

Structural studies of the human epidermal growth factor receptor family

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INTRODUCTION

The human Epidermal Growth Factor Receptor (EGFR) family is a family of receptor tyrosine kinases consisting of 4 members: EGFR, HER2, HER3, and HER4. These receptors are expressed on a wide variety of tissue types, and have been implicated in many human cancers, including breast, gastric, colon, and prostate cancers [1,2]. In particular, the overexpression of HER2 is an important diagnostic indicator for poor patient prognosis [1]. Furthermore, recombinant humanized antibodies which recognize the HER2 extra-cellular domain (ECD), such as Mab4D5 (Herceptin™), are proving especially effective in combination therapy regimens for HER2 overexpressing breast tumors, which represent the most aggressive form of the disease [3,4].

Signalling via the EGFR family appears to involve the combinatorial dimerization of the four known family members [5,6]. This dimerization is mediated by at least 8 different hormones, including EGF (EGFR, EGFR:HER2, EGFR:HER3, and EGFR:HER4), and the heregulins (HER3:HER2, EGFR:HER3, HER3:HER4 and HER4:HER2) [5]. Thus far, no known ligand binds specifically to HER2. However, heregulin binding and tyrosine phosphorylation by HER3 or HER4 is enhanced by the presence of HER2, suggesting that for these receptors the physiologically relevant species is a HER3:HER2 or HER4:HER2 heterodimer [7,8].

Little is known about the structures of the EGFR family members. The full-length receptors are 1210-1342 amino acids in length, with the ~650 amino ECDs being extensively glycosylated. The ECDs are highly homologous (44-59% identity/ 61-71% similarity) and contain 4 subdomains: an L1 domain (EGFR amino-acids 25 to 189), a cysteine rich domain (CRD1) (amino-acids 217 to 334), a second L2 domain (residues 338 to 501), and a final CRD2 (504 to 645). The L domains and CRDs are homologous to the first two subdomains, respectively, of the type 1 insulin-like growth factor receptor (IGFR) [9]. The L1 domain of the IGFR forms a right handed β -helix structure, while the CRD is composed of disulfide-linked modules reminiscent of the TNFR superfamily [10].

In order to begin to understand the complicated signalling mechanisms exhibited by this family of receptors, we have undertaken the crystal structure determination of the ECDs of two of the family members: HER3 and HER2.

RESULTS

The ECD of HER3 was produced as an IgG Fc fusion protein in CHO cells, and cleaved with papain [8]. The resulting protein is monomeric in solution, and was crystallized by the vapor diffusion method. A single crystal was flash cooled in liquid nitrogen, and a data set was collected at ALS beamline 5.0.2. The crystals belong to Laue group P6/m with unit cell axes $a=b=121.2$ Å, $c=115.8$ Å, and the systematic absences suggest that the space group is $P6_2$ or $P6_4$. The data were integrated with Denzo and scaled with Scalepack [11]. Data statistics are reported in Table 1.

The ECD of HER2 was produced as a secreted, monomeric protein in CHO cells, and purified by immunoaffinity chromatography from the concentrated cell-culture fluid. The HER2 ECD was crystallized in complex with recombinant humanized Fab4D5 [12], which is the corresponding Fab fragment of Herceptin™. The complex crystallizes readily in space group P2₁2₁2₁ and we have collected multiple data sets at ALS beamline 5.0.2. The crystals diffract to at least 3.2 Å resolution, with unit cell axes a=63 Å, b=115Å, and c=206 Å. Data statistics are reported in Table 2.

Thus far, all attempts at molecular replacement using the coordinates of either the IGFR or Fab4D5 [12] have failed. This is not surprising, given the multi-domain structure of both of these search models. Even for Fab4D5, significant differences, especially in the elbow angle, are likely to exist between the free and complexed forms of the molecule. A search for heavy atom derivatives is currently under way.

Table 1. HER3 ECD data statistics.

Resolution (Å)	30-3.95	4.02-3.95
Rmerge	0.070	0.308
Chi ²	0.991	1.192
Completeness (%)	99.8	100.0
% <2σ(I)	9.5	27.5
% redundancy >4	62.3	54.6

Table 2: Native HER2/Fab4D5 data statistics.

Resolution (Å)	30-3.3	3.36-3.30
Rmerge	0.072	0.279
Chi ²	0.919	1.107
Completeness (%)	99.9	99.9
% <2σ(I)	12.2	31.6
% redundancy >4	29.9	29.6

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