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Epidermal growth factor, from gene organization to bedside

Fenghua Zeng[1] and **Raymond C. Harris**[1,2]

[1]Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

[2]Department of Veterans Affairs, Nashville, Tennessee

Abstract

In 1962, epidermal growth factor (EGF) was discovered by Dr. Stanley Cohen while studying nerve growth factor (NGF). It was soon recognized that EGF is the prototypical member of a family of peptide growth factors that activate the EGF receptors, and that the EGF/EGF receptor signaling pathway plays important roles in proliferation, differentiation and migration of a variety of cell types, especially in epithelial cells. After the basic characterization of EGF function in the first decade or so after its discovery, the studies related to EGF and its signaling pathway have extended to a broad range of investigations concerning its biological and pathophysiological roles in development and in human diseases. In this review, we briefly describe the gene organization and tissue distribution of EGF, with emphasis on its biological and pathological roles in human diseases.

Keywords

Epidermal growth factor; expression; cell proliferation; regeneration; ion transport; cancer

1. Introduction

EGF was discovered by Dr. Stanley Cohen more than half a century ago [1]. He found that injection of a submaxillary gland extract into newborn mice induced precocious eyelid opening and incisor eruption due to a direct stimulation of epidermal growth and keratinization. Consequently, EGF was isolated, purified, and characterized. It is a single-chain polypeptide consisting of 53 amino acids that is derived from the cleavage of a large precursor, prepro-EGF. Urogastrone, an inhibitor of gastric acid secretion, was independently isolated from human urine and was subsequently found to be structurally and functionally identical to mouse EGF and was proven to be human EGF [2,3]. EGF is now

Correspondence: Dr. Raymond C. Harris, S-3223 Medical Center North, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232. Tel#: 615-322-2150. Fax #:615-343-2675. ray.harris@vanderbilt.edu.

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known as the prototype of the group I EGF family that also includes transforming growth factor-α (TGF-α), heparin-binding EGF (HB-EGF), amphiregulin, betacellulin, epiregulin and epigen [4,5]. Structurally, they all contain one or more EGF repeats (EGF motif) in their extracellular domain, which is a sequence of 35–40 amino acids spaced by six conserved cysteines in the following pattern: $CX_7CX_{3–5}CX_{10–12}CXCX_5GXRC$ (C, cysteine; G, glycine; R, arginine; X, other amino acids). One glycine and one arginine in this sequence are also conserved in all EGF-related growth factors but not in proteins that contain EGF motifs without growth factor activity [6]. The six cysteines pair and form three intramolecular disulfide bonds with the following interactions: C1–C3, C2–C4 and C5–C6 (numbered according to their order in the sequence), which are important for maintaining their biological activities [7]. Functionally, these growth factors share the ability to bind the same receptor, the EGF receptor (EGFR, ErbB1), activate its intrinsic tyrosine kinase activity, and couple the receptor to downstream signaling pathways controlling cell proliferation, differentiation, survival, or motility [6,8,9]. Studies of the EGF family /EGFR continue to provide insights into roles for this axis in development, physiology and disease. As for EGF *per se*, the studies have shifted from its basic characterization to its role in biology, pathology and clinical application in human diseases. Therefore, this review will describe briefly the gene organization and tissue distribution of EGF, with emphasis on its biological and pathological roles in human diseases.

2. Gene organization

Followed its discovery in mouse salivary glands, genes encoding both mouse and human EGF were cloned and sequenced [10,11]. It was found that EGF is derived from a large precursor, prepro-EGF (Fig. 1). The genes encoding prepro-EGF were mapped to chromosome 4q25–q27 in humans and chromosome 3 (GRCm38) in mice (Table 1). There is 66% homology between these two sequences and both consist of 24 exons. The prepro-EGF gene is a mosaic, as 15 of its exons (exons 6–15, 17–19 and 20–21) encode sequences that are homologous to exon-encoded regions in other proteins. Exons 8–15 are homologous to a region of the low density lipoprotein (LDL) receptor gene. Eight individual cysteine-rich EGF-like repeats (EGF-motif) are encoded by exons 6–9, 15 and 17–19, as introns interrupt the coding sequence and mark the end of each repeat. EGF is encoded by two exons, 20 and 21. In contrast to EGF-like repeats, introns do not mark the end of the EGF-coding region and exon 21, which codes for the COOH-terminal portion of EGF, also encodes the transmembrane (TM) domain of the prepro-EGF. In addition, exons 20 and 21 are also homologous to the TGF-α and transmembrane domains of the TGF-α percursor gene [11,12]. Therefore, the prepro-EGF gene belongs to three gene families: one that includes proteins that have the EGF-like repeat motif; a growth factor family that includes the TGF-α precursor; and a receptor family that includes the LDL receptor [11].

The 4.7 to 5.6 Kb cDNA sequence has a long open reading frame that encodes the prepro-EGF of 1,207 amino acids in human and 1,217 amino acids in mouse. There is 75% homology between the coding regions of the human and mouse cDNA sequences. The homology between the 5’- and 3’-untranslated regions of the two sequences is 66% and 60%, respectively. Prepro-EGF is N-glycosylated and contains two prominent hydrophobic regions, one of which represents the signal peptide and the other that anchors the precursor

in the plasma membrane. Mature EGF lies immediately external to the hydrophobic transmembrane domain and can be released from the precursor by cleavage of Arg-Asn and Arg-His bonds at its NH2- and COOH-termini, respectively [13]. In cells that do not cleave this precursor, such as kidney cells, the membrane-bound prepro-EGF may function through paracrine and/or juxtacrine growth control mechanisms [14]. It may also serve as a receptor for as yet unknown ligands.

3. EGF expression

EGF has been detected in a variety of body fluids, such as milk [15–17], saliva [18], urine [19], plasma [18], intestinal fluid [20], amniotic fluid [21], and others [22], which is locally produced and secreted by the lactating breast, submaxillary gland, kidney, Brunner's glands of the duodenum, and placenta, respectively. Submaxillary gland is the major EGF producing site in mice, where it is synthesized, processed and stored in granules of the tubular duct cells. Consequently, EGF concentrations are high in mouse saliva [18]. Interestingly, only mature and diffusible 6 kDa EGF was detected in those secretory granules, where prepro-EGF is not detectable [23]. The release of EGF into saliva involves exocytosis by fusion of the secretory granule membrane with the apical cellular membrane (exocrine). A small amount of the EGF accumulated in submaxillary glands ends up in the blood (endocrine). The production and secretion of EGF in submaxillary gland are dependent on androgen levels and sympathetic system status. The concentration of EGF in submaxillary gland is1000 times higher in adult male mice than that in female mice (1000 ng/mg vs 70 ng/mg wet tissue) [24,25]. EGF concentration in submaxillary gland is low in newborn or immature male mice and gradually increases that parallels the androgen levels [22,24]. On the other hand, the androgen-dependent EGF contents in the submaxillary gland do not reflect its level in the plasma, which is indicated by the observations that there are no significant differences in plasma EGF levels (about 1ng/ml) between adult male and female mice [26]. The release of EGF from this site is highly regulated and may be achieved at least in part through activation of adrenergic receptors expressed by submaxillary glands [27]. Adrenergic stimulation such as phenylephrine injection or emotional stress will dramatically increase EGF levels in the saliva and blood [28,29].

Unlike mice, EGF concentrations in salivary gland and saliva are much lower in humans [30] and rats [31]. In humans, kidney is the predominant source for EGF production and urine contains high levels of EGF [19], although the highest EGF concentration was detected in the prostate fluids [32]. There are several features of kidney EGF production: 1) EGF accumulates as prepro-EGF associated with the apical membrane of epithelial cells. The fully mature 6 kDa EGF is not detectable [33]. 2) EGF-containing peptides of varying molecular masses, from 6 kDa to 160 kDa, can be detected in urine, which indicates that EGF can be released from membrane-anchored prepro-EGF [19,34]. 3) There is a sexual dimorphism of EGF concentrations in both kidney and urine, with higher EGF levels in females. It is not affected by the menstrual cycle, oral contraceptives, or postmenopausal estrogen therapy [35]. However, urine EGF concentrations increase during pregnancy, reaching peak levels at 19–22 gestational weeks [36].

Similar to other members in the EGF family, EGF detected in biological and pathological states exhibits different levels of expression [37–39]. Its expression levels also vary at different stages of development [40–42]. In newborn rat kidneys, positive EGF immunostaining was observed in the proximal tubules and decreased slowly with age. In adult kidneys, high concentrations of prepro-EGF mRNA are found in thick ascending limb of Henle's loop and distal convoluted tubules [14,40].

4. Animal models

To understand the developmental, physiological, and pathological roles of EGF, transgenic mice with either EGF overexpression [43–47] or deletion [48] have been developed.

4.1 EGF overexpression

4.1.1. Transgenic mice with widespread human EGF expression—By insertion of human EGF cDNA under the beta actin promoter, transgenic mice with human EGF widespread expression were generated. Those transgenic mice showed low birth weight and stunted growth, which was associated with altered chondrocyte development in the growth plate and abnormal osteoblast accumulation in the endosteum and periosteum. The mechanism involved a reduction of serum insulin-like growth factor-binding protein-3 (IGFBP3). In addition, adult male transgenic mice were sterile and exhibited hypospermatogenesis. Interestingly, no signs of tumor formation were found in these transgenic animals [45,49].

4.1.2. Transgenic mice with mouse EGF overexpression—To generate transgenic mice with mouse EGF overexpression, full-length mouse EGF cDNA was inserted under a ubiquitous transcription promoter (cytomegalovirus). As a result, markedly increased levels of EGF mRNA and protein expression were detected in various tissues of the transgenic mice [43,44]. In line with the transgenic mice with human EGF overexpression, transgenic mice with mouse EGF overexpression also showed stunted growth, which was much more severe in homozygous than heterozygous progenies. In addition, those transgenic mice exhibited: 1) Hair follicle deficits and thin fur that could be rescued by inhibiting the EGFR signaling by crossing with waved-2 mice, which harbor a point mutation in EGFR that greatly diminishes its tyrosine kinase activity [50]; 2) Hypersensitivity to psychostimulants, such as cocaine, which may due to the neurotrophic action of EGF on dopamine neurons; 3) Behavioral deficits relevant to schizophrenia [43]. In humans, EGF A61G polymorphism in the 5' untranslated region (UTR), a single nucleotide substitution (G to A) at position 61 of the EGF gene that results in increased EGF expression (G/G > G/A > A/A) has been associated with lower birth weight and fetal growth restriction in individuals from Western Europe [51,52]. In several meta-analysis, A61G polymorphism was correlated with cancer development in individuals carrying the G alleles, which led to the highest EGF expression [51,53–56].

4.1.3. Transgenic mice with tissue-specific mouse EGF overexpression—Compared to global gene overexpression, transgenic mice with tissue-targeted gene overexpression more directly test the effect of a candidate factor on that particular tissue without directly affecting other organ systems. For example, studies have shown that

systemic administration of EGF attenuates intestinal tissue damage and improves mortality in a variety of animal models of noninfectious inflammation and intestinal injury, and work on targeted-transgenic mice made it clear that those healing effects of EGF are directly through the intestine. In studies to test the direct role of EGF on intestinal injury, transgenic mice with EGF overexpression exclusively in enterocytes were generated by ligating murine preproEGF cDNA to the rat intestinal fatty acid-binding protein (I-FABP) promoter [47]. These transgenic mice had improved post-resection adaptation compared to wild-type mice. The transgenic mice appeared phenotypically normal albeit with slightly shorter intestines in the mouse line with the greatest production of EGF. The enterocyte-specific overexpression of EGF also conferred a survival advantage in mice subjected to septic peritonitis. The beneficial effects of EGF were intestine-specific and were associated with prevention of peritonitis-induced intestinal hyper-permeability via a claudin-2-mediated mechanism [46]. The major limitation of those studies was lack of examination of phospho-EGFR levels.

4.2. EGF null mice

EGF null mice were generated by deletion of EGF exon 20. In contrast to EGFR null mice, which exhibit several dermal, gastrointestinal, pulmonary, renal and neurological abnormalities, EGF null mice display no overt phenotype and no gross or histological abnormalities in lung, kidney and gastrointestinal tract, which may indicate overlapping or compensatory functions among EGF family members [57].

5. EGF biological functions

Both prepro-EGF and EGF have been documented to be biologically active by binding to its receptor, EGFR, in a variety of tissues [58]. EGFR, also known as ErbB1 or HER1, is a type I transmembrane receptor tyrosine kinase (RTK) that belongs to the EGFR/ErbB superfamily, which includes three other RTKs: ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4. Upon ligand binding, EGFR forms homodimers with another EGFR or heterodimers with other EGFR/ErbB family members. The receptor dimerization is a critical step for the activation of intrinsic tyrosine kinases and autophosphorylation of the c-terminal specific tyrosine-containing residues that serve as docking sites for a variety of signaling molecules harboring Src homology 2 (SH2) or phosphotyrosine binding (PTB) motifs, whose recruitments lead to the activation of Ras/Raf/MEK/ERK, JAK/STAT, PI3K/AKT/mTOR, and PLCγ/PKC signaling pathways that affect cell proliferation, differentiation, and apoptosis and consequently regulate many physiological processes, such as organ development, growth, regeneration, ion transportation, etc. [4,14,59–61].

5.1. EGF in embryo development

As a mitogenic growth factor, EGF plays an important role in embryo development from as early as pre-implantation [62,63]. After fertilization, the one cell egg undergoes a series of cleavage divisions, progressing through 2-cell, 4-cell, 8-cell, 16-cell, mulberry-shaped 20- to 30-cell mass (morula) to the formation of blastocyst, which is composed of trophoblast and inner cell mass (embryonic stem cells, ESC) that eventually develop into placenta and embryo respectively. Accumulated evidence suggests that growth factors are necessary components in early embryonic development *in vivo* and *in vitro* [64]. Among these growth

factors, EGF has been shown to promote pre-implantation embryo growth [62,65,66], as well as trophoblast invasion and post-implantation embryo growth [67,68]. In an *in vitro* fertilized (IVF) porcine embryo developmental study, the rate of blastocyst formation from either 2-cell or morula stage was significantly improved by EGF addition with the minimal addition of re-crystallized bovine serum albumin in culture media. However, EGF alone was not able to elicit any stimulatory effects on embryo development without protein supplementation [62]. Similarly, EGF treatment significantly increased the blastocyst formation rate, the total number of cells per blastocyst, the cell ratio of the inner cell mass and the trophectoderm, and EGFR protein expression in cloned mouse embryos, and these effects were enhanced when EGF and TGF-α were combined [63]. In pregnant mice, reduction of maternal EGF by sialoadenectomy results in growth restriction of embryos [69]. Those studies indicated that EGF plays an important synergistic effect with other growth factors in embryonic development. In addition, EGF produced by uterine tissues and macrophages can enhance trophoblast outgrowth and regulate urokinase plasminogen activator (uPA) and matrix metalloproteinase 9 (MMP-9) expression [70,71]. PA and MMPs have been implicated in mammalian gametogenesis [72], ovulation [73], fertilization [74], early development and embryo implantation [75].

Consistently, the role of EGFR on embryonic and placental development is much more prominent. EGFR expression has been detected in the apical blastomere membrane of the 4-cell stage mouse embryo [62,76]. In EGFR null mice, placentas have fewer proliferative trophoblasts than wild-type and exhibit strain-specific defects in the spongiotrophoblast and labyrinth layers that can result in mid-gestational embryonic lethality [77–79]. Moreover, increasing the levels of EGFR signaling by using hypermorphic EGFR (Dsk5) allele results in larger placental size with a more prominent spongiotrophoblast layer and increased expression of glycogen cell-specific genes [80]. This study also demonstrated that mice with increased levels of EGFR signalling exhibit an extensive level of genetic background-dependent phenotypic variability, and EGFR expressed in the uterine stroma may play an underappreciated role in preparation of the uterus for embryo implantation [80]. These differences between the relative importance of EGFR and EGF *per se* once again illustrates that it is sometimes difficult to ascribe biological functions solely to one EGF family member because of redundancy and compensation of expression and activity.

5.2. EGF in tissue regeneration

Stem cells have emerged as one of the fundamental underpinnings in tissue biology and in regenerative medicine [81]. In addition to embryonic stem cells, stem/progenitor cells have been found in various adult tissues, such as skin, blood, gut, heart, brain, etc. [82,83], where they not only replace differentiated cells during normal tissue turnover (homeostasis), but are also capable of massive lineage expansion following injury or transformation (tissue repair). Stem cells are undifferentiated biological cells with a high potential for proliferation and the capacity for self-renewal with retention of multipotency. Therefore, stem cells hold great promise in regenerative medicine and tissue engineering. However, due to their low numbers in a tissue, either *in vivo* or *in vitro* expansions without biasing future differentiation for optimal utility are often needed. Studies have shown that *in vivo* stem cells are maintained and regulated by the local tissue microenvironments surrounding the

stem cells, called stem cell niches [84–86]. Yet, precisely how a core niche program is regulated to selectively control the behavior of distinct stem cell populations remains poorly understood [83]. Over the last decade, considerable progress has been made in identification of microenvironmental factors favoring the growth and expansion of the stem cell pool [87]. Among them, EGF has emerged as a powerful regulator of stem cells in different tissues, such as neural stem/progenitor cells [88,89], neural crest stem cells [90], germline stem cells [91], cardiac stem cells [87], bone marrow multipotential stromal cells (MSCs) [92], brain tumor stem cells [93], mouse embryonic stem cells [94], gut stem cells [83,95], keratinocyte stem cells [96], and multipotent stromal cells in the heart [97].

A stage-specific impact of EGF signaling pathway on the stem cell activity has been proposed recently [91]. Studies of the division frequency of germline stem cells (GSCs) in testes of Drosophila melanogaster indicated that GSC division frequency is under genetic control of the highly conserved EGF signaling pathway. When EGF signaling was attenuated, a two-fold increase in the percentage of GSCs in mitotic division was detected. Interestingly, EGF attenuation specifically increased the GSC division frequency in adult testes, but not in larval testes, which indicates an inhibitory effect of EGF on stem cell division in adult testes. This stage-specific requirement for EGF may reflect the different functions of GSCs in immature versus mature tissues. In larval testes, entire germline cell populations from GSC may be required, while in adult testes GSCs may be only used to replenish differentiated cells when needed. In larval testes, nutrient availability and cell growth may be the primary factors governing the frequency of GSC divisions. Conversely, soon after eclosion, *Drosophila* males reach sexual maturity, and spermatogenesis may rely on EGF-mediated signaling to regulate GSC divisions.

EGF may also impose dose-dependent effects on stem cell function [88,92]. Human neural progenitor cell (hNPC) cultures treated with 100 ng/ml EGF showed significantly increased growth rates compared with traditional level of 20 ng/ml. Interestingly, this was through increased survival of dividing cells rather than increased proliferation and was associated with prolonged activation of ErbB2 and phosphorylated Akt. Furthermore, high EGF levels selectively protect a large proportion of elongated radial glial-like cells within the growing neurospheres, which maintain the capacity to generate neurons upon differentiation. Therefore, the exact concentrations of growth factors added to growing stem and progenitor cell culture systems should be carefully evaluated to maintain specific populations of dividing cells [88].

Different forms of EGF, soluble versus surface-tethered, may affect stem cell expansion differently [92,98,99]. Soluble EGF was shown to augment adult bone marrow multipotential stromal cell (MSC) proliferation while preserving early progenitors within the MSC population, and thus did not induce differentiation. However, the tethered form of EGF was shown to promote osteogenic differentiation. Soluble EGF was also shown to increase paracrine secretion of other growth factors from MSC, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). Thus, soluble EGF can be used not only to expand MSC *in vitro*, but also to enhance paracrine secretion through drug-releasing MSC-encapsulated scaffolds *in vivo*. Tethered EGF may be utilized to direct MSC towards osteogenic lineage both *in vitro* and *in vivo* [92].

Finally, EGF has been shown to impose synergistic effects on stem cell expansion with other growth factors in combined therapy or through EGF-initiated upregulation of other growth factors, such as VEGF, HGF, HB-EGF, etc. [63,92,100]. On the other hand, EGF may also play distinct roles compared to other growth factors on certain kinds of stem cell expansion [90]. For example, in a study to investigate the effect of microenvironmental factors on quail trunk neural crest (NC) stem cell development, EGF was found to induce differentiation of NC to neuronal and melanocytic phenotypes (neurogenesis and melanogenesis), while fibroblast growth factor 2 (FGF2) promoted NC differentiation to Schwann cells (gliogenesis). In the presence of both EGF and FGF2, the neuronal differentiation predominated. These findings suggest that these two growth factors may play distinct roles in the fate decision of NC progenitors and in the development of the peripheral nervous system [90].

5.3 EGF in ion transport

A number of studies have shown that EGF and its related growth factors are involved in regulation of various epithelial ion channels such as epithelial sodium channel (ENaC) [101], $Na^+/K^+/2Cl^-$ co-transporter (NKCC1) [102], transmembrane protein 16A (TMEM16A, Ca^{2+}-dependent Cl^- channel) [103], calcium-activated K^+ channels (KCa3.1) [104], transient receptor potential melastatin 6 (TRPM6), and cation-nonselective transient receptor potential channel 5 (TRPC5) [105], that govern ion homeostasis of Na^+, K^+, Cl^- or Mg^{2+}, in tissues like kidney, intestine, colon, and lung. The role of EGF on sodium and magnesium transport has been shown to be of particular physiological importance.

5.3.1. EGF and sodium transport—Sodium transport and homeostasis in the kidney connecting segment and collecting duct is mediated by the apical sodium-selective channel, ENaC, and the intracellular gradient driving cellular entry of sodium is dependent upon the Na^+/K^+-ATPase on the baslolateral membrane of the cell. ENaC is also located in the apical membrane of polarized epithelial cells of lung, and colon. Dysfunction or aberrant regulation of this channel is associated with a spectrum of diseases, such as hypertension, polycystic kidney disease and cystic fibrosis [106–108]. Various stimuli, including hormones and growth factors, modulate ENaC and fine-tune Na^+ absorption. EGF has been known for almost two decades to regulate sodium transport. However, several investigations have reported contradictory results indicating opposite effects of EGF on ENaC activity and sodium transport. EGF appears to affect sodium transport and ENaC in a context-dependent manner: it increases sodium absorption in the airways [109] and intestine [110], but decreases sodium transportation in the renal collecting ducts (CDs) [111,112]. The CDs are the final sites for renal regulation of Na^+ and water balance. Within the CD, Na^+ diffuses from the lumen into principal cells through ENaC and is extruded at the basolateral membrane in exchange for uptake of K^+ by the Na^+/K^+-ATPase. In the kidney, EGF expression can be found in the thick ascending limb of Henle's loop and distal convoluted tubules (DCTs) [113]. Studies have shown that EGF and its related growth factors TGF-α, HB-EGF, and amphiregulin have a biphasic effect on sodium absorption in cultured murine $mpkCCD_{c14}$ principal cells [114]. Basolateral application of the EGF family growth factors to polarized $mpkCCD_{c14}$ principal cells grown on permeable supports acutely increases Na^+

reabsorption, whereas chronic treatment of the monolayers with EGF and its related growth factors leads to significant inhibition of ENaC-mediated transport.

Studies have shown that infusion of high EGF levels decreased renal ENaC activity, prevented the development of hypertension, and attenuated glomerular and renal tubular damage. Conversely, deficiency of renal cortical EGF increases ENaC activity and contributes to salt-sensitive hypertension. Therefore, clinical application of EGF receptor inhibition either with antibodies such as Cetuximab or small molecule tyrosine kinase inhibitors may increase the risk of renal injury, especially in patients with a predisposition for salt-sensitive hypertension [106].

5.3.2. EGF and magnesium transportation—Magnesium is a versatile electrolyte known to be involved in many cellular processes. It functions as a cofactor in energy metabolism, nucleotide and protein synthesis, and as a regulator of Na^+, K^+, and Ca^{2+} channels. To maintain these cellular functions, both plasma and cellular Mg^{2+} concentrations have to be tightly controlled [115–117]. The systemic balance of Mg^{2+} and its intracellular concentration are determined by intestinal absorption and renal reabsorption/excretion. Under physiologic conditions, about 10% of filtered Mg^{2+} is excreted. The DCT is responsible for reabsorbing only 5–10% of the filtered Mg^{2+}, but is critical for fine-tuning Mg^{2+} reabsorption to determine the final urinary Mg^{2+} concentration and thus plays a key role in the regulation of Mg^{2+} homeostasis [115,118]. For this reabsorption to occur, active transcellular Mg^{2+} transport requires passive Mg^{2+} entry across the luminal membrane. Recent studies have shown that TRPM6, an Mg^{2+}-permeable channel that is expressed in the luminal membrane of the intestinal epithelium and the DCT, is a likely candidate for influx of Mg^{2+} across the luminal membrane [119]. Inactivating mutations of TRPM6 both impair gut absorption of Mg^{2+} and produce renal wasting [115].

EGF has been identified as a novel autocrine/paracrine magnesiotropic hormone that stimulates Mg^{2+} reabsorption in the DCT via engagement of its receptor on the basolateral membrane of DCT cells and subsequent activation of the Mg^{2+} channel TRPM6 in the apical surface [116]. By use of whole-genome linkage analysis and a subsequent candidate gene approach, *EGF* was shown to be the affected gene in patients with autosomal recessive isolated renal hypomagnesemia. As noted above, the *EGF* gene is highly expressed along the DCT [113], an important site for regulating urinary magnesium excretion. By activating the EGFR receptor, EGF stimulates the trafficking of TRPM6 channels to the luminal membrane, increasing the reabsorption of Mg^{2+} through TRPM6 [120]. A point mutation in prepro-EGF that retains EGF on the apical but not the basolateral membrane, or inhibition of EGFR by anti-EGFR antibodies, Cetuximab, or by EGFR tyrosine kinase inhibitors, such as Erlotinib, were shown to lead to suppressed activity of TRPM6 and renal Mg^{2+} wasting [116,121]. In this regard, use of Cetuximab in patients with head, neck or metastasis colorectal cancer is associated with a high incidence of symptomatic hypomagnesemia [122]. Erlotinib-injected mice also failed to reduce fractional excretion of Mg^{2+} in response to a decreased serum Mg^{2+} concentration [121]. In addition, simultaneous TRPM6 and EGF mRNA downregulation were seen in the rat kidneys with cisplatin - or cyclosporin A (CsA) - induced hypomagnesemia, which might indicate that EGF also influences TRPM6 mRNA synthesis [117,123]. EGF-mediated stimulation of TRPM6 may occur via signaling through

Src kinases and the small GTPase Rac1, thereby redistributing endomembrane TRPM6 to the plasma membrane [120].

6. EGF clinical relevance

Through binding and activating EGFR, EGF itself or combined with other growth factors triggers many biological responses, including cell proliferation, differentiation and migration, which supporting a regulative role for the EGF/EGFR signaling in normal development [20] as well as pathophysiological events such as tissue repair including ulcer/wound healing [124–127], tissue repair after ischemia/reperfusion injury [128–130], etc. Exogenous EGF administration has shown beneficial antiapoptotic and antioxidant effects and has been found to reduce the tissue injury caused by IRI in different organs, such as heart [129], intestine [128], and kidney [130].

6.1. EGF and ulcer/wound healing

As for ulcer/wound healing, the effect of EGF may vary according to the course of the wound and the routes or forms of EGF being applied. First, the healing action of EGF appears to differ in acute versus chronic wounds. Based on *ex vivo* research, EGF is upregulated after acute wounding injury, resulting in increased expression of keratins K6 and K16, thereby enhancing re-epithelialization and increasing the tensile strength in wounds [131]. Conversely, downregulation of EGF and its receptor as well as a mislocalization of EGF receptor in the cytoplasm of keratinocytes instead of the membrane are seen in chronic wounds. This probably contributes to an inhibition of epithelialization. Exogenous EGF is readily degraded in the chronic wound environment, which limits its effect on the chronic wound healing process. Secondly, the effect of EGF is reduced due to its poor transdermal permeability and biological stability if applied topically to the wounds. Many studies have suggested that the local concentration of EGF needs to be sufficiently high and prolonged for effective wound healing [126,132,133]. Therefore, different drug delivery systems that are able to protect and stabilize the protein have been investigated, including hydrogels, sponges, polymeric pellets, nanofibers, microspheres, a biomimetic delivery system that incorporated EGF with biocompatible components such as hyaluronic acid, collagen, vitronectin or liposome, and more recently, a poloxamer gel formulation of recombinant low-molecular-weight protamine conjugated EGF (rLMWP-EGF) by conjugating a highly positively charged rLMWP to the N-terminal of EGF [134–140]. The latter has shown better healing effects on burn injuries [139]. Thirdly, the effect of EGF on wound healing is likely dose-dependent [132,141,142]. The higher dose of EGF has been shown to achieve higher healing rates and shorter time to heal than the lower dose. This brings up safety concerns, as repeated application of EGF can induce hyperplasia and hypertrophy of skin keratinocytes and fibroblasts, as well as promote angiogenesis, which may predispose to cancer development, especially in patients who are immune-incompetent [143]. However, the clinical data so far have shown that EGF treatments have been well tolerated, and no significant adverse reactions have been observed [141].

6.2. EGF and cancer

Increased EGFR activity due to more EGF synthesis, EGFR overexpression, and/or EGFR mutation, has been detected in a variety of tumors including glioblastoma (GBM), non-small cell lung cancer (NSCLC), head and neck, breast, colorectal, ovarian, prostate and pancreatic cancers, etc. [144]. The expression levels of EGF and EGFR are correlated with progressive tumor growth and metastasis [59,145–147] through: 1) increasing tumor cell proliferation and migration through EGFR-Ras/Raf/MEK/ERK and EGFR-PI3K/AKT pathways [146]; 2) localization of EGFR to the nucleus to promote cell proliferation through its tyrosine kinase activity or by acting as a transcriptional regulator [144,146,148]; 3) dysregulation of autophage activity [149,150]; 4) stimulation by EGF of the expression of several matrix metalloproteinases (MMPs, such as MMP1 and MMP9) that facilitate cancer invasion and metastasis; and/or 5) EGF-mediated decrease in the abundance of microRNAs that restrain oncogenic transcription factors [151]. Therefore, specific EGFR inhibition is one of the key targets for cancer therapy. The three most common agents are: 1) monoclonal anti-EGFR antibodies (mAbs), such as cetuximab and panitumumab, that inhibit ligand binding; 2) small molecule receptor tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib, and lapatinib, that block the activation and phosphoraylation of EGFR; 3) anti-EGFR vaccines that elicit an immune response against EGFR-expressing tumor cells. For example, CDX-110 is a peptide vaccine that induces anti-tumor immune responses to EGFR variant III (EGFRvIII) positive cells [152]. Recently, many other agents such as antisense oligonucleotides, microRNA, affibodies, and nanobodies have been investigated, and some of them have begun to show efficacy in targeting and inhibiting EGFR [144,153].

7. Conclusion

EGF is widely expressed in the body and plays a fundamental role in development, tissue regeneration and ion transport, which occurs either by EGF itself or synergistically with other members of EGF family through binding/activating their receptor, EGFR. It is a pivotal factor in the healing cascade, and has been widely used clinically to accelerate ulcer/wound repair. Several carriers or delivery systems aimed to retard EGF degradation and facilitate EGF to the targeted area have been developed and dramatically improved its healing efficiency. On the other hand, dysregulation of EGF/EGFR axis has been linked to different pathogenesis and diseases and cancer development. Therefore, personal history and family genetic background should be considered when using EGF as a treatment therapy.

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Highlights

1. Submaxillary gland and kidney are predominant source of EGF production.
2. EGF/EGFR signaling promotes embryonic development and stem cell regeneration and regulates ion transport.
3. EGF plays pivotal role in ulcer/wound healing.
4. Dysregulation of EGF expression may contribute to the cancer development.

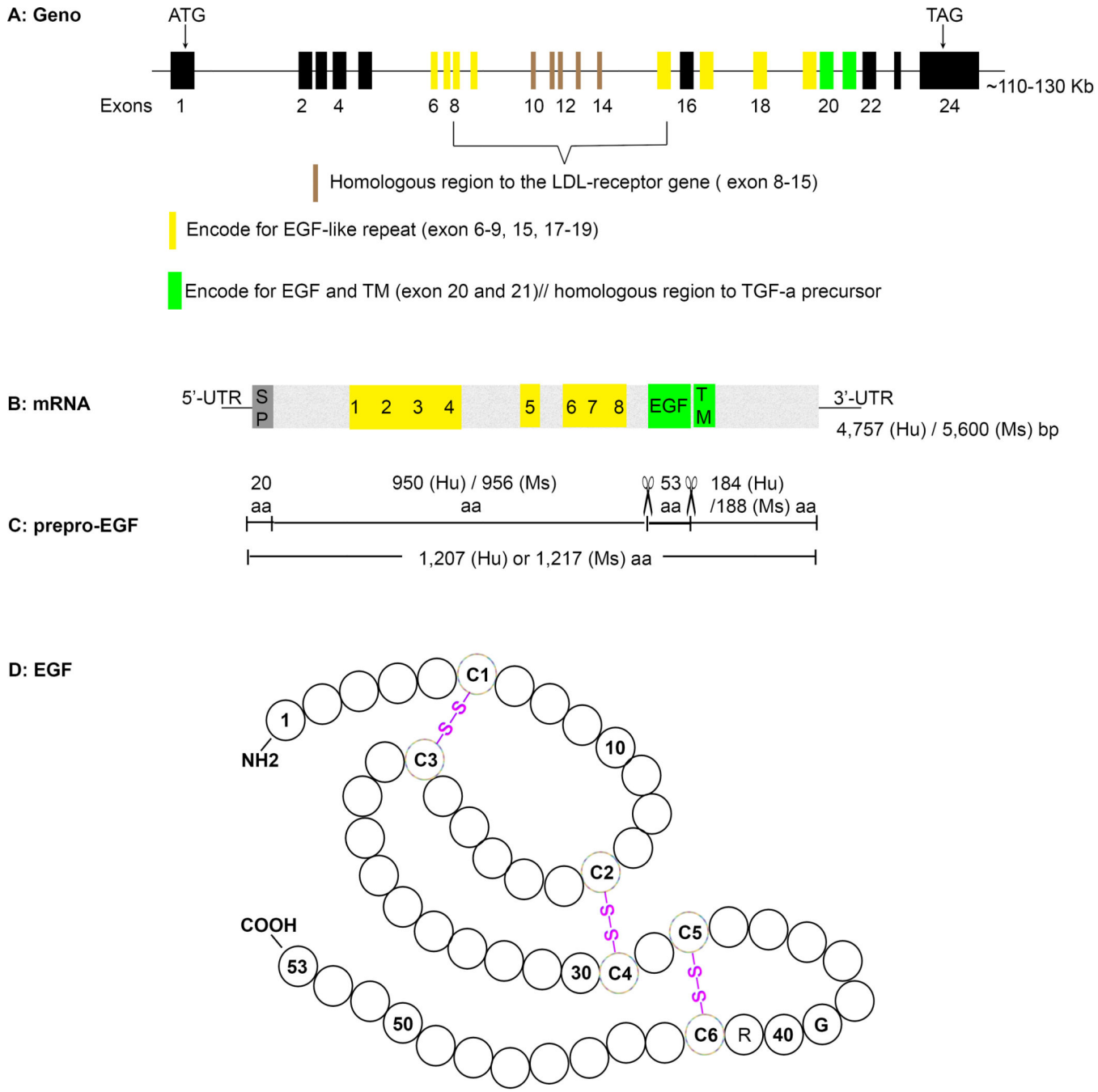

Fig. 1.

Schematic representation of EGF gene, mRNA and protein. **A)** The EGF precursor gene has 110–130 kb pairs and 24 exons. Some exons encoded protein segments that are homologuous to sequences in other proteins, like LDL receptor, EGF-like repeats, and TGF-α precursor, are noted. EGF was coded by exon 20 and 21. The start codon ATG lies in the first exon and stop codon TAG the last exon. **B)** EGF precursor mRNA is 4,757 bp long in human and 5,600 bp in mice. Protein coding regions are highlighted. The positions of signal peptide (SP), EGF-like repeats (1–8, highlighted yellow), EGF, and transmembrane (TM)

domain (green) are noted. UTR, untranslated region; Hu: human; Ms: mouse. **C)** Prepro-EGF protein sequence. It has 1,207 amino acids (aa) in human and 1,217 aa in mouse. The first 20–29 aa functions as signal peptide. Mature EGF (53 aa) is flanked by 950/184 aa in human and 956/188 aa in mice and lies immediately external to the hydrophobic transmembrane domain. Cleavage sites for releasing mature EGF are marked with scissor icons. **D)** The amino acid sequences of EGF with placement of disulfide bonds. The position of conserved cysteines (C1 to C6), glycine (G), and arginine (R) are specified. Modified from Savage CR, et al. [7].

Table 1

Characterization of EGF gene, DNA, and protein

	Human	**Mouse**
Location in chromosome	4q25	3, GRCm38
Gene ID	NC_000004.11	NC_000069.6
DNA size	130 Kb	101 Kb
Number of Exons	24	24
mRNA ID	NM_001963.4	NM_010113.3
mRNA size	5,600 bp	4,757 bp
Protien ID	NP_001954.2	NP_034243.2
Protein size	1,207 aa (Mr* 130–160 kDa)	1,217 aa (Mr 130–160 kDa)
Mature EGF size	53 aa (Mr* 6–8 kDa)	53 aa (Mr* 6–8 kDa)

*Mr: relative molecular mass