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Short communication

Matching of trastuzumab (Herceptin®) epitope mimics onto the surface of Her-2/neu – a new method of epitope definition

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Abstract

As seen with the proto-oncogene Her-2/neu, antibodies targeting different parts of a receptor can have opposing effects. Depending on epitope specificity, in this case, tumor growth can be inhibited – but also enhanced. Therefore, the definition of molecular binding sites is of increasing importance in modern medicine. We here introduce a novel approach for binding site localization, utilizing information obtained by the phage display technique. This is a high throughput screening method for identification of peptide mimics, so called mimotopes, of any binding structure of interest. All target molecules whose structure is available in the RCSB Protein Data Bank can be scanned for mimotope matches on their surface. In this study, we present the matching results of five mimotopes defined for the epitope recognized by trastuzumab (Herceptin®), a humanized monoclonal antibody inhibiting tumor growth, on Her-2/neu. The localization thus obtained corresponds to the known trastuzumab epitope. We therefore suggest the algorithm as a novel way of binding site definition, circumventing co-crystallization experiments.

Keywords: Trastuzumab epitope; Mimotope; Surface matching; Phage display

1. Introduction

The human epidermal growth factor receptor 2 (HER-2, also known as Her-2/neu, ErbB2) is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases, which in humans includes EGFR (HER-1, ErbB1), HER-2, HER-3 (ErbB3) and HER-4 (ErbB4) (Yarden and Sliwkowski, 2001). HER receptors are essential in the regulation of cell proliferation and differentiation, therefore, their overexpression and uncontrolled activation is associated with essentially all of the key features of cancer, such as autonomous cell growth, invasion, angiogenic potential and development of distant metastases (Alroy and Yarden, 1997; Olayioye et al., 2000). Her-2/neu is overexpressed in ∼30% of invasive breast cancers and ∼70% of ductal carcinomata in situ (Slamon et al., 1989), but also in ovarian (Slamon et al., 1989), renal and colon cancers (Brossart et al., 1998). As the molecule contains a large extracellular domain and is thus accessible to components of the immune system, a series of monoclonal antibodies targeting Her-2/neu were generated (Fendly et al., 1990). The most prominent example is trastuzumab (Herceptin®), a humanized monoclonal antibody (Carter et al., 1992), which inhibits growth of Her-2/neu overexpressing tumor cells in vitro and in vivo (Sliwkowski et al., 1999). It proved to be successful as monotherapy of even heavily pretreated breast cancer patients (Cobleigh et al., 1999), monotherapy as first-line treatment in metastatic disease (Vogel et al., 2002), and especially in combinations with various cytotoxic drugs (Slamon et al., 2001; Pegram et al., 2004). Consequently, trastuzumab was approved by the U.S.

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Food and Drug administration for passive immunotherapy of breast cancer patients in 1998. In contrast to these successes, other antibodies targeting Her-2/neu were found to promote tumor growth (Yarden, 1990). It was demonstrated that these opposing effects were due to epitope specificity (Yip et al., 2001). Thus, the definition of inhibitory epitopes became important for identifying sites as targets for anticancer therapies. The trastuzumab binding site on Her-2/neu was characterized by co-crystallization experiments (Cho et al., 2003). Here we report a new method for epitope definition. Using the phage display technique, which allows to characterize short peptides structurally mimicking an epitope of interest, we defined five such mimotopes of the epitope recognized by trastuzumab on Her-2/neu (Riemer et al., 2004). The candidate peptides were extensively characterized for structural equivalency with the trastuzumab binding site and experimentally proven to be true mimics before being termed mimotopes. These peptides can be used for immunizations, aiming at a precise induction of tumor-inhibitory antibodies. We now aimed to utilize the obtained mimotope sequences for epitope definition through computer-aided pattern matching, performed by a newly developed algorithm (Kraml et al., submitted for publication) that compares the structural and chemical characteristics of the mimotope peptide and the surface exposed amino acids of the original antigen.

2. Materials and methods

2.1. Definition and characterization of mimotopes

Definition and characterization of mimotopes have been performed as described elsewhere (Riemer et al., 2004). In short, three rounds of biopanning with trastuzumab (Herceptin®, Roche, Hertfordshire, UK) were carried out with a random peptide phage library, expressing cysteine-flanked decapeptides, circularized by disulfide bridging, fused to pIII of the filamentous phage M13 (Library CL10, kindly provided by PD Dr. Luca Mazzucchelli, University of Bern, Switzerland). After each round of biopanning, colony screenings for selection of trastuzumab-specific phage clones were done according to Barbas et al. (1991). Positive clones were DNA sequenced and further subjected to a second specificity test in the ELISA format. Finally, they were examined for mimicry with the original antigen, Her-2/neu, in two inhibition experiment setups. Inserts fulfilling the criteria of positivity in both the colony screening and the specificity ELISA, and showing antigen mimicry, were considered to be true epitope mimics, i.e. mimotopes.

2.2. Mimotope matching

Obtained mimotope primary sequences were subjected to the surface matching algorithm developed by Kraml et al. (submitted for publication). Localization of epitope regions on antigen surfaces is achieved utilizing mimotope amino acid sequence information and antigen molecule spatial structure as given in the RCSB Protein Data Bank (http://www.rcsb.org/pdb/). The surface area localization scheme consists of a $M \times N$ variable linear program, where M and N are the numbers of mimotope and antigen atoms, respectively, each variable representing the distance between one mimotope atom and one antigen atom in a notional superimposition of the two molecules. The objective function, to be minimized, measures how close mimotope atoms are aligned to antigen atoms, which special consideration being given to charged atoms. Distances between antigen atoms and distances between mimotope side chain atoms are used to define the initial set of constraints. This linear program is solved repeatedly, each time with additional constraints harvested from previous runs having been added, until the closest superimposition not violating mimotope bond length and bond angle requirements is identified.

3. Results

3.1. Mimotopes

As reported previously, five circular decapeptide mimotopes, falling into three groups, were obtained for the epitope recognized by trastuzumab on Her-2/neu (Riemer et al., 2004) (Table 1). Sequence analysis revealed no homology to Her-2/neu or any other member of the EGFR family in database alignments (EMBL Data Library). As the trastuzumab epitope is described to be conformational, this finding was expected.

3.2. Surface matching

Mimotopes C-QMWAPQWGPD-C, C-KLYWADGELT-C and C-KLYWADGEFT-C matched to aa 595–602 of Her-2/neu. Mimotopes C-VDYHYEGTIT-C and C-VDYHYEGAIT-C matched to an overlapping area, just moved by one position, i.e. to aa 596–603 (Table 1). These residues belong to the juxtamembrane extracellular region of the receptor (Fig. 1), and form part of the described trastuzumab binding site (Cho et al., 2003). This site on Her-2/neu is composed of three loop regions that contact the antibody: loop 1 (aa 557–561), loop 2 (aa 570–573) and loop 3 (aa 593–603). The mimotopes match exactly to loop 3 (Fig. 2), which comprises the largest part of the epitope.

Table 1
Mimotope sequences and respective matching sites

Insert sequence	Match on Her-2/neu primary sequence (residues)
C-QMWAPQWGPD-C	595–602
C-KLYWADGELT-C	595–602
C-KLYWADGEFT-C	595–602
C-VDYHYEGTIT-C	596–603
C-VDYHYEGAIT-C	596–603