

Short communication

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

Angelika B. Riemer^a, Georg Kraml^a, Otto Scheiner^a,
Christoph C. Zielinski^b, Erika Jensen-Jarolim^{a,*}

^a Center of Physiology and Pathophysiology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

^b Department of Medicine I, Clinical Division of Oncology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

Received 27 September 2004

Available online 8 January 2005

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.

© 2004 Elsevier Ltd. All rights reserved.

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.

* Corresponding author. Tel.: +43 1 40400 5103; fax: +43 1 40400 5130.

E-mail address: erika.jensen-jarolim@meduniwien.ac.at
(E. Jensen-Jarolim).

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

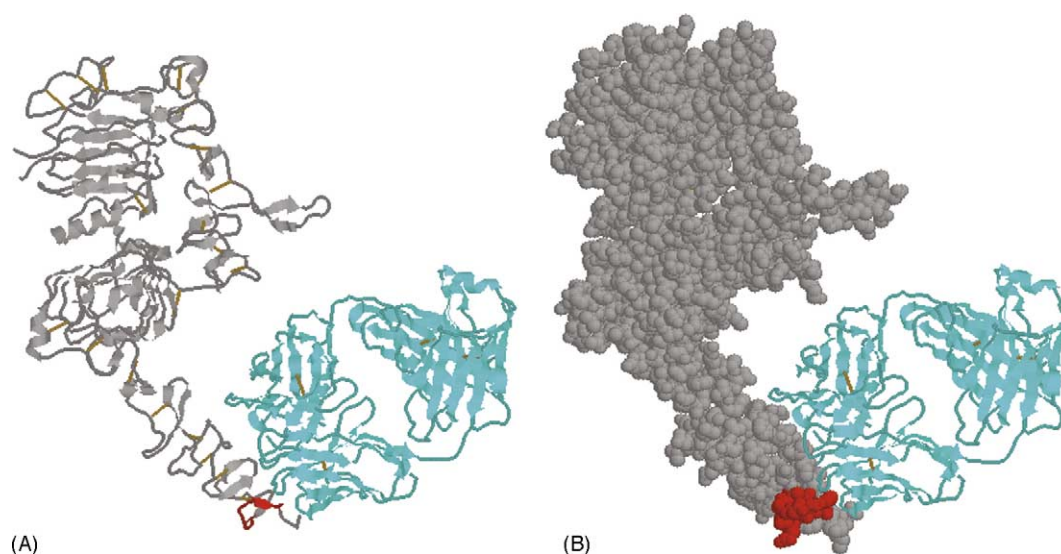


Fig. 1. Trastuzumab mimotopes match to the antibody's binding site on Her-2/neu. The mimicked area is shown in red. Panel A: Ribbon diagram of the extracellular domain of Her-2/neu (gray) and trastuzumab Fab (cyan). Panel B: Her-2/neu is shown as spacefill model. This figure was made with the program Protein Explorer 2.411 Beta[®].

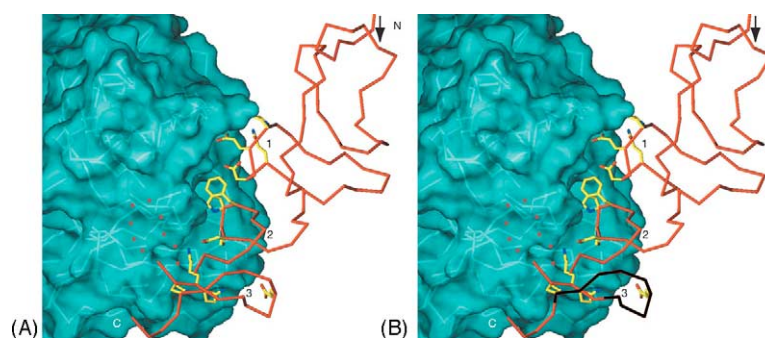


Fig. 2. Mimotopes match to loop three of the trastuzumab binding site. The backbone of Her-2/neu is shown in red, with side chains of residues contacting the antibody added in yellow. The surface of the trastuzumab Fab is shown in cyan. Panel A: The whole binding site, consisting of loop 1 (aa 557–561), loop 2 (aa 570–573) and loop 3 (aa 593–603). Panel B: The mimotope matching area (aa 595–603) is indicated in black on loop 3. (Panel A is reproduced with permission from Cho et al., 2003).

4. Discussion

Definition of molecular binding sites is of increasing importance in modern medicine. Tailor-made inhibitors are modeled to fit exactly in known ligand binding grooves or enzyme reactive centers, thus replacing the actual binding partners due to higher affinity (e.g. as in Moulder et al., 2000). In humoral immunology, the concept of epitope-specific targeting has gained importance, as antibodies were shown to both ameliorate or aggravate disease symptoms (Hurwitz et al., 1995; Lewis et al., 1993; Stancovski et al., 1991; Yip et al., 2001). To determine interaction sites, so far co-crystallization experiments had to be carried out. Unfortunately, not all proteins are capable of crystallizing; furthermore, even with those that are, highly purified samples of the binding partners must be available in considerable amounts for this approach to work. On the other hand, many three-dimensional data are already available for proteins, and are accessible in

the RCSB Protein Data Bank. Utilizing the phage display technique one is able to identify peptide mimics of virtually any binding structure of interest. There are several programs for matching surface patches of molecules (Durand et al., 1999; Poirrette et al., 1997; Li et al., 2003), but so far none was developed explicitly for mimotope matching. We devised an algorithm that makes use of given .pdb files and mimotope sequences, allowing for relative flexibility of short peptides when scanning a given protein surface for matching sites (Kraml et al., submitted for publication). As we previously defined five mimotopes for the epitope recognized by trastuzumab on Her-2/neu (Riemer et al., 2004), we applied the new approach to this oncogenic receptor. There was the additional advantage of the trastuzumab epitope on Her-2/neu already being known (Cho et al., 2003), so we were able to immediately verify obtained results. The mimotopes, though differing considerably in amino acid composition, all matched to the same area on the receptor, i.e. loop three of

the described trastuzumab binding site. This strengthens the postulate that mimotopes mimic an epitope solely by their biochemical and structural properties, and not necessarily by sequence similarities. The fact that all five mimotopes obtained in the biopanning procedure match to the same loop point out that this part of the trastuzumab epitope may be the immunologically most dominant. It is also the largest of the three loops involved. Our results also indicate that a circular decapeptide mimotope mimics about a third of the area of an antibody's epitope.

In conclusion, we here present a novel method for epitope definition. The Her-2/neu extracellular domain scanned in this study has a molecular weight of 110 kDa and consists of 632 amino acids, showing that the algorithm can successfully be applied even to large proteins of interest.

Acknowledgements

We thank Prof. Daniel J. Leahy, The Johns Hopkins University School of Medicine, Baltimore, MA, USA, for his permission to use his figure 'The Herceptin-binding site', *Nature* 421 (6924): 756–60, 2003. This work was supported in part by BioLife Science GmbH, Vienna, Austria and the Center of Excellence in Clinical and Experimental Oncology of the Medical University of Vienna (Austrian Federal Ministry of Education, Science and Culture, GZ 200.062/2-VI/1/2002).

References

- Alroy, I., Yarden, Y., 1997. The ErbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand–receptor interactions. *FEBS Lett.* 410, 83–86.
- Barbas 3rd., C.F., Kang, A.S., Lerner, R.A., Benkovic, S.J., 1991. Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. *Proc. Natl. Acad. Sci. U.S.A.* 88, 7978–7982.
- Brossart, P., Stuhler, G., Flad, T., Stevanovic, S., Rammensee, H.G., Kanz, L., Brugger, W., 1998. Her-2/neu-derived peptides are tumor-associated antigens expressed by human renal cell and colon carcinoma lines and are recognized by in vitro induced specific cytotoxic T lymphocytes. *Cancer Res.* 58, 732–736.
- Carter, P., Presta, L., Gorman, C.M., Ridgway, J.B., Henner, D., Wong, W.L., Rowland, A.M., Kotts, C., Carver, M.E., Shepard, H.M., 1992. Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl. Acad. Sci. U.S.A.* 89, 4285–4289.
- Cho, H.S., Mason, K., Ramyar, K.X., Stanley, A.M., Gabelli, S.B., Denney Jr., D.W., Leahy, D.J., 2003. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 421, 756–760.
- Cobleigh, M.A., Vogel, C.L., Tripathy, D., Robert, N.J., Scholl, S., Fehrenbacher, L., Wolter, J.M., Paton, V., Shak, S., Lieberman, G., Slamon, D.J., 1999. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* 17, 2639–2648.
- Durand, P., Pasari, R., Baker, J., Tsai, C., 1999. An efficient algorithm for similarity analysis of molecules. *Int. J. Chem.* 2, 1–12.
- Fendly, B.M., Winget, M., Hudziak, R.M., Lipari, M.T., Napier, M.A., Ullrich, A., 1990. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer Res.* 50, 1550–1558.
- Hurwitz, E., Stancovski, I., Sela, M., Yarden, Y., 1995. Suppression and promotion of tumor growth by monoclonal antibodies to ErbB-2 differentially correlate with cellular uptake. *Proc. Natl. Acad. Sci. U.S.A.* 92, 3353–3357.
- Kraml, G., Riemer, A. B., Jensen-Jarolim, E., Computational mimotope surface matching, submitted for publication.
- Lewis, G.D., Figari, I., Fendly, B., Wong, W.L., Carter, P., Gorman, C., Shepard, H.M., 1993. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. *Cancer Immunol. Immunother.* 37, 255–263.
- Li, C.H., Ma, X.H., Chen, W.Z., Wang, C.X., 2003. A protein-protein docking algorithm dependent on the type of complexes. *Protein Eng.* 16, 265–269.
- Moulder, S.L., Yakes, F.M., Muthuswamy, S.K., Bianco, R., Simpson, J.F., Arteaga, C.L., 2000. Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells in vitro and in vivo. *Cancer Res.* 60, 3384–3388.
- Olayioye, M.A., Neve, R.M., Lane, H.A., Hynes, N.E., 2000. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.* 19, 3159–3167.
- Pegram, M.D., Konecny, G.E., O'Callaghan, C., Beryt, M., Pietras, R., Slamon, D.J., 2004. Rational combinations of trastuzumab with chemotherapeutic drugs used in the treatment of breast cancer. *J. Natl. Cancer Inst.* 96, 739–749.
- Poirrette, A.R., Artymiuk, P.J., Rice, D.W., Willett, P., 1997. Comparison of protein surfaces using a genetic algorithm. *J. Comput. Aided Mol. Des.* 11, 557–569.
- Riemer, A.B., Klinger, M., Wagner, S., Bernhaus, A., Mazzucchelli, L., Pehamberger, H., Scheiner, O., Zielinski, C.C., Jensen-Jarolim, E., 2004. Generation of peptide mimics of the epitope recognized by trastuzumab on the oncogenic protein Her-2/neu. *J. Immunol.* 173, 394–401.
- Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A., et al., 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244, 707–712.
- Slamon, D.J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., Norton, L., 2001. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344, 783–792.
- Sliwkowski, M.X., Lofgren, J.A., Lewis, G.D., Hotaling, T.E., Fendly, B.M., Fox, J.A., 1999. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin. Oncol.* 26, 60–70.
- Stancovski, I., Hurwitz, E., Leitner, O., Ullrich, A., Yarden, Y., Sela, M., 1991. Mechanistic aspects of the opposing effects of monoclonal antibodies to the ERBB2 receptor on tumor growth. *Proc. Natl. Acad. Sci. U.S.A.* 88, 8691–8695.
- Vogel, C.L., Cobleigh, M.A., Tripathy, D., Gutheil, J.C., Harris, L.N., Fehrenbacher, L., Slamon, D.J., Murphy, M., Novotny, W.F., Burchmore, M., Shak, S., Stewart, S.J., Press, M., 2002. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.* 20, 719–726.
- Yarden, Y., 1990. Agonistic antibodies stimulate the kinase encoded by the neu protooncogene in living cells but the oncogenic mutant is constitutively active. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2569–2573.
- Yarden, Y., Sliwkowski, M.X., 2001. Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.* 2, 127–137.
- Yip, Y.L., Smith, G., Koch, J., Dubel, S., Ward, R.L., 2001. Identification of epitope regions recognized by tumor inhibitory and stimulatory anti-ErbB-2 monoclonal antibodies: implications for vaccine design. *J. Immunol.* 166, 5271–5278.