An efficient system to generate monoclonal antibodies against membrane-associated proteins by immunisation with antigen-expressing mammalian cells

JournalArticle (Originalarbeit in einer wissenschaftlichen Zeitschrift)

ID 524394
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Year 2010
Title An efficient system to generate monoclonal antibodies against membrane-associated proteins by immunisation with antigen-expressing mammalian cells
Journal BMC biotechnology
Volume 10
Pages / **Article-Number** 87
ABSTRACT: BACKGROUND: The generation of monoclonal antibodies specific for protein antigens usually depends on purified recombinant protein for both immunisation and hybridoma screening. Purification of recombinant protein in sufficient yield and purity is a tedious undertaking and can be demanding especially in the case of membrane proteins. Furthermore, antibodies generated against a purified recombinant protein are frequently incapable of binding to the endogenous protein in its native context. RESULTS: We describe a strategy to generate monoclonal antibodies against membrane or membrane-associated proteins that completely bypasses any need for purified recombinant antigen. This approach utilises stably transfected mammalian cells expressing recombinant antigens on their cell surface for immunisation of mice. The transfected cells are also used for measuring seroconversion, hybridoma selection and antibody characterisation. By presenting the antigen in its native conformation for immunisation and hybridoma selection, this procedure promotes the generation of antibodies capable of binding to the endogenous protein. In the present study, we applied this approach successfully for three predicted GPI-anchored proteins of the malaria parasite Plasmodium falciparum. CONCLUSIONS: The described entirely cell-based technology is a fast and efficient approach for obtaining antibodies reactive with endogenous cell-surface proteins in their native conformation
Publisher BioMed Central
ISSN/ISBN 1472-6750
edoc-URL http://edoc.unibas.ch/dok/A5842808
Full Text on edoc No;
Digital Object Identifier DOI 10.1186/1472-6750-10-87
PubMed ID http://www.ncbi.nlm.nih.gov/pubmed/21159168
ISI-Number WOS:000286095600001
Document type (ISI) Journal Article