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Review

Epidermal growth factor, from gene organization to bedside

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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.

Contents

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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 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].

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	5600 bp	4757 bp
Protien ID	NP_001954.2	NP_034243.2
Protein size	1207 aa (Mr* 130–160 kDa)	1217 aa (Mr 130–160 kDa)
Mature EGF size	53 aa (Mr* 6–8 kDa)	53 aa (Mr* 6–8 kDa)

* Mr: relative molecular mass.

The 4.7 to 5.6 kb cDNA sequence has a long open reading frame that encodes the prepro-EGF of 1207 amino acids in human and 1217 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 NH^{2-} 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 1 ng/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