Session 4: Molecular Biology

Wednesday 14th April 2010. Moderator: Jim W. Larrick

[16.10–16.30]
‘Synthetic antibodies: New tools for new biology’
Sachev Sidhu
University of Toronto, Canada

Abstract not provided.

[16.30–16.50]
‘Two-in-One antibodies: The generation of high affinity dual specific antibodies’
Germaine Fuh
Genentech Inc., South San Francisco, CA, USA

We explore the ability of an antibody-combining site to interact with two unrelated protein antigens by an engineering approach using phage display. We mutate the light chain CDRs of Herceptin to generate a repertoire for selection and isolate the variants that retain HER2 binding and bind to secondary antigens. Crystallographic and mutagenesis studies of a HER2/VEGF dual specific Fab revealed an extensive overlap of the antibody surface areas contacting the two antigens, but distinct sets of residues are engaged energetically with the two antigens. The high dual affinity is achieved and translated to dual action in vitro and in vivo. The results demonstrate 1) the capability of antibody-combining site to interact with two unrelated antigens with high affinity, and 2) a strategy that can be applied to generate other dual specific antibodies toward two defined targets.

[17.10–17.30]
‘Novel human anti-ErbB2 immunoagents’
Claudia De Lorenzo, G. Riccio, P. Laccetti and G. D’Alessio
University “Federico II”, Naples, Italy

Overexpression of ErbB2 receptor is associated with progression of malignancy of breast cancer, and is a sign of a poor prognosis. Herceptin, a humanized anti-ErbB2 antibody, has proved to be effective in the immunotherapy of breast carcinoma. However, it can engender cardiotoxicity and a high fraction of breast cancer patients are resistant to Herceptin-treatment.

Two novel human antitumor immunoconjugates were engineered in our laboratory by fusion of a human anti-ErbB2 scFv, termed Erbicin, with either a human RNase or the Fc region of a human IgG1, called Erb-hRNase and Erb-hcAb (human anti-ErbB2-compact Antibody), respectively. Both immunoagents are selectively cytotoxic for ErbB2-positive cancer cells in vitro and vivo.

The Erbicin-Derived Immunoagents (EDIA) target on breast cancer cells an ErbB2 epitope different from that of Herceptin. As the cardiotoxic side effects of Herceptin have no obvious basis in its interactions with the low levels of ErbB2 in cardiomyocytes, we tested whether these two novel anti-ErbB2 immunoagents might not present this effect.

ISSN 1093-2607/10/$27.50 © 2010 – IOS Press and the authors. All rights reserved
We report that Erb-hRNase and Erb-hcAb did not show cardiotoxic effects both in vitro on rat and human cardiomyocytes and in vivo on a mouse model, whereas Herceptin was strongly toxic. This difference was found to be due to their different mechanism of action, which can explain their different effects: Herceptin, at difference with Erb-hcAb, induces apoptosis in cardiac cells.

More interestingly, we found that EDIA are active on Herceptin-resistant cells both in vitro and in vivo. The sensitivity of these cells to treatment with EDIA is likely due the different epitope recognized by Erbicin derived immunoagents, since Erb-hcAb, at difference with Herceptin, was found able to inhibit the signalling pathway downstream ErbB2.

These results suggest that EDIA are immunoagents which could fulfill the therapeutic need of patients ineligible to Herceptin treatment due to cardiac dysfunction and could prove to be effective for treatment of some breast cancer patients which cannot be cured with Herceptin.

References


‘Preparation of human antibody light chain possessing catalytic activity’
Taizo Uda, Emi Hifumi and Akira Nishizono
Oita University, Oita-shi, Japan

By immunizing ground-state peptides or proteins into mice, we have successfully produced catalytic antibody light chains (antigenases) possessing serine protease-like characteristics. The unique feature of antigenase is the ability to enzymatically decompose a target molecule that is being killed. The authors have established some antigenases that could destroy, (i) the HIV-1 envelope protein gp41 [1], (ii) chemokine receptor CCR5 peptide [2], and (iii) Helicobacter pylori urease etc [3]. Based on their structural analysis, we proposed a concept [4], in which the antigenase encodes a catalytic triad composed of Ser, His and Asp in the germline gene.

Based on above concept, we examined human antibody light chains over 50 reported so far. Surprisingly, in kappa chain, the catalytic triad was mostly encoded in Subgroup II which is considered a minor group. Statistical data we have investigated will be given in the presentation.

In order to verify the above concept, we amplified an antibody germline gene as the cDNA belonging to Subgroup II, which was taken from human leukocyte. The cDNA was inserted in pET system, followed by a transformation to express the protein (human antibody light chain) in E.coli. We could recover the protein with very high purity by passing two affinity columns. Its catalytic activity was measured using synthetic substrate (peptide-MCA) to monitor the fluorescence generated by the cleavage reaction of the peptide-MCA bond, suggesting the human antibody light chain exhibited the amidase activity.

Conclusively, the preparation method mentioned above will be useful to obtain a catalytic antibody light chain.

‘Fab-Fv: An antibody fragment format with extended serum half-life’
David P. Humphreys
UCB Group, Slough, UK

Although antibody fragments have found clinical utility in both imaging and therapeutic applications, they often require a longer serum half-life than that of the native fragment. Achieving the appropriate serum half-life with antibody fragments has thus far been achieved through PEGylation and various protein and peptide fusions. We have developed a format called Fab-Fv, whereby a vL and vH is fused to each C-terminus of a Fab fragment. We will describe and exemplify a Fab-Fv in which the Fv binds with high affinity to serum albumin in order to confer a long serum half-life.

‘The path to platinum: The evolution of human combinatorial antibody libraries (HuCAL)’
Stefanie Urlinger
MorphoSys AG, Martinsried, Germany

Over the years the HuCAL antibody library concept, characterized by modular design and high quality trinucleotide synthesized CDR regions, was continu-
ally improved. The latest library is HuCAL Platinum which comprises many new features, such as gene-optimized framework regions and improved CDR design, and thus offers a significantly increased functional sequence space delivering large numbers of high-quality antibodies. In addition to selecting a broad diversity of high affinity, functionally active and well-expressing antibodies we strive to develop assays allowing us to assess the physico-chemical characteristics of promising antibodies early on in the project flow. This is mandatory in order to (1) select final lead candidates with high chances for success during the development process and (2) learn about potential risk factors of a molecule at an early stage.