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Research paper

Mimtags: The use of phage display technology to produce novel protein-specific probes

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ABSTRACT

In recent times the use of protein-specific probes in the field of proteomics has undergone evolutionary changes leading to the discovery of new probing techniques. Protein-specific probes serve two main purposes: epitope mapping and detection assays. One such technique is the use of phage display in the random selection of peptide mimotopes (mimtags) that can tag epitopes of proteins, replacing the use of monoclonal antibodies in detection systems. In this study, phage display technology was used to screen a random peptide library with a biologically active purified human interleukin-4 receptor (IL-4R) and interleukin-13 (IL-13) to identify mimtag candidates that interacted with these proteins. Once identified, the mimtags were commercially synthesised, biotinylated and used for in vitro immunoassays. We have used phage display to identify M13 phage clones that demonstrated specific binding to IL-4R and IL-13 cytokine. A consensus in binding sequences was observed and phage clones characterised had identical peptide sequence motifs. Only one was synthesised for use in further immunoassays, demonstrating significant binding to either IL-4R or IL-13. We have successfully shown the use of phage display to identify and characterise mimtags that specifically bind to their target epitope. Thus, this new method of probing proteins can be used in the future as a novel tool for immunoassay and detection technique, which is cheaper and more rapidly produced and therefore a better alternative to the use of monoclonal antibodies.

1. Introduction

Protein-specific probes used for epitope mapping and protein-tagging studies have been the focal point of research during recent years employing various methods of detection of proteins, such as antibodies and labelled proteins. Currently, protein analysis consists of immunoassay techniques such as ELISA, polyacrylamide gel electrophoresis and related blotting techniques that use antibodies for detection and interactions with antigens (Westermeier and Marouga, 2005). The advent of monoclonal antibodies with the development of hybridoma technology by Kohler and Milstein in 1975 was another remarkable milestone, which has revolutionised the way we conduct research today (Borrebaeck, 2000; Chames et al., 2009; Kennett, 1979; Kohler and Milstein, 1975). Monoclonal antibodies are generally specific to the target antigen and are often used for epitope mapping of proteins for exploring the human proteome for diagnostics and therapeutic purposes (Rockberg et al., 2008). The epitope region can either be linear or conformational in their structure and the amino acid sequence can be continuous or discontinuous (Siddiqui, 2010). The ability of monoclonal antibodies to detect a single antigenic determinant (epitope) has earned it a major advantage over the use of polyclonal antisera. However, there are disadvantages of using antibodies where precise recognition of epitopes is seldom accurate (Rowley et al., 2004) and there are limitations and

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disadvantages with these techniques (Attarwala, 2010). The hybridoma technology involves many phases from the fusion of the primed B cells with myeloma cell line to the large-scale production of monoclonal antibodies from these highly selective hybrid cell lines (Falkenberg, 1998; Kohler and Milstein, 1975). For researchers working in the field, the use of this available in vitro technique is often problematic and has found to be very labour intensive and expensive (Falkenberg, 1998). Animal ethics is a major concern when it comes to development of monoclonal antibodies, which causes unnecessary suffering of animals (Falkenberg, 1998; Festing and Wilkinson, 2007). Another disadvantage of larger protein based probes, such as biotin binding proteins, is the size of these antibodies that are tagged with a detectable label. Larger probes present a challenge of inefficiently penetrating the conformational structures of target proteins as opposed to short peptides. For example, a large protein such as an antibody may interact with random functional groups on a single protein to the exclusion of thousands of other competing cytoplasmic proteins, nucleic proteins, carbohydrates and small molecules (Chen and Ting, 2005). It is therefore imperative to find new techniques that are particularly important for quantitative proteomics, less tedious and cost-effective.

A more contemporary pattern of research has brought short peptide sequences under the spotlight as target specific probes for in vitro and in vivo analysis of protein ligands such as transmembrane proteins. This type of epitope tagging has led to very successful epitope mapping of target antigens. The methodology has been used for protein localization, immunoprecipitation, and protein–protein interaction (Hernan et al., 2000). Briefly, an epitope is a sequence of amino acids in any structure that is recognised by an antibody. A single protein may carry multiple epitopes that can be recognised by antibodies. A mimotope (peptides mimicking epitopes) is a structure that mimics the structure of an epitope. Due to this structural similarity, a mimotope can elicit the same antibody response as an epitope via the paratope region of the antibody. Two decades ago it was realised that using peptide phage libraries, mimotopes could be isolated which mimic the epitope on a given protein. Using this technique, the 3-dimensional structure of the epitope can be determined along with the critical residues that make up the epitope (Xu et al., 2004). Hence, in this case the mimotope acts as a probe against the desired epitope on a protein or antibody as a therapeutic agonist or antagonist.

A new term introduced in this paper is 'Mimtags' which are the same short sequences of amino acids that play a critical role in the advancement of phage technology from a very different perspective. These short sequences of peptides are a part of fused proteins on the surfaces of phage used in phage display libraries. These mimotopes (Knittelfelder et al., 2009) that can be tagged (mimtags) on to protein sequence can effectively identify the epitope region of a target antigen as protein-specific probes. Hence, the term "mimtag" will now be used throughout the entire body of this paper replacing the term peptide mimotope.

The technique has been used in various studies to prove its effectiveness. A recent study conducted by our NeuroAllergy Research Laboratory (NARL) has shown remarkable results (Ahmed et al., 2011, 2012, 2013). In our study, random peptide library on M13 phage was used to screen IL-4R and IL-13. An affinity selection in this way allows the identification of mimtags that bind to the cytokine and its receptor, and hence, will most likely target epitopes more effectively due to the small size and nature of the mimtag (12-mer peptide). The mimtag also has the ability to conform to 3-Dimensional configuration, increasing its binding efficiency (Bredehorst and David, 2001). The bound peptide was sequenced and synthesised for further characterisation by immunoassay techniques. The study shows the potential use of biotinylated peptide mimtags as examples and their advantage over complex antigens and antibodies being applied as novel protein specific probes.

2. Materials and methods

To reveal the methodology involved in the isolation and characterisation of novel mimtags, we have divided the methods between two mimtags denoted N1 as an antagonist for IL-4R and P9 for IL-13. Phage display along with ELISA immunoassay (for the N1 mimtag) and dot-blot assay (for the P9 mimtag) were used for immunological characterisation of mimtags. Our research group identified both mimtags using phage display technology and inhibited the proteins from binding to their corresponding proteins in the case of IL-4Rα (BioScientific Pty Ltd, NSW, Australia) with cytokine interleukin-4 (IL-4) and IL-13 (Abcam, Cambridge, UK) with interleukin-13 receptor (IL-13R). Two different techniques have been used with 2 different proteins to show a range of method applications with this technology.

2.1. General description of the M13 phage library kit

Phage display libraries, PhD-12, in which 12-mer random mimtags are expressed at the amino terminus of protein pIII of the filamentous bacteriophage were purchased from New England BioLabs Inc. (Beverly, MA USA), as a kit. The peptide mimtag is followed by a short spacer sequence Gly-Gly-Gly-Ser, followed by the wild-type pIII sequence. Each of the libraries had a complexity of 2×10^9 virions and was amplified to give a final titer of 2×10^{11} plaque-forming units (pfu). All experiments were carried out under strict sterile conditions using a Class II biological safety cabinet.

Phage display peptide library (on arrival, the kit components were stored at −20 °C):

- 100 μl, ~1 × 1013 pfu/ml. Supplied in TBS with 50% glycerol;
- 96 gIII sequencing primer (5′-HO CCC TCA TAG TTA GCG TAA CG-3′, z100 pmol, 1 pmol/μl);
- 28 gIII sequencing primer (5′-HO GTA TGG GAT TTT GCT AAA CAA C-3′, 100 pmol, 1 pmol/μl);
- *Escherichia coli* (*E. coli*) ER2738 host strain; F′ proA + B + lacq-(*lacZ*) M15 zzf::Tn10 (TetR)/*fhuA2 glnV thi*-(*lac-proAB*)-(*hsdMS-mcrB*)*5* (rk mk McrBC). Host strain supplied as 50% glycerol culture: not competent. Stored at −20 °C.

2.2. Immobilization of target IL-4Rα and biopanning

The target solution was prepared using 0.1 M sodium hydrogen carbonate ($NaHCO_3$, pH 8.6), mixed with 5 mg/ml