of fibroblasts,[25] and was found to be bearing tyrosin-kinase activity,[26] and phosphorylated itself (autophosphorylation) in response to EGF treatment in A-431 epidermoid carcinoma cells.[27] In the 1980s, the overexpression of EGFR in various epithelial tumors was widely reported and it has been substantiated that deregulation of EGFR itself and its signaling pathway plays an important role in human cancers. For example, EGFR mutations in human cancers have been analyzed intensively and found that the mutations resulted in increased tyrosine kinase activity of EGFR[28] and the increased stimulation of EGFR through autocrine growth factor loops, in particular, through TGF-α.[29] Excessive signaling of EGFR is a hallmark of a wide variety of solid tumors. The continuous massive studies have revealed four members of the EGFR family and 13 members of their ligand family (the EGF family) described above. Amplification of EGFRs, for instance, was found in the majority of carcinomas, and amplification of the ErbB2d gene can be found in 20–30% of metastatic breast lesions. Thus, EGFRs are attractive candidates for targeted therapy, and, to date, anti-EGFR and anti-ErbB2 therapeutics have been developed, and some of them are in clinical use by using humanized neutralizing antibodies and synthetic small compounds of tyrosine kinase inhibitors (TKIs) (Table 1).[30–32] The recent clinical application of EGFR-TKIs was prospective well for the patients with the high levels of EGFR expression in non-small cell lung cancers (NSCLCs). Unexpectedly, the response for

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Table 1. Approved epidermal growth factor receptors (EGFRs) targeted therapeutics

Drug	Target	Type	Application
Cetuximab (Erbitux)	EGFR	Chimeric mAb	CRC, and head and neck cancer
Panitumumab (Vecitibix, ABX-EGF)	EGFR	Fully human mAb	CRC
Gefinitib (Iressa, ZD1839)	EGFR	TKI	NSCLC
Erlotinib (Tarceva)	EGFR	TKI	NSCLC and panvreas cancer
Trastuzumab (Herceptin)	ErbB2	Humanized mAb	Breast cancer

CRC, colorectal cancer; mAb, monoclonal antibody; NSCLC, non-small cell lung carcinoma; TKI, tyrosine kinase inhibitor.

EGFR-TKIs in patients was quite low. These results have shown that the response for EGFR-TKIs is not correlated to the expression level of EGFR. However, correlation analyses between mutations in the EGFR gene and response for an EGFR-TKI gefitinib in clinical samples are providing intriguing results showing that the patient response was correlated with somatic mutations (either small, in-frame deletions or amino acid substitutions) in the EGFR kinase domain to enhance kinase activity,[33,34] and that in contrast, a single somatic mutation, T790M, of the EGFR kinase domain confers resistance to gefitinib treatment in NSCLC.[35] Subsequently, it was found that this mutation is associated with inherited susceptibility to lung cancer.[36] These reports strongly indicate that *EGFR* mutation analysis is helpful to predict sensitivity to gefitinib.

On the other hand, accurate prediction of the side-effects of TKIs is a big issue for developing more potent and safe drugs. For this purpose, it is becoming a focal point to profile the specificity of newly developed TKIs using kinase protein panels that are commercially available (http://www.carnabio.com/japanese/).

Ectodomian shedding of the ligands and EGFR transactivation in cancer

All members of the EGF family are type I transmembrane proteins and are expressed on cell surfaces that can be cleaved by cell surface proteases.[1–19,37,38] However, in some instances (NRG-1beta1 cleavage in Golgi apparatus),[39] there is a step that leads to the release of soluble ligands. This cleavage, termed ectodomain shedding, is a crucial step in the control of ligand availability and receptor activation. EGFRs are often constitutively stimulated in cancer owing to the presence of EGF ligands in the tumors.[40] The production of soluble EGF family ligands through ectodomain shedding occurs in response to various physiological and pharmacological agonists, including 12-O-tetradecanoylphorbol-13-acetate,[41,42] calcium ionophores,[43] GPCR ligands such as bombesin,[44] angiotensin II,[45] and bacterial lipoteicchoic acid,[46] and cytokines and growth factors.[47,48] These shedding stimuli evoke cleavage and release of soluble ligands, mostly HB-EGF, leading to the rapid phosphorylation of EGFR and the following activation of intracellular signaling pathways. This process, termed EGFR transactivation, has important biological implications in normal and cancer cells.[30,31] Therefore, it is essential to understand the mechanisms and functional potency of ectodomain shedding in the following three aspects: (i) what kinds of proteases catalyze the ectodomain shedding; (ii) how the responsible proteases are regulated by extracellular and intracellular signals; and (iii) what are the priority proteases and regulatory molecules for molecular-based therapeutic drug development? Most proteases involved in the ectodomain shedding belong to the metalloproteases ADAMs family, and have also been examined in tumor cells. ADAMs, including ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17, ADAM 19, ADAM 28, ADAMTS1, ADAMTS4 and ADAMTS5 have been associated with various types of cancer.[49,50] Most of them are involved in the shedding of distinct EGFR ligands. Activation mechanisms of ADAMs are still largely unknown, but some clues to elucidate the mechanisms have been raised from the aspects regarding phosphorylation and interactive proteins of ADAM tails,[51] and G-protein regulation.[52] Protease activities of ADAMs are multiple-regulated, not only by regulatory domains such as the pro-domain and cyotoplasmic domains, but also by intrinsic metalloprotease inhibitors such as tissue inhibitor of metalloprotease,[53] and reversion-inducing-cysteine-rich protein with kazal motifs.[54] Small compound inhibitors of ADAMs have been developed in several industries, and proved to be potent drugs for some diseases including cancer in mouse models. However, it is still far away from clinical application for cancer treatment. As an alternative way, a siRNA strategy for targeting ADAMs might be favorable. Much more precise studies of intracellular mechanisms underling ADAM activation by extracellular stimuli would provide us with more fascinating ways to develop ADAM inhibitors as a drug in the future. The molecular mechanism underlying the ligand shedding-dependent EGFR transactivation mediated by ADAM activation is summarized in Fig. 1.

Pathophysiology in disruption of proHB-EGF shedding

Heparin-binding EGF-like growth factor is a leading molecule that has been linked to ligand shedding and EGFR transactivation, and is most intensively analyzed in its biological relevance in shedding *in vitro* and *in vivo*. Ligand shedding-dependent EGFR transactivation was first described following activation of GPCR,[55] and proHB-EGF itself has been proved to be a crucial ligand and plays a central role in EGFR transactivation under various extracellular stimuli so far.[56,57]

Keratinocytes are the source of numerous growth factors (among which HB-EGF is prominent) and play a central role in re-epithelialization in skin wound healing in an autocrine manner as a migration factor, as well as a mitogen.[58,59] Wounding stimuli evoked proHB-EGF shedding enough for healing induction, which was abrogated by metalloprotease inhibitors to block the shedding.[59] Aberration of the shedding of proHB-EGF in soluble HB-EGF and uncleavable proHB-EGF knock-in mice resulted in the severely abnormal developments of skin and heart.[60] We also observed that skin wound healing is obviously retarded by the absence of keratinocyte migration in conditional *Hb-egf–/–* mice.[61] Over half of HB-EGF-null mice die during the first postnatal week, and survivors have dysfunctional hearts with grossly enlarged ventricular chambers and reduced life spans.[62,63] Newborns lacking HB-EGF have enlarged and malformed semilunar and atrioventricular heart valves. Furthermore, these phenotypes are similar to those observed in mice expressing an uncleavable form of proHB-EGF (HB^{uc} mice) generated by targeted gene replacement.[60] The HB^{uc} mice showed dilated cardiomyopathy in adult. Interestingly, dysregulated secretion of HB-EGF in mice carrying transmembrane domain-truncated mutant of proHB-EGF (HB^{Dtm} mice) caused ventricular hypertrophy in heart.[60] These findings indicate that the balanced shedding of proHB-EGF is essential for the heart development and maintenance of the cardiac function,[57,64] and that proHB-EGF shedding is strictly controlled during development.

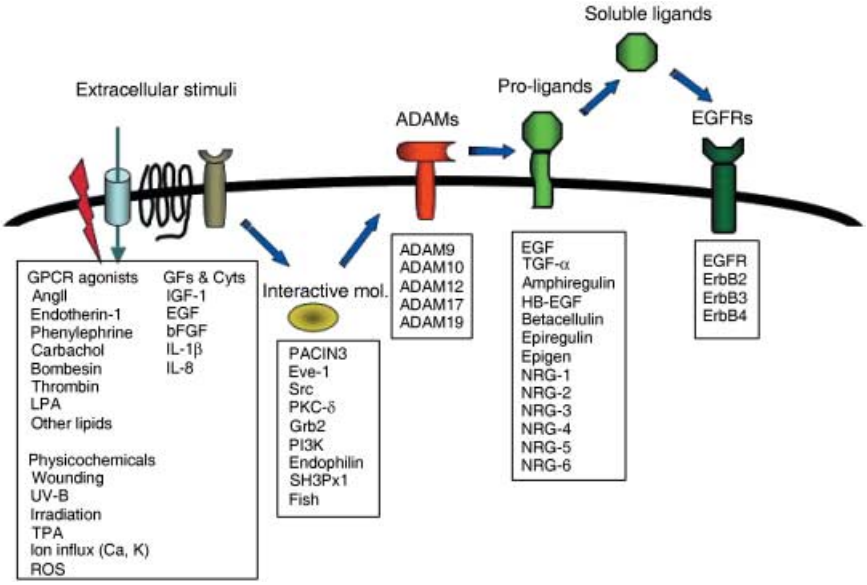

Fig. 1. Ligand-shedding dependent epidermal growth factor receptor (EGFR) transactivation. Disintegrin and metalloprotease (ADAM) proteins are activated by various stimuli including wounding, ion influx, G-protein coupled receptor (GPCR) signaling, growth factor and cytokine signaling, protein kinase C (PKC) activation, and binding of cytoplasmic interactive proteins. EGFR ligand molecules are proteolytically cleaved by specific metalloprotease-activity of ADAMs, resulting in the production of soluble ligands and stimulation of EGFR in autocrine and paracrine manners. AngII, angiotensin II; Cyts, cytokines; GFs, growth factors; HB-EGF, Heparin-binding epidermal growth factor carboxy-terminal fragment; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; NRG, neuregulin; PI3K, phosphatidyl inositol 3-kinase; PKC, protein kinase C; ROS, reactive oxygen species; TGF-α, transforming growth factor-α; TPA, 12-O-tetradecanoylphorbol-13-acetate.

Ectodomain shedding of proHB-EGF is induced by various stimuli including phorbol ester TPA,[42,65] calcium ionophore,[43] and various growth factors and cytokines.[47,48] Protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) are involved in the intracellular signaling pathway for proHB-EGF processing.[66,67] GPCR agonists also stimulate proHB-EGF processing, which mediates EGFR transactivation by GPCR signaling.[44] The transactivation of EGFR induced, for example, by endothelin I, thrombin, lysophosphatidic acid (LPA), carbacol,[44] insulin-like growth factor-1 (IGF-1),[68] basic fibroblast growth factor (bFGF), EGF,[48] interleukin-8 (IL-8),[47] estrogen,[69,70] angiiotensin II, phenylephrine,[45,71,72] *Helicobacter pylori*,[73] α_2-adrenergic receptor agonists,[74] bacterial lipoteichoic acid,[46] epoxyeicosatrienoic acid,[75] and ultraviolet-B,[76] also apparently depends on proHB-EGF shedding.

Metalloproteases are responsible for the proteolytic cleavage of proHB-EGF as well as the other EGF family members because the ectodomain shedding of proHB-EGF is efficiently inhibited by various metalloprotease inhibitors. Multiple members of the ADAM family are mainly implicated as shedding enzymes of proHB-EGF. The ADAMs are characterized by a conserved domain structure, consisting of an N-terminal signal sequence followed by pro-domain, metalloprotease and disintegrin domains, a cycteine-rich region, usually containing an EGF repeat, and finally a transmembrane domain and cytoplasmic tail.[77,78] The overexpression of ADAM9 resulted in the increased shedding of proHB-EGF without TPA and ADAM9 mutants of the metalloprotease domain inhibited TPA-induced shedding in VeroH cells.[67] TPA-dependent proHB-EGF shedding, however, remains unaffected in embryonic fibroblasts derived from mice lacking ADAM9.[79] ADAM12 expression promoted proHB-EGF shedding and the overexpression of dominant-negative (metalloprotease domain deleted) ADAM12 but not ADAM9 abrogated the shedding by TPA treatment in HT1080 cells. Exogenous expression of dominant-negative ADAM12 inhibited phenylephrine-induced proHB-EGF shedding in cardiomyocytes.[45] TPA-dependent proHB-EGF shedding was largely impaired in embryonic fibroblasts derived from mice lacking ADAM12,[80] and ADAM17.[81,82] Transfection of wild-type ADAM10, but not metalloprotease domain deleted ADAM10 stimulated the release of soluble HB-EGF in COS7 cells,[83] and ADAM10 was implicated in proHB-EGF shedding and EGFR transactivation mediated by IL-8,[46] and bacterial lipoteichoic acid induced GPCR signaling.[47] Reintroduction of ADAM17 into the immortalized fibroblasts derived from the mice eliminated zinc-binding domain of ADAM17 resulted in the increased shedding of proHB-EGF.[81,82]

The mechanism of ADAM activation has not yet been elucidated. However, we have identified two ADAM12-docking proteins; Eve-1,[84] and PACSIN3,[85] that upregulate TPA-induced ADAM12 activation. Both Eve-1 and PACSIN3 contain Src homology 3 (SH3) domains that can interact with the proline-rich motifs of the ADAM12 cytoplasmic domain. Knockdown of each docking protein significantly reduces TPA- and angiotensin II-induced proHB-EGF shedding. Further, it has been reported that nardilysin (*N*-arginine dibasic convertase (NRDc)), a metalloendopeptidase of the M16 family, specifically binds proHB-EGF and enhances its shedding in cooperation with ADAM17.[86,87]

HB-EGF-CTF signaling beyond RTK activation

The ectodomain shedding of proHB-EGF actually produces two fragments: an extracellular fragment (HB-EGF) and a remnant fragment (HB-EGF-CTF). Recently we have identified promyelocytic leukemia zinc finger (PLZF) and B-cell leukemia 6 (Bcl6) proteins as a binding protein of the cytoplasmic tail of proHB-EGF.[23,88] PLZF and Bcl6 are transcriptional repressors with structure domain homology of BR-C, Itk, and kab and C2H2-type Zn-finger suppress cyclin A, c-myc and HoxD, and macrophage inflammatory protein 1-α, CD69 and CyclinD2 expression, respectively.[89–94] These repressors negatively regulate the cell cycle. PLZF and Bcl6 produce transcriptional repression through recruitment of a repressor complex that contains N-CoR, SMRT, Sin3a, and histone deacetylases.[95–102] HB-EGF-CTF generated by proHB-EGF shedding still contains a transmembrane region and is translocated from the plasma membrane into the reticular network of the endoplasmic reticulum (ER) and the nuclear envelope by retrograde membrane trafficking (Hieda and Higashiyama, 2007, unpublished observation). Since this process is impaired by the inhibition of metalloprotease activity, translocation of HB-EGF-CTF into the nuclear envelope is a shedding-dependent event. HB-EGF-CTF has been visualized in the inner nuclear envelope under an electron microscope. Internalized HB-EGF-CTF associates with nuclear PLZF and Bcl6, which might occur at the nuclear periphery. One intriguing paper has just been published showing that proHB-EGF itself upregulated E-cadherin expression through suppression of ZEB1, a Zn-finger typed transcriptional repressor, in pancreatic cells[103] although the mechanism underlying such regulation was not

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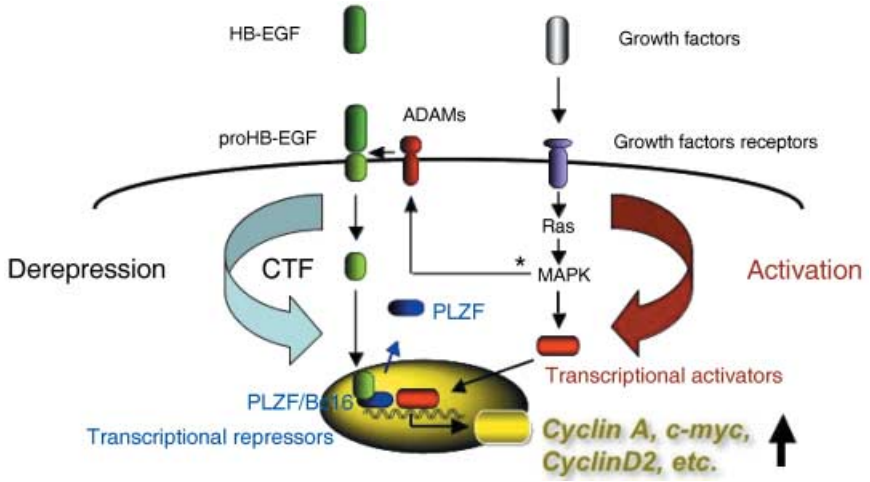

Fig. 2. Heparin-binding epidermal growth factor carboxy-terminal fragments (HB-EGF-CTF) signaling couples with growth factor receptor signaling. ProHB-EGF is shed by specific disintegrin and metalloproteases (ADAMs) and yield amino- and carboxy-terminal fragments (HB-EGF and HB-EGF-CTF). Produced HB-EGF binds and activates epidermal growth factor receptor (EGFR), resulting in the activation of mitogen-activated protein kinase (MAPK) cascade and various gene transcription. HB-EGF-CTF translocates into the inner nuclear membrane. HB-EGF-CTF and HB-EGF generated by proHB-EGF shedding mediate signaling into the nucleus directly (CTF signaling) and indirectly via EGFR signaling, respectively. Derepression signaling by HB-EGF-CTF and activation signaling through EGFR are coupled, regulate gene transcription and result in cellular responses to various stimuli. PLZF, promyelocytic leukemia zinc finger. *Activation of ADAMs by MAPK pathways has been reported in Gechtman *et al.*[66] and Umata *et al.*[116]

discussed at all. We showed a possibility that proHB-EGF itself translocated into the inner nuclear membrane and regulated transcriptional repressor activities (Hieda and Higashiyama, 2007, unpublished observation). We speculate that suppression of ZEB1 might be directly regulated by the cytoplasmic domain of the nuclear-translocated proHB-EGF.

Various recent studies have shown transcriptionally silent genes are located at or translocated to the nuclear periphery upon silencing.[104,105] Indeed, interaction of HB-EGF-CTF with PLZF or Bcl6 results in the reversal of decreased expression of their target genes.[23,88] Whether HB-EGF-CTF containing a transmembrane domain would directly regulate genes silenced by PLZF and Bcl6 at the nuclear periphery is still under investigation.

Coupling of derepression signaling with growth signaling

In parallel with HB-EGF-CTF production, proHB-EGF shedding also generates HB-EGF, a soluble ligand of EGFR. EGFR signaling promotes G1-phase progression in the cell cycle by regulating the expression of cyclin D and c-Myc via the Ras-MAPK signaling cascade. Therefore, proteolytic cleavage of proHB-EGF by ADAMs generates two types of mitogenic signaling molecules, and the coordination of the dual intracellular signals mediated by HB-EGF and HB-EGF-CTF may be important for cell cycle progression.[23,57] In fact, EGFR and FGFR-mediated *c-Myc* induction and cell cycle progression in primary cultured mouse embryonic fibroblasts are abrogated by knockout of the *Hb-egf*, or by a metalloprotease inhibitor, although molecules downstream of the receptors are activated.[48] Induction of *c-Myc* expression by EGF or bFGF is recovered in *Hb-egf*-depleted mouse embryonic fibroblasts by overexpression of wild-type proHB-EGF, but no recovery was observed with an uncleavable mutant of proHB-EGF. The uncleavable mutant also inhibited EGF-induced acetylation of histone H3 at the mouse *c-Myc* first intron region, which could negatively affect transcriptional activation. Thus, the signal transduction initiated by generation of HB-EGF-CTF in the shedding event plays an essential intermediary role in growth factor induced cell cycle progression.

Promyelocytic leukemia zinc finger and Bcl-6 as well as HB-EGF are expressed in a large number of tissues including the heart.[106–108] This suggests that deregulation of PLZF and Bcl6 repressional activities by proHB-EGF shedding is involved in regulation of cell proliferation and differentiation in various signaling cascades during the development and maintenance of adult tissues. Heart failure observed in HB-EGF-null, HB^{uc} and HB^{Dtm} mice[60,62] might in part be due to the loss of HB-EGF-CTF-PLZF/Bcl6 signaling in the heart, where PLZF and Bcl6 are highly expressed and are suggested to be involved in maintaining cardiac function.[108] HB-EGF-CTF has the ability to interact with these transcriptional repressors, which suggests that the integration of EGFR members and CTF signaling leads to various cellular responses by controlling gene transcription (Fig. 2).

BAG-1 has been reported as the binding protein of the cytoplasmic domain of proHB-EGF, and its interaction with proHB-EGF leads to decreased cell adhesion, increased resistance to apoptosis, and rapid secretion of soluble HB-EGF.[109] BAG-1 is a multifunctional protein that interacts with a diverse array of molecular targets including the Bcl-2 apoptosis regulator, the 70 kDa heat shock proteins, Hsc70 and Hsp70, nuclear hormone receptors, the RAF kinase, components of the ubiquityinylation/proteasome machinery, and DNA.[110] While BAG-1 was first identified as a proHB-EGF cytoplasmic binding protein in the cytoplasm and plasma membrane,[109] BAG-1 L, an isofom of BAG-1, is localized in the nuclei of some types of cells.[111,112] These findings suggest that BAG-1 is a binding protein of nuclear HB-EGF-CTF and mediates the CTF signaling by proteolytic processing of proHB-EGF.

Potential roles of cytoplasmic domains of EGF family members

Membrane-anchored growth factors and their receptors provide the potential for bidirectional signaling, with a forward signal (receptor activation) mediated by the ectodomain and a reverse signal mediated by the intracellular domain of the ligand precursor. In the EGF family molecules, proTGF-α activates an unidentified kinase in cells expressing it, when the precursor engages EGFR on neighboring cells.[113] Recently, it has been reported that NRG-1 is cleaved at the transmembrane domain and the released intracellular domain (NRG-1-ICD) enters the nucleus to repress the expression of several regulators of apoptosis.[114] Currently, the same group has shown that in the mouse cochlea, synaptic activity increases the level of nuclear NRG-ICD and upregulates postsynaptic density protein-95 (PSD-95), a scaffolding protein that is enriched in postsynaptic structures. NRG-ICD enhances the transcriptional activity of the PSD-95 promoter by binding to a zinc-finger transcription factor Eos. The NRG-ICD-Eos complex induces endogenous PSD-95 expression *in vivo* through a signaling pathway that is mostly independent of gamma-secretase regulation. This upregulation of PSD-95 expression by the NRG-ICD-Eos complex provides a molecular basis for activity-dependent synaptic plasticity.[115]

While the machinery of CTF signaling of proHB-EGF and NRG-1 seems to be different, these findings suggest that the

CTFs of the EGF family precursors have the ability to interact with transcriptional regulators in the nucleus and control gene expression in cells expressing the EGF family ligands. Indeed, we currently observed that other members of the EGF family are able to interact in many ways with transcriptional repressors (Morimoto and Higashiyama, 2007 unpublished observation).

Conclusion

Since EGF was discovered, EGF and its related ligands have been a driving force of the research of EGFRs and their signaling pathways for more than 40 years, which provides us tremendous information to understand growth mechanisms in normal and cancer cells. Therefore, we have paid little attention to the fact that all members of the EGF family are membrane-anchored proteins, because their properties as ligands to activate receptor tyrosine kinases are fascinating enough. However, the interest of researchers in CTFs produced by ectodomain shedding are revealing ingenious molecular mechanisms of the EGF family ligands as growth factors, and soon we will able to fully understand why the EGF family ligands are membrane-anchored factors. We believe that the knowledge obtained from the EGF family also provides new insights into the function of other membrane-anchored growth factors and cytokines, and is quite helpful in unveiling unique properties of cancer.

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