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The epidermal growth factor receptors and their family of ligands: Their putative role in atherogenesis

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Abstract

The epidermal growth factor receptor is a member of type-I growth factor receptor family with tyrosine kinase activity that is activated following the binding of multiple cognate ligands. Several members of the EGF family of ligands are expressed by cells involved in atherogenesis. EGF receptor mediated processes have been well characterised within epithelial, smooth muscle and tumour cell lines in vitro, and the EGF receptor has been identified immunocytochemically on intimal smooth muscle cells within atherosclerotic plaques. There is also limited evidence for the expression of the EGF receptor family on leukocytes, although their function has yet to be clarified. In this review, we will discuss the biological functions of this receptor and its ligands and their potential to modulate the function of cells involved in the atherosclerotic process.

Keywords: EGF; Receptor; ErbB1; Atherogenesis; SMC; Monocyte; Macrophage

1. Introduction

The initiation and development of the atherosclerotic plaque is driven by the interaction between several growth factors and cytokines that stimulate cell migration, proliferation and activation (reviewed by Ross in 1993 [1]). The cellular response to these factors is predicated by the presence of appropriate receptors on cells involved in atherogenesis. These cells include vascular smooth muscle cells, endothelial cells and leukocytes. The latter are thought to be particularly important in the initial phases of lesion development [2–5] and it is therefore likely that factors contributing to monocyte/macrophage and T cell accumulation and activation are major modulators of the process. Among the many factors implicated in atherogenesis are several ligands for the EGF receptor family. The epidermal growth factor receptor is the prototype of the type-I growth factor receptor family with tyrosine kinase activity. In this review, we will discuss the biological functions of this receptor and its ligands and their potential to modulate the function of cells involved in the atherosclerotic process.

2. The epidermal growth factor family

The EGF family consists of several peptide growth factors (Table 1) that act as ligands for the EGF family of receptors (Table 2). Among these are: epidermal growth factor (EGF), transforming growth factor-α (TGFα), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin (AR), betacellulin (BTC), epiregulin (EPR), epigen and the four neuregulins. NRG-1 is also known as Neu differentiation factor (NDF), heregulin (HRG), acetylcholine receptor-inducing activity (ARIA) and glial growth factor (GGF) [6–8].

EGF family members are commonly grouped with respect to their structural homology and biological activity (Table 1). All members contain one or more repeats of a conserved six cysteine-containing motif in their extracellular domain. These six cysteine residues are contained within a sequence of 35–40 amino acids $CX_7CX_{4–5}CX_{10–13}CXCX_8GXRC$ (C, cysteine; G, glycine; R, arginine; X, any amino acid), and

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Table 1
The epidermal growth factor (EGF) family, isolation and shared sequence homology between members

EGF family	Isolated from conditioned medium of cells/or tissues	Shared sequence homology (%)	References
EGF	Mouse submaxillary gland human urine		[9–12]
TGFα (SGF)	Murine 3T3 fibroblasts	35% with EGF and BTC	[7,13–15]
HB-EGF	Macrophage-like cell line (U-937)	EGF motif shows 41% with TGFα/EGF	[16,7]
BTC	Pancreatic β cell tumour line		[17]
AR	Human breast adenocarcinoma cell line (MCF-7)	38% with EGF, 32% with TGFα	[18,19]
EPR	Mouse fibroblasts-derived tumour cell line (NIH 3T3)	24–50% with all the members	[20,21]
Epigen	Mouse keratinocytes	24–37% with other members	[22]

Table 2
Cognate ligands for EGF receptor family members

Growth factor	Chromosome location	Precursor length (aa)	Mature growth factor (aa)	Molecular weight (Da)	Receptor	References
EGF	4 (4q25)	1207	53	6045	ErbB1	[6,7]
AR	4 (4q13–21)	252	84	9759	ErbB1	[6,27]
TGFα	2 (2q13)	160	50	–	ErbB1	[23]
HB-EGF	5 (5q23)	208	87	2200	ErbB1, ErbB4	[16,24]
BTC	4 (4q13–21)	162	46 (80)	3200	ErbB1, ErbB4	[6,28]
EPR	4 (4q13.3)	163	–	500	ErbB1, ErbB4	[20]
Epigen	–	152	–	–	ErbB1	[22]
NRG-1	8 (8p21–12)	–	–	4400	ErbB3, ErbB4	[29]
NRG-2	–	–	–	–	ErbB3, ErbB4	[30,31]
NRG-3	–	–	–	–	ErbB4	[32]
NRG-4	–	–	–	–	ErbB4	[33]

have the potential to form three intra-molecular disulfide bond pairings between C1–C3, C2–C4 and C5–C6 to produce three loops that are essential for high-affinity binding to the receptor [6,7,23]. HB-EGF and amphiregulin also contains a region rich in basic amino acids residues within their N-terminal regions that is responsible for their heparin-binding ability [24–26].

2.1. Processing of the EGF ligand precursors

Members of the EGF family are derived from type-I trans-membrane glycoprotein precursors. These precursor molecules comprise an extracellular region containing the growth factor sequence, a hydrophobic trans-membrane domain and a cytoplasmic domain. Within the plasma membrane they may undergo proteolytic cleavage, resulting in the release of soluble, biologically active growth factors [7].

All six EGFR ligands can be cleaved by members of the family of a disintegrin and metalloproteinase (ADAMs) (Table 3). ADAMs are intregral membrane proteins with extracellular metalloproteinase and integrin-binding sites. They have a wide tissue distribution and are essential for mammalian development [34,35]. It is still unclear as to how the ligand shedding occurs. It has been speculated that the ADAM may initially adhere to its substrate or a substrate-associated protein using its disintegrin domain and subsequently cleave the substrate proteolytically using its metalloproteinase domain. Research is ongoing to test the idea that both the metalloproteinase and disintegrin domains are active at the same time [34]. The precursor of EGF (ProEGF) contains nine EGF-like subunits in its extracellular domain. Cleavage occurs between the first and second motifs. The EGF subunit closest to the plasma membrane is released as a mature EGF molecule, the fate of the other eight EGF-like subunits is unknown [36]. TGFα is cleaved at two sites within its extracellular domain. The well characterised TACE/ADAM17 and ADAM10 are capable of cleaving TGFα at its N-terminal site but as yet it is unclear what has C-terminal cleavage activity [6,37]. HB-EGF is also capable of being cleaved by matrix metalloproteinases (MMPs), notably MMP-3 and MMP-7 [38,39]. Matrix metalloproteinases are members of a family of at least 15 endopeptidases that function extracellularly at neutral pH. While the majority of MMPs are secreted, a recently described subclass (MT-MMPs) remains anchored in the plasma membrane.

Table 3
The EGFR ligands and the associated ADAMs responsible for their cleavage

EGFR ligand	ADAM	References
EGF	ADAM10	[40]
HB-EGF	ADAM9, 10, 12, 17	[6,40–43]
TGFα	ADAM10, 17	[37,40]
BTC	ADAM10	[40]
AR	ADAM17	[40,44]
EPR	ADAM17	[40]

Certain factors are capable of enhancing growth factor shedding. Treatment of cells with the phorbol ester TPA, an activator of protein kinase C (PKC), induces a strong increase in the level of TGFα synthesis in keratinocytes and other cells as well as increased cleavage of the membrane bound form [23]. PKC, along with increases in cytosolic calcium