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Development of intracellular delivery strategies for antibody therapeutics

(Supervision: Dr Christian Fercher)

Monoclonal antibodies (mAb) have been utilised widely in clinical and basic research settings for the treatment of various diseases. Whilst all therapeutically approved monoclonal antibodies or fragments thereof are directed against cell surface receptors or proteins of the human secretome, intracellular antigen targeting strategies still await translation into the clinic. Despite significant advances in protein delivery technologies, reports of highly efficient transport vehicles are still sparse when systemically delivered in vivo. According to recent estimations, only several hundred proteins of the human proteome are suitable targets for small molecule drugs, providing an excellent opportunity for intracellular antibody therapeutics.

This project aims to develop suitable delivery strategies to translocate mAbs and various antibody fragments across the mammalian cell membrane. Potential strategies include the use of virus-like particles (VLPs), proteolipid and synthetic nanoparticles, liposomes and an in-house developed bispecific antibody platform. Cargo encapsulation will be tested using a variety of methods such as analytical HPLC-SEC, UV-Vis, DLS, BLI, SPR, immunostaining, flow cytometry etc. Intracellular delivery will be assessed using in vitro cell culture models. The successful applicant will be further trained in all aspects of recombinant mammalian and bacterial protein expression and purification techniques to enable in-house production of required antigens and antibodies.

Enrolling school: School of Chemistry & Molecular Biosciences (SCMB)

Suitable academic background: BSc in Molecular Biology, Biotechnology, Biochemistry etc.

Skills obtained in project: molecular cloning methods, protein expression and purification, protein analysis, biomaterial science, mammalian cell culture

Publication & postgraduate career potential: All our projects will lead to refereed publications and will provide a solid foundation for postgraduate studies.

To discuss further details of this project please contact
Dr Christian Fercher
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Discovery of novel functional antibodies against a human transcription factor cancer target

(Supervision: Dr Christian Fercher)

Transcription factors (TFs) are DNA binding proteins responsible for regulating gene expression in all forms of life. In embryonic development of complex eukaryotes, some TFs are expressed in a spatiotemporal manner, giving rise to cell differentiation and proliferation, tissue specification and directional growth and remodelling. Their biological functions oversee the biological specification and

cellular differentiation in a wide range of cell embryonic cell lines in development. Mutations can have potentially severe phenotypic consequences and can be embryonically lethal. This is indicative of their crucial roles in organogenesis throughout embryonic development. The TF Sox18 is involved in lymphangiogenesis and is upregulated in certain cancers, contributing to metastatic tumour transition. Its redundant roles in fully developed humans makes for an attractive therapeutic target. Consequently, the discovery of functional antibodies against Sox18 and related TFs (e.g. Sox2, Sox9) presents an opportunity to develop a potential therapeutic modality for preclinical in vitro and in vivo animal studies.

Methodologies in this project include recombinant bacterial expression and affinity purification of truncated TF variants. Successful isolation of these proteins will be assessed via SDS-Page and immunoblotting, and their structural integrity will be analysed by analytical HPLC-SEC and ELISA. The purified proteins will be used as antigens in a phage display biopanning campaign using an in-house human single-chain variable fragment (scFv) antibody library. Isolated phage pools will be assessed by phage ELISA and screened for high affinity binders to the corresponding TF. The most promising candidates will be reformatted into full length monoclonal antibodies via in-fusion cloning methods. Functional testing of antibodies expressed and purified from CHO cells will be carried out using in vitro binding and cell culture assays.

Enrolling school: School of Chemistry & Molecular Biosciences (SCMB)

Suitable academic background: BSc in Molecular Biology, Biotechnology, Biochemistry, immunology

Skills obtained in project: protein expression and purification, protein analysis, antibody discovery, functional antibody screening

Publication & postgraduate career potential: All our projects will lead to refereed publications and will provide a solid foundation for postgraduate studies.

To discuss further details of this project please contact
Dr Christian Fercher
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Discovery of blocking antibodies for Angiotensin type II receptor.

(Supervision: Dr Lucia Zacchi)
ARC Training Centre for Biopharmaceutical Innovation

Monoclonal antibodies (mAbs) are a major class of biologics used for the treatment of chronic disease indications, principally cancer, inflammatory diseases and infectious disease. The angiotensin II type 2 receptor is a clinically-validated target for the relief of peripheral neuropathic pain. The small molecule drug EMA401, that binds the receptor, can effectively relieve peripheral neuropathic pain. Due to some advantages mAbs have as therapeutic entities over small molecules, the goal of this project is to isolate blocking mAbs against the angiotensin II type 2 receptor. We will use state-of-the-art antibody engineering and antibody discovery methodologies to find new mAbs that bind the angiotensin II type 2 receptor, and to fully characterize the mAbs, including specificity and affinity. After extensive analysis of the isolated panels of antibodies, a suitable lead mAb will be selected for further development along the drug development pipeline.

Methodology: transformation of plasmids in bacteria, plasmid extraction and verification by restriction digestion. Maintenance and transfection of mammalian cell cultures. Antibody phage display biopanning of stably expressing or transiently transfected mammalian cells. FACS to enrich for better

binders and ELISA to identify best candidates. Reformatting of most promising candidates into full length monoclonal antibodies. Expression of candidate antibodies in CHO cells, mAb purification, and mAb functional testing through cell culture assays.

Enrolling school: School of Chemistry & Molecular Biosciences (SCMB).

Suitable academic background: BSc in Molecular Biology, Biotechnology, Biochemistry, Microbiology, Immunology.

Skills to be obtained in the project: antibody discovery, protein expression and purification, functional antibody screening. Writing and oral presentation techniques.

Publication & postgraduate career potential: All our projects will lead to refereed publications and will provide a solid foundation for postgraduate studies.

To discuss further details of this project please contact
Dr Lucia Zacchi
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Expression of antibodies in yeast

(Supervision: Dr Lucia Zacchi)
ARC Training Centre for Biopharmaceutical Innovation

One novel class of therapeutic antibodies with enormous potential are bi and tri-specific antibodies. However, these type of antibodies suffer from major biosynthetic bottlenecks in CHO cells that lead to poor yield, increasing industrial production cost. We will use the yeast Saccharomyces cerevisiae to develop a suitable expression system for antibodies and help identify and solve production bottlenecks. We will then test in CHO cells if the targets identified in yeast translate into increased antibody production in mammalian systems.

Methodologies: Recombinant protein engineering. Transformation of plasmids in bacteria, plasmid extraction and verification by restriction digestion. Diverse genetic, molecular, cellular, and biochemical techniques in yeast. SDS-PAGE, mass spectrometry proteomics and HPLC characterization of antibodies. Mammalian cell protein expression.

Enrolling school: School of Chemistry & Molecular Biosciences (SCMB).

Suitable academic background: BSc in Molecular Biology, Biotechnology, Biochemistry, Microbiology.

Skills to be obtained in the project: genetic and protein engineering, protein expression and purification, yeast protein expression and genetic manipulation, antibody characterization, mammalian cell protein expression and purification, mass spectrometry proteomics and HPLC. Writing and oral presentation techniques.

Publication & postgraduate career potential: All our projects will lead to refereed publications and will provide a solid foundation for postgraduate studies.

To discuss further details of this project please contact
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