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A tale of the epidermal growth factor receptor: The quest for structural resolution on cells

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ABSTRACT

The challenge of determining the architecture and geometry of oligomers of the epidermal growth factor receptor (EGFR) on the cell surface has been approached using a variety of biochemical and biophysical methods. This review is intended to provide a narrative of how key concepts in the field of EGFR research have evolved over the years, from the origins of the prevalent EGFR signalling dimer hypothesis through to the development and implementation of methods that are now challenging the conventional view. The synergy between X-ray crystallography and cellular fluorescence microscopy has become particularly important, precisely because the results from these two methods diverged and highlighted the complexity of the challenge. We illustrate how developments in super-resolution microscopy are now bridging this gap. Exciting times lie ahead where knowledge of the nature of the complexes can assist with the development of a new generation of anti-cancer drugs.

1. Introduction

The human epidermal growth factor receptor (hEGFR; aka HER1) is the founding member of the growth factor receptor tyrosine kinase (RTK) super-family [1]. This family also comprises 18 sub-groups of cell surface receptors for many growth factors, cytokines and hormones [2]. The evolution of the EGFR family can be traced from one receptor/ligand pair in *C. elegans*, through one receptor with multiple ligands in *D. melanogaster*, to a family comprising four receptors (HER1 and ErbB2-4, known as HER2-4 in humans) and at least 13 ligands in mammals [3,4].

The EGFR family are key regulators of cell-to-cell inductive processes and cell fate [5]. Their function is to transmit growth factor signals from the outside to the inside of the cell where changes in gene expression allow the cell to respond to the new circumstances. Deregulated signalling by cell surface HER1 receptors (e.g. via activating mutations in the *HER1* gene) is implicated in a substantial percentage of lung cancers [6]. As activation of these receptors has been shown to result in the growth and progression of the malignancy, there have been considerable research efforts directed toward the development of effective inhibitors of HER1. Several of these cancer drugs are in different stages of preclinical and clinical trials [7].

Here we review the efforts towards understanding the structure–function relationships underlying the transduction of the EGF signal and the activation of EGFR across the plasma membrane. This understanding requires the determination of structure at high resolution. Because biological function usually requires the interaction of many molecules within a complex system, a full understanding of the structure–function relationship is only possible if we are able to obtain structural detail of molecules within the context of their natural environment; the cell. Although this inevitably leads us to discussions of how fluorescence based microscopy techniques have been used for this purpose, we have restricted the scope of this review to efforts to elucidate EGFR macromolecular structure. For an excellent review that discusses the imaging of other aspects of EGFR biology such as interaction dynamics and the events subsequent to receptor activation please see [8]. Ultimately the goal would be to obtain atomic resolution structures from molecules in living cells, in real time, but this is likely to remain out of reach for the foreseeable future.

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2. A brief history of 50+ years of EGFR biology

EGFR research can be traced back to the early 1960s, when Stanley Cohen purified the epidermal growth factor (EGF) from mouse submaxillary glands and showed that it induced precocious eyelid opening and tooth eruption in mouse embryos [9]. Given that fibroblast cells in culture responded to a treatment with ^{125}I-labelled EGF by enhancing DNA synthesis and proliferation, Cohen and co-workers began to ask questions about how EGF induces cell growth. Initial experiments showed that EGF specifically binds to a cell surface receptor, the EGFR, identified as a 170-kDa plasma membrane component [10] that is subsequently internalised and proteolytically degraded [11,12]. Typical early representations of the EGF and its receptor in the membrane are shown near the start the timeline, (Fig. 1).

The concept of intracellular receptor signals generated via protein phosphorylation on tyrosine residues gained momentum after the discovery that the EGF, insulin and platelet-derived growth factor receptors are protein tyrosine kinases that can be activated by their respective ligands [13]. The molecular architecture of EGFR became available after cloning and sequencing of the complete *c*DNA of EGFR from epidermal carcinoma cells in which the EGFR gene was amplified 25-fold [14]. The mature receptor is composed of 1186 amino acid (aa) residues, is glycosylated, and has a modular structure composed of a ligand-binding extracellular domain of 621 aa residues tethered to the plasma membrane by a single transmembrane region of 23 hydrophobic aa and is attached to a cytoplasmic domain composed of 542 amino acids. The latter contains a 300 aa tyrosine kinase catalytic region flanked by a juxtamembrane domain and a C-terminal domain. EGF binding results in autophosphorylation of the C-terminal domain tyrosine residues [5,15,16]. This phenomenon regulates the capacity of EGFR to phosphorylate exogenous substrates [17].

Seminal studies using retroviruses like the Rous sarcoma tumour virus v-SRC gene, which encodes the protein tyrosine kinase v-Src [18], led to the identification of genetic sequences capable of transforming normal cells into cancer cells [19]. One is the retroviral oncogene v-ErbB, a transforming gene of avian erythroblastosis virus which encodes a truncated homologue of the human chicken EGFR in which most of the extracellular domain and 32 aa residues at the C-terminal end are deleted [5]. The catalytic domain of human EGFR is homologous to the protein tyrosine kinase encoded by the SRC gene family of oncogenes [20], so the discovery of an in-frame deletion of 267 aa within the EGF binding site (known as EGFRvIII) expressed most frequently in high-grade human glioblastoma [21], revealed that the transforming competence of v-ErbB resulted from the truncation of the EGFR extracellular domain. This suggested that deregulation of protein tyrosine phosphorylation was of general importance in cellular transformation and tumorigenesis [22] and highlighted the importance of EGFR in cancer biology.

Given the primary structure of the EGFR, the mechanism of EGF signal transduction across the plasma membrane must include an intramolecular conformational change and/or an intermolecular allosteric process involving the interaction between neighbouring cytoplasmic domains. Evidence for the involvement of EGFR dimerisation in the receptor's activation was provided by a number of methods including chemical cross-linking binding assays on immobilised extracellular domains, the migration patterns of EGFR in native gels [23–26] and by the second order dependence on EGFR concentration of the activation kinetics of solubilised receptors. Important studies of a chimeric receptor made by combining the extracellular domain of the constitutively dimeric insulin receptor and the intracellular domain of EGFR showed that this chimeric protein is activated by insulin binding [27]. This suggested that receptor tyrosine kinases use similar mechanisms of signal transduction.

In 2002, two seminal papers finally provided the first X-ray crystallographic structures of an EGFR dimer fragment. These structures included three of the four domains of each receptor's extracellular region [28,29] and showed that, against expectations, the ligands were not directly involved in dimerisation. Instead, in this so called back-to-back extracellular dimer, the ligand–receptor interface, i.e. the 'receptor front', points away from the dimerization interface (Fig. 1). A year later, a structure of a nearly complete extracellular domain of a receptor monomer revealed that without ligand the receptor forms a conformation in which an intramolecular tether between domain II and IV buries the crucial dimerisation

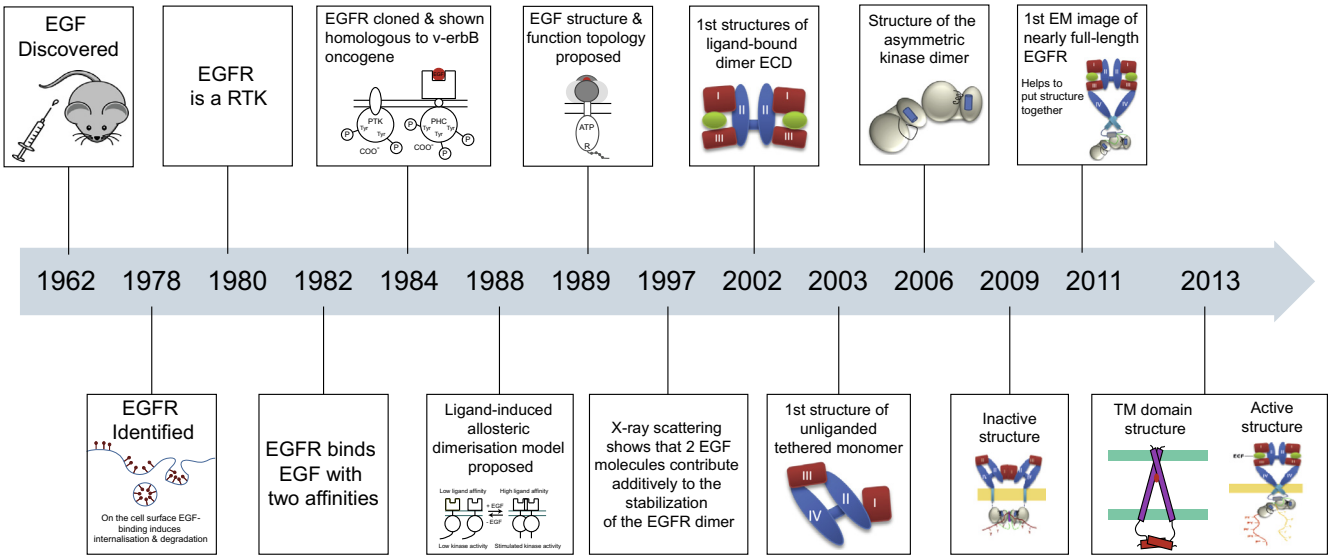

Fig. 1. Time line of breakthrough discoveries of the mechanism of EGFR activation. The time line shows the evolution of the structure and function of the EGFR. The notions of tyrosine kinase ligand-induced dimerization, allosteric signalling, association with dysregulated signal and cell transformation and receptor topology precede crystallographic information. Crystal structures available from 2002 onward revealed the structures of receptor fragments which were assembled into an almost complete picture with the help of pioneering electron microscopy images of nearly full length receptors. Recently, long atomistic simulations using cutting-edge technology have revealed putative interactions between receptor complexes and the membrane.

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