Published in final edited form as:

Semin Cell Dev Biol. 2014 April ; 0: 57–61. doi:10.1016/j.semcdb.2013.12.011.

Structure and function of epigen, the last EGFR ligand

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Abstract

Epigen is the latest addition to the mammalian family of EGFR ligands. Epigen was initially identified as a novel expressed sequence tag with homology to the EGF family by high throughput sequencing of a mouse keratinocyte complementary DNA library, and received its name for its ability to act as an epithelial mitogen. In vitro studies attributed to epigen several unique features, such as persistent and potent biological actions involving low affinity receptor binding, as well as sub-maximal receptor activation and inactivation. Similarly to the other EGFR ligands, the expression of epigen is up-regulated by hormones and in certain cancer types. While the biological functions of epigen remain to be uncovered, it appears to play a role in epidermal structures, such as the mammary gland and the sebaceous gland. The latter organ, in particular, was greatly enlarged in transgenic mice overexpressing epigen. Interestingly, mice lacking epigen develop and grow normally, probably due to functional compensation by other EGFR ligands. Future studies are likely to reveal the biological roles of the unique receptor binding properties of epigen, as well as its potential harnessing during disease.

Keywords

cancer; ERBB family; epigen; EGFR; gene knockout

1. Introduction

The first lines of evidence implicating secreted molecules in the regulation of cell growth emerged from studies performed in the 1950s by Rita Levi-Montalcini and Stanley Cohen [1]. Grafting a lump of a mouse sarcoma onto a chick embryo resulted in extensive attraction of nerve fibers to the lump. This observation led to the isolation of the first growth factor, nerve growth factor (NGF). Later studies by Stanley Cohen isolated, and fully sequenced, the epidermal growth factor (EGF) from the murine submaxillary gland [2, 3].

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EGF contains three disulfide bonds and an evolutionary conserved core structure of approximately 50 amino acids, which is shared by 10 additional mammalian growth factors, as well as by multiple virus-encoded molecules [4]. Later studies revealed that EGF, like other mammalian family members, is synthesized as a transmembrane precursor containing additional EGF-like motifs [5], however only the one adjacent to the membrane acts as a receptor-binding growth factor upon proteolytic processing of the large precursor [6]. Growth factor synthesis and secretion have ever been linked to pathological disorders, especially cancer. For example, early on Cohen and George Todaro reported that cells infected by the feline sarcoma virus lost their ability to bind EGF [7], an observation that led to the isolation from a murine sarcoma of two “transforming growth factors”, TGF-alpha (TGFA), the first kin of EGF, and TGF-beta, the founder of the TGF-beta/BMP (bone morphogenetic protein) family [8]. Another outcome of these studies has been the realization that growth factor secretion might be classified as a mechanism allowing activation of neighboring cells (paracrine loops), for example in embryonic inductive processes, and a mechanism of self-activation (autocrine loops), which is considered a major process supporting tumor progression [9].

In a similar manner, the cell surface receptors specific to EGF-like polypeptides have been linked to the initiation and progression of human malignancies and other pathological conditions. These are four transmembrane molecules that comprise the type I receptor tyrosine kinases, also called ERBB or HER (human EGF receptor) family [10]. The extracellular domains of the ERBB proteins are able to extend a dimerization arm, once they are occupied by a growth factor [11], thereby receptor dimers are induced upon binding of specific EGF-like ligands. Unlike the ectodomains, the more conserved cytoplasmic portions of ERBB proteins share a catalytic region, a tyrosine-specific kinase able to auto-phosphorylate and trans-phosphorylate other proteins. Kinase activation involves relieving the intrinsically auto-inhibited domain through intermolecular interactions and the formation of an asymmetric kinase dimer [12]. Importantly, both homodimerization and heterodimerization of ERBB proteins occur, such that non-catalytic regions that flank the tyrosine kinase domain and formation of distinct receptor dimers dictate the identity of proteins that undergo trans-phosphorylation and physical recruitment to the activated ERBB dimers [13]. Especially important is ERBB2/HER2 (also called NEU), which binds no ligand but can form relatively potent receptor heterodimers [14, 15]. Biased formation of ERBB2-containing heterodimers, along with their ability to evade negative feedback, such as receptor ubiquitination and degradation, are thought to underlay the transforming ability of the *ERBB2* gene in breast, gastric and other tumors that amplify the gene and/or overexpress the respective protein [16]. Similarly, heterodimer formation and constitutive, ligand-independent kinase activation, might explain the transforming function of certain EGFR/ERBB1 mutant proteins in lung, brain and other tumors [17–19].

2. Identification and characteristics of epigen

Epigen is the latest addition to the family of mammalian EGFR ligands [20]. Hence, it is the 11th member of the EGF-like family and the 7th ligand of EGFR. Epigen was first identified in 2001 by Lorna Strachan and colleagues [21]. Their high throughput sequencing of a mouse keratinocyte complementary DNA library revealed a novel expressed sequence tag

with homology to the EGF family. They named the encoded growth factor epigen, for its ability to act as an epithelial mitogen. The 152 amino acids murine pro-epigen molecule contains the characteristic signal sequence and a transmembrane domain. Northern blotting indicated that epigen is present in testis, heart, and liver. Interestingly, in order to induce comparable proliferation of HaCaT keratinocytes, Strachan and collaborators needed to increase epigen concentration by 10- or 100-fold higher than TGFA or EGF, respectively. Bose Kochupurakkal and colleagues re-discovered epigen while addressing the potential existence of a direct ligand for ERBB2 [22]. To this end, they applied algorithms based on genomic and cDNA structures and re-identified all known EGF-like growth factors, including epigen, but failed to identify novel, ERBB2-specific factors. In line with the results obtained by Strachan et al., recombinant epigen stimulated proliferation of cells engineered to express EGFR, either alone or in combination with ERBB2. Strikingly, when tested at high concentrations epigen's activity was more potent than the maximal mitogenic action obtained with EGF or TGFA. Moreover, ligand displacement analyses attributed to epigen an approximately 100-fold less potent binding to EGFR. The anomalous mitogenic and binding activities of epigen were attributed by the authors to inefficient receptor ubiquitination and endocytosis.

The above-described initial studies of epigen clearly distinguished it from the high-affinity group of EGF-like peptides, and characterized it as a low-affinity ligand. The notion that the eleven mammalian EGF-like ligands actually fall into two functionally distinct groups emerged from studies performed with several synthetic variants of pox viral ligands [23], although the initial isolation of amphiregulin already noted a discrepancy between bioactivity and binding affinity [24]. The causative agents of smallpox, DNA poxviruses, depend on virus-encoded EGF-like growth factors able to bind with relatively low-affinity to mammalian ERBB proteins. Interestingly, the growth factors of Shope fibroma virus, myxoma virus and vaccinia virus (SFGE, MGF and VGF, respectively) display unique patterns of receptor specificity; whereas SFGE is a broad-specificity ligand, VGF binds primarily to EGFR homodimers, and the exclusive receptor for MGF is a heterodimer comprised of ERBB2 and ERBB3. In spite of 10- to 1000-fold lower binding affinity to the respective receptors, the viral ligands are mitogenically equivalent or even more potent than their mammalian counterparts, and as in the case of epigen, the anomaly might be ascribed to attenuation of receptor degradation and ubiquitination. As a result, the low-affinity ligands induce sustained signal transduction downstream of the cognate receptor, but their extracellular concentration remains relatively high due to ineffective endocytosis.

3. Chromosomal localization and gene structure

In similarity to all other EGF-like genes, but the genes encoding for neuregulins 1 and 2, the open reading frame of epigen is spread into two exons: the first encodes the amino-terminal part (four cysteines) and the other encodes for the rest of the molecule (cysteines 5 and 6). The open reading frame of epigen is most related to that of another low-affinity ligand, epiregulin. In support of a common origin and late duplication of an ancestral chromosomal region, the two genes are co-aligned, co-locate at the long arm of human chromosome 4 (4q21), next to the amphiregulin locus, and their open reading frames are separated by a

mere 25 kilobase pairs [22]. This pattern is conserved in the mouse genome [25], in line with a gene duplication that preceded diversion of primates from rodents.

4. Messenger RNA and protein synthesis and processing

Due to alternative splicing, several *EPGN* transcript isoforms have been described. The canonical sequence encompasses 2,695 nucleotides and the protein coding sequence is located within nucleotides 65-526. Translation produces a 154 amino acid-long human protein, composed of a signal peptide, an extracellular region (containing the EGF-like domain), a transmembrane region, and a 58 amino acid cytoplasmic tail (Figure 1). The EGF-like domain of epigen consists of 41 amino acids (residues 56-96) and, in similarity to all EGFR ligands, it is characterized by three disulfide bonds formed by six cysteines (the pairs are 60-73, 68-84, and 86-95). Two potential N-glycosylation sites have been identified at positions 37 and 41, but the significance of such a posttranslational modification of epigen remains unexplored. Another modification, cleavage by ADAM17, a member of the family of membrane-anchored metalloprotease, releases the extracellular portion of pro-epigen from its transmembrane anchor [26]. Proteolytic processing by an ADAM (a disintegrin and metalloprotease) regulates the bioavailability of several other EGFR-ligands. In the case of epigen, ectodomain shedding can be stimulated by phorbol esters, phosphatase inhibitors and calcium influx (see Figure 2).

5. Receptor binding characteristics

A blocking monoclonal antibody to EGFR/ERBB1 was able to inhibit epigen-induced growth of human keratinocytes, and treatment of cells with epigen induced tyrosine phosphorylation of EGFR [21]. Consistent with the conclusion that EGFR acts as a direct receptor for epigen, testing this ligand on a series of interleukin-3-dependent myeloid cells ectopically expressing single ERBB family members, or pairs of two receptors, confirmed specificity to EGFR [22]. Moreover, neither ERBB3 nor ERBB4 displayed responses to epigen when singly expressed, and in line with the augmenting role for ERBB2, co-expression of EGFR and ERBB2 allowed epigen to exert more potent mitogenic signals in the set of engineered myeloid cells. Importantly, the binding affinity of epigen is 10- to 100-fold lower than that of EGF and TGFA, and this translated to weaker auto-phosphorylation of EGFR. Nevertheless, epigen exerted more potent mitogenic effects than EGF. These properties of epigen are shared with epiregulin [27] and with pox viral, EGF-like ligands [23]. The enhanced potency of epigen might be due, in part, to dissociation of the ligand at the acidic environment of endosomes, as proposed for TGFA [28]. In addition, the underlying mechanism might entail ineffective uptake of the ligand and long half-life in the circulation, in similarity to an engineered long-lasting mutant of EGF [29]. This feature is likely enhanced by ineffective receptor ubiquitination and degradation of epigen-stimulated EGFRs [22].

6. Context-dependent expression and putative biological functions

Initially, epigen was shown to be expressed in the liver, heart, and testes, and at lower levels in lung and kidney [21]. Further studies indicated a wider expression pattern, including the endocrine pancreas [30], the outer and inner root sheaths of the hair follicle and in dorsal

root ganglia [22]. In the latter study, EPGN expression was observed in infiltrating epithelial cells of invasive adenocarcinomas of the breast and prostate [22]. Additional studies also showed that epigen is overexpressed in human bladder cancer [31] and in breast cancer specimens [32]. A recent study revealed that the combined expression level of epigen and TGFA had the highest predictive potential for the growth inhibitory activity of cetuximab, a monoclonal antibody used to treat colorectal and head/neck tumors, when tested on a panel of cell lines derived from head and neck squamous cell tumors [33]. If confirmed in clinical samples, this finding may be of importance for anticipating the therapeutic success of cetuximab in cancer patients. In contrast, among the family of EGF-like ligands, epigen was shown to play a minor role in the pathogenesis of peripheral nerve sheath tumors [34]. Interestingly, epigen expression was up-regulated by ectodysplasin in skin [35] and in mammary glands [36], in the hyperplastic skin of fatty acid transport protein 4-deficient mice [37], and in the enlarged sebaceous glands of fatty acid 2-hydroxylase-deficient mice [38]. Supporting the latter findings, we reported that overexpression of epigen in transgenic mice results in giant sebaceous glands (see below). While epigen seems to play a major role in skin, the available experimental data indicate that additional organs may be under the influence of this growth factor. Epigen was identified as a target of the luteotropic hormone in ovarian granulosa cells [39] and of interleukin-13 in primary airway epithelial cells [40].

7. Transgenic and knockout animal models

In order to study the biological functions of epigen, one of us generated transgenic mice overexpressing this EGFR ligand under the control of the ubiquitously active chicken β -actin promoter. The most prominent phenotype of mice overexpressing epigen was an enlargement of sebaceous glands and increased sebum production [41]. A role for epigen in sebaceous gland pathophysiology has been supported by the significant up-regulation of epigen in an independent mouse model showing enlarged sebaceous glands [38]. In addition to sebaceous gland hyperplasia, transgenic mice overexpressing epigen exhibited a progressive demyelinating neuropathy, pointing to a role in the peripheral nervous system [41]. Mice lacking epigen develop and grow normally, probably due to functional compensation by other EGFR ligands [41].

8. Conclusions

While the well-established redundancy of EGFR-specific ligands [42] is reflected also in the phenotype of epigen-depleted animals [41], in vitro studies attributed to epigen several unique features, such as persistent and potent biological actions involving low affinity receptor binding, as well as sub-maximal receptor activation and inactivation [22]. It is presently unclear how the lasting actions of epigen are harnessed by normal physiology and possibly during pathological processes. Presumably, the identity of amino acid sequences flanking the EGF-like domain of epigen determine accessibility to enzymes that process pro-epigen, in similarity to other family members [43], and both the promoter and 3'-untranslated region of the respective gene confer specific attributes to the encoded growth factor. These features might not be revealed unless a pathological situation is encountered. For example, another low affinity ligand, amphiregulin, displays specific functions in liver

regeneration [44] and in fibrosis [45]. Future studies will likely reveal similar disease-related harnessing of the long lasting biological actions of epigen.

Acknowledgments

Yosef Yarden is the incumbent of the Harold and Zelda Goldenberg Professorial Chair in Molecular Cell Biology. His research is carried out at the Marvin Tanner Laboratory for Research on Cancer, and his studies are supported by the European Research Council (ERC), the National Institutes of Health (NIH-NCI), the Seventh Program of the European Commission, the German-Israel Project Cooperation (DIP), the Israel Cancer Research Fund (ICRF), the US-Israel and Germany-Israel Binational Foundations (BSF and GIF), the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, and the M.D. Moross Institute for Cancer Research.

Marlon R. Schneider is at the Ludwig-Maximilians-University in Munich, Germany. His research is supported by the German Research Foundation (DFG), the Else-Kröner-Fresenius Stiftung, the Fritz Thyssen Foundation, and the Cicatricial Alopecia Research Foundation.

References

1. Cohen S, Levi-Montalcini R, Hamburger V. A nerve growth-stimulating factor isolated from sarcoma AS 37 and 180. *Proc Natl Acad Sci USA*. 1954; 40:1014–8. [PubMed: 16589582]
2. Savage CR Jr, Hash JH, Cohen S. Epidermal growth factor. Location of disulfide bonds. *The Journal of biological chemistry*. 1973; 248:7669–72. [PubMed: 4750422]
3. Savage CR Jr, Inagami T, Cohen S. The primary structure of epidermal growth factor. *The Journal of biological chemistry*. 1972; 247:7612–21. [PubMed: 4636327]
4. Brown JP, Twardzik DR, Marquardt H, Todaro GJ. Vaccinia virus encodes a polypeptide homologous to epidermal growth factor and transforming growth factor. *Nature*. 1985; 313:491–2. [PubMed: 3871510]
5. Gray A, Dull TJ, Ullrich A. Nucleotide sequence of epidermal growth factor cDNA predicts a 128,000-molecular weight protein precursor. *Nature*. 1983; 303:722–5. [PubMed: 6304537]
6. Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, Sela M, et al. The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96:4995–5000. [PubMed: 10220407]
7. Todaro GJ, De Larco JE, Cohen S. Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor to cells. *Nature*. 1976; 264:26–31. [PubMed: 187944]
8. Roberts AB, Anzano MA, Lamb LC, Smith JM, Frolik CA, Marquardt H, et al. Isolation from murine sarcoma cells of novel transforming growth factors potentiated by EGF. *Nature*. 1982; 295:417–9. [PubMed: 6276769]
9. Sporn MB, Todaro GJ. Autocrine secretion and malignant transformation of cells. *The New England journal of medicine*. 1980; 303:878–80. [PubMed: 7412807]
10. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001; 2:127–37. [PubMed: 11252954]
11. Burgess AW. EGFR family: structure physiology signalling and therapeutic targets. *Growth Factors*. 2008; 26:263–74. [PubMed: 18800267]
12. Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell*. 2006; 125:1137–49. [PubMed: 16777603]
13. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol*. 2009; 21:177–84. [PubMed: 19208461]
14. Wada T, Qian XL, Greene MI. Intermolecular association of the p185neu protein and EGF receptor modulates EGF receptor function. *Cell*. 1990; 61:1339–47. [PubMed: 1973074]
15. Goldman R, Levy RB, Peles E, Yarden Y. Heterodimerization of the erbB-1 and erbB-2 receptors in human breast carcinoma cells: a mechanism for receptor transregulation. *Biochemistry*. 1990; 29:11024–8. [PubMed: 1980216]

16. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987; 235:177–82. [PubMed: 3798106]
17. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:13306–11. [PubMed: 15329413]
18. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004; 304:1497–500. [PubMed: 15118125]
19. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *The New England journal of medicine*. 2004; 350:2129–39. [PubMed: 15118073]
20. Schneider MR, Wolf E. The epidermal growth factor receptor ligands at a glance. *J Cell Physiol*. 2008; 218:460–6. [PubMed: 19006176]
21. Strachan L, Murison JG, Prestidge RL, Sleeman MA, Watson JD, Kumble KD. Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *The Journal of biological chemistry*. 2001; 276:18265–71. [PubMed: 11278323]
22. Kochupurakkal BS, Harari D, Di-Segni A, Maik-Rachline G, Lyass L, Gur G, et al. Epigen, the last ligand of ErbB receptors, reveals intricate relationships between affinity and mitogenicity. *The Journal of biological chemistry*. 2005; 280:8503–12. [PubMed: 15611079]
23. Tzahar E, Moyer JD, Waterman H, Barbacci EG, Bao J, Levkowitz G, et al. Pathogenic poxviruses reveal viral strategies to exploit the ErbB signaling network. *EMBO J*. 1998; 17:5948–63. [PubMed: 9774339]
24. Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ. Structure and function of human amphiregulin: a member of the epidermal growth factor family. *Science*. 1989; 243:1074–6. [PubMed: 2466334]
25. Pathak BG, Gilbert DJ, Harrison CA, Luetke NC, Chen X, Klagsbrun M, et al. Mouse chromosomal location of three EGF receptor ligands: amphiregulin (Areg), betacellulin (Btc), and heparin-binding EGF (Hegfl). *Genomics*. 1995; 28:116–8. [PubMed: 7590736]
26. Sahin U, Blobel CP. Ectodomain shedding of the EGF-receptor ligand epigen is mediated by ADAM17. *FEBS Lett*. 2007; 581:41–4. [PubMed: 17169360]
27. Shelly M, Pinkas-Kramarski R, Guarino BC, Waterman H, Wang LM, Lyass L, et al. Epiregulin is a potent pan-ErbB ligand that preferentially activates heterodimeric receptor complexes. *The Journal of biological chemistry*. 1998; 273:10496–505. [PubMed: 9553109]
28. Ebner R, Derynck R. Epidermal growth factor and transforming growth factor- α : differential intracellular routing and processing of ligand-receptor complexes. *Cell Regul*. 1991; 2:599–612. [PubMed: 1777504]
29. Reddy CC, Niyogi SK, Wells A, Wiley HS, Lauffenburger DA. Engineering epidermal growth factor for enhanced mitogenic potency. *Nature biotechnology*. 1996; 14:1696–9.
30. South JC, Blackburn E, Brown IR, Gullick WJ. The neuregulin system of ligands and their receptors in rat islets of langerhans. *Endocrinology*. 2013; 154:2385–92. [PubMed: 23610133]
31. Amsellem-Ouazana D, Bieche I, Tozlu S, Botto H, Debre B, Lidereau R. Gene expression profiling of ERBB receptors and ligands in human transitional cell carcinoma of the bladder. *The Journal of urology*. 2006; 175:1127–32. [PubMed: 16469638]
32. McIntyre E, Blackburn E, Brown PJ, Johnson CG, Gullick WJ. The complete family of epidermal growth factor receptors and their ligands are co-ordinately expressed in breast cancer. *Breast cancer research and treatment*. 2010; 122:105–10. [PubMed: 19760033]
33. Oshima G, Wennerberg J, Yamatodani T, Kjellen E, Mineta H, Johnsson A, et al. Autocrine epidermal growth factor receptor ligand production and cetuximab response in head and neck squamous cell carcinoma cell lines. *Journal of cancer research and clinical oncology*. 2012; 138:491–9. [PubMed: 22193422]

34. Byer SJ, Brossier NM, Peavler LT, Eckert JM, Watkins S, Roth KA, et al. Malignant peripheral nerve sheath tumor invasion requires aberrantly expressed EGF receptors and is variably enhanced by multiple EGF family ligands. *J Neuropathol Exp Neurol*. 2013; 72:219–33. [PubMed: 23399900]
35. Lefebvre S, Fliniaux I, Schneider P, Mikkola ML. Identification of ectodysplasin target genes reveals the involvement of chemokines in hair development. *J Invest Dermatol*. 2012; 132:1094–102. [PubMed: 22277947]
36. Voutilainen M, Lindfors PH, Lefebvre S, Ahtiainen L, Fliniaux I, Rysti E, et al. Ectodysplasin regulates hormone-independent mammary ductal morphogenesis via NF-kappaB. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:5744–9. [PubMed: 22451941]
37. Lin MH, Chang KW, Lin SC, Miner JH. Epidermal hyperproliferation in mice lacking fatty acid transport protein 4 (FATP4) involves ectopic EGF receptor and STAT3 signaling. *Dev Biol*. 2010; 344:707–19. [PubMed: 20513444]
38. Maier H, Meixner M, Hartmann D, Sandhoff R, Wang-Eckhardt L, Zoller I, et al. Normal fur development and sebum production depends on fatty acid 2-hydroxylase expression in sebaceous glands. *The Journal of biological chemistry*. 2011; 286:25922–34. [PubMed: 21628453]
39. Carletti MZ, Christenson LK. Rapid effects of LH on gene expression in the mural granulosa cells of mouse periovulatory follicles. *Reproduction*. 2009; 137:843–55. [PubMed: 19225042]
40. Taniguchi K, Yamamoto S, Aoki S, Toda S, Izuhara K, Hamasaki Y. Epigen is induced during the interleukin-13-stimulated cell proliferation in murine primary airway epithelial cells. *Experimental lung research*. 2011; 37:461–70. [PubMed: 21867383]
41. Dahlhoff M, Schafer M, Wolf E, Schneider MR. Genetic deletion of the EGFR ligand epigen does not affect mouse embryonic development and tissue homeostasis. *Experimental cell research*. 2013; 319:529–35. [PubMed: 23142483]
42. Luetke NC, Qiu TH, Fenton SE, Troyer KL, Riedel RF, Chang A, et al. Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development*. 1999; 126:2739–50. [PubMed: 10331984]
43. Hinkle CL, Sunnarborg SW, Loiselle D, Parker CE, Stevenson M, Russell WE, et al. Selective roles for tumor necrosis factor alpha-converting enzyme/ADAM17 in the shedding of the epidermal growth factor receptor ligand family: the juxtamembrane stalk determines cleavage efficiency. *The Journal of biological chemistry*. 2004; 279:24179–88. [PubMed: 15066986]
44. Mitchell C, Nivison M, Jackson LF, Fox R, Lee DC, Campbell JS, et al. Heparin-binding epidermal growth factor-like growth factor links hepatocyte priming with cell cycle progression during liver regeneration. *The Journal of biological chemistry*. 2005; 280:2562–8. [PubMed: 15536070]
45. Zhou Y, Lee JY, Lee CM, Cho WK, Kang MJ, Koff JL, et al. Amphiregulin, an epidermal growth factor receptor ligand, plays an essential role in the pathogenesis of transforming growth factor-beta-induced pulmonary fibrosis. *The Journal of biological chemistry*. 2012; 287:41991–2000. [PubMed: 23086930]

Highlights

1. Epigen is the latest addition to the mammalian family of EGFR ligands, which includes six other growth factors.
2. Despite low affinity binding to EGFR, sub-maximal receptor activation by epigen delivers strong mitogenic signals.
3. Similarly to the other EGFR ligands, the expression of epigen is up-regulated by hormones and in certain cancer types.
4. Epigen functions in epidermal structures, such as the sebaceous gland, which was enlarged in mice overexpressing epigen.
5. Mice lacking epigen develop and grow normally, probably due to functional compensation by other EGFR ligands.

Figure 1. Domain structure of pro-epigen

The various domains of epigen are represented by boxes and the respective numbers of amino acids are indicated. These are the signal peptide at the N-terminus (residues 1-22), the extracellular domain (residues 23-110), which includes a glycosylation sub-domain, the EGF-like core of six cysteines and a short juxtamembrane domain. The cytoplasmic and transmembrane domains are also shown.

Figure 2. Processing of pro-epigen

The transmembrane precursor of epigen is schematically presented, including the three cysteine-cysteine bridges of the EGF-like domain (green circles). The site of cleavage by ADAM17 is indicated, but the identity of the protease that presumably cleaves at a site N-terminally to the EGF-like domain is yet unknown. The two putative glycosylation sites are marked by CHO.