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Review

Interaction of antibodies with ErbB receptor extracellular regions

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ABSTRACT

Antibodies to the extracellular region of the ErbB receptors have played key roles in the development of a mechanistic understanding of this family of receptor tyrosine kinases. An extensively studied class of such antibodies inhibits activation of ErbB receptors, and these antibodies have been the focus of intense development as anti-cancer agents. In this review we consider the properties of ErbB receptors antibodies in light of the current structure-based model for ErbB receptor homo- and hetero-dimerization and activation. Crystal structures of the Fab fragments from five different inhibitory antibodies in complex with the extracellular regions of EGFR and ErbB2 have been determined. These structures highlight several different modes of binding and mechanisms of receptor inhibition. Information about antibody interactions with the structurally well-characterized soluble extracellular regions of ErbB receptors can be combined with the rich knowledge of the effects of these antibodies in cultured cells, and *in vivo*, to provide insights into the conformation and activation of ErbB receptors at the cell surface.

Contents

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Introduction

Antibodies have played a crucial role in understanding ErbB receptors since the early mechanistic studies of this family of receptor tyrosine kinases (RTKs). Antibodies were essential for the generation of purified EGFR that was used to demonstrate that ligand-induced dimerization is a critical first step in receptor activation [1]. Other antibodies have provided clues as to the nature of the two ligand affinity "classes" of receptors that exist at the cell surface [2,3]. For ErbB2 (also known as HER2/*neu*), antibodies have also played a key role in establishing the identity and role of this receptor [4,5]. ErbB2 antibodies were found to reverse the phenotype of transformed cells by binding to and down-modulating this oncogenic protein [6]. This confirmed the link between the *neu* oncogene and malignancy, and provided proof of concept that antibodies could have antitumor activity [7].

In the early 1980s, a number of groups generated monoclonal antibodies to the extracellular region of EGFR using as immunogen A431 epidermoid carcinoma cells, which express high levels of cell surface epidermal growth factor receptor (EGFR). The resulting antibodies display various properties. Some had no effect on growth factor activation [8], while others induced receptor aggregation and mimicked the effects of ligand stimulation [9,10]. A third class of antibodies blocked the ability of growth factor to activate the receptor [11,12]. Antibodies of this third class have received substantial attention as potential inhibitors of EGFR activation in human tumors [11,12]. It has been clear for many years that there is a correlation between aberrant activation of members of the ErbB receptor family and the development and progression of cancers [13,14]. Both the extracellular and intracellular regions of EGFR and ErbB2 are targets of therapeutic agents in active clinical use and/or development [15,16]. In 2003, when the first special issue on the ErbB/EGF system was published in *Experimental Cell Research*, the ErbB2 targeted antibody drug trastuzumab/Herceptin had been approved for use in ErbB2 positive breast cancers, and several anti-EGFR directed antibody drugs were in clinical trials. There are now three EGFR antibodies approved for use in various clinical settings (Table 1), and numerous other antibodies against this family of receptors are the focus of active clinical trials. Many excellent reviews focus on the development and clinical application of monoclonal antibodies against the extracellular regions of ErbB receptors [7,15–18]. In this review we consider the interactions of antibodies with the extracellular region of ErbB receptors in light of structure-based models of ErbB receptor homo- and hetero-dimerization.

Structural studies in the past 6 years have led to a model for ligand-induced homo- and hetero-dimerization and activation of ErbB receptors, the details of which have been extensively reviewed [19–21]. Recent developments in understanding mechanisms of ErbB receptor activation are discussed in an accompanying review in this issue [22]. The salient points of this model for the discussion of antibody mediated inhibition of ErbB receptors are summarized in Fig. 1. For three of the four members of the ErbB receptor family, EGFR/ErbB1, ErbB3/HER3 and ErbB4/HER4, the unliganded extracellular region adopts a "tethered" conformation in which domains II and IV interact [23–25]. The ligand-bound form of the extracellular region has only been crystallographically observed for EGFR [26,27] and, in this state, the extracellular region of the receptor adopts a very different conformation. Ligand binds between domains I and III of the receptor molecule, and drives exposure and remodeling of a dimerization interface on domain II. The fourth member of this family of receptors, ErbB2, has no known soluble ligand and is a structural outlier among this family of four receptors. The unliganded conformation of ErbB2 does not adopt the tethered conformation. Rather, ErbB2 adopts an extended arrangement of domains similar that of the ligand-bound form of EGFR [28,29]. Based on these structures a generalized mechanism of ligand-induced homo- and hetero-dimerization has been proposed [19] and is shown schematically in Fig. 1. This mechanism of activation of ErbB receptors has substantial implication for possible modes of binding by antibodies that could inhibit or modulate receptor activation.

Antibodies against the extracellular region of ErbB receptors

In the following sections we briefly review a selection of the antibodies against the extracellular regions of ErbB receptors that are important inhibitors of receptor function, or that have provided opportunities to probe receptor activation mechanisms. Properties of key antibodies are also summarized in Table 1.

EGFR antibodies

mAb 225 (chimerized to IMC-C225/cetuximab/Erbitux)

Monoclonal antibody 225 was one of several antibodies raised by inoculation of mice with A431 epidermoid carcinoma cells by a group led by Prof. John Mendelsohn (University of Texas M.D. Anderson Cancer Center). Three antibodies (mAbs 225, 528 and 579) were characterized that inhibited EGF binding to the receptor,