Aiming a blow against tissue ageing: Targeting the αVβ8 integrin

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Overview

Ageing is associated with a progressive degeneration of tissues, which has a negative impact on the structure and function of vital organs and is a key risk factor for most chronic diseases. Since the proportion of the world's population aged >60 years will double in the next four decades, this will be accompanied by an increased incidence of chronic age-related diseases that will place a huge burden on healthcare resources. There is increasing evidence that the tissue damage caused by chronic inflammatory disorders, such as chronic obstructive pulmonary disease (COPD) and inflammatory bowel disease (IBD), models the effects of accelerated tissue ageing [1,2]. Hence, the study of these illnesses not only aids in their treatment but also increases our understanding of how tissues age. Such disorders also epitomise the multiple chronic debilitating diseases, which are increasingly prevalent and a major cause of morbidity and mortality in the elderly (COPD is now the fourth leading cause of death in the UK).

Diseases such as COPD and IBD involve both inflammatory and fibrotic responses. The inflammatory response is mediated by chronic activation of the immune system causing tissue injury, while the fibrotic response leads to deposition of extracellular matrix components and stiffening of the affected tissue, resulting in organ malfunction. There are currently no viable therapies to reverse the tissue destruction caused by these diseases.

A master regulator of both inflammatory and fibrotic responses is the protein Transforming growth factor-β (TGF-β). TGF-β is always produced as an inactive complex that must be activated to enable binding to its receptor and subsequent function. Recent evidence highlights a crucial role for members of the integrin receptor family in controlling the activation of TGF-β [3]. The integrin αVβ8 is found on immune regulatory cells (such as T-regs and dendritic cells) and can also be highly expressed on fibroblasts (cells that deposit ECM proteins). Several studies show a central role for this receptor in both the inflammatory and fibrotic elements of the disease process [4-8]. Not only is αVβ8 a biomarker for disorders such as COPD [4,5] but also agents that regulate the activity of αVβ8 can have a strong therapeutic benefit [4].

Although αVβ8 represents an attractive therapeutic target, the study of αVβ8 function in disease models (e.g. in mouse) has been greatly hampered by the lack of suitable monoclonal antibodies to detect, or modulate the function of, this integrin. This deficit is mainly a result of the very high degree of sequence homology between αVβ8 from different species, leading to a poor response after immunisation. Recently, alternative approaches to generating antibody-like reagents have become available, which are not limited by the immune response. Affimers are protein scaffolds that offer a highly promising alternative to antibody-based technologies [9,10]. Affimers that bind to the target protein can be selected from a large library, and then produced in bacteria at low cost. The small size of affimers (~14 kDa) also endows them with improved tissue penetration and localisation relative to monoclonal antibodies. Although a very new technology, affimers have the potential to supersede therapeutic monoclonal antibodies [11], and have already been used for diagnostics [12].

The proposed project is to fund an interdisciplinary collaboration with Dr Darren Tomlinson (from the internationally renowned Astbury Centre for Structural Molecular Biology at the University of Leeds) to develop affimers recognising mouse αVβ8. Dr Tomlinson has the library and the expertise for affimer screening, which we do not have in Manchester. Dr Tomlinson's laboratory was the first to develop these reagents [9,10], and has successfully raised affimers against many different proteins.

Project details

Recombinant integrins will be purchased from Bio-techne. Screening of affimer libraries on mouse αVβ8 will take place in Dr Tomlinson's laboratory. Purified affimers will then be further selected by negative screening [13] on a closely related integrin (mouse αVβ1). The selected affimers will then undergo characterisation in Manchester, first *in vitro* (using the biophysics technique Surface Plasmon Resonance, SPR) to determine the affinity and specificity of binding to mouse αVβ8 using the ProteOn XPR-36 instrument, and then preliminary testing *in vivo* (using wild-type mice and mouse cell lines). Our first priority is to generate affimers that bind mouse αVβ8 with high affinity, which work well for histology in frozen sections etc.; however, we also hope to obtain some affimers capable of modulating αVβ8 function, and this will also be tested by SPR and in cell-based assays.

Project Time Line: Months 1-3: Affimer screening. Months 4-5: Affimer characterisation. Month 6: *In vivo* testing. (Note: we do not currently anticipate being able to test affimers in disease models in the time frame and costs of the project but will do so if circumstances permit.)

We are a multi-disciplinary team with expertise in biophysics, cell biology, immunology and *in-vivo* disease models. We have also established links with Scott Levison (Consultant Gastroenterologist) and Angela Simpson

(Professor of Respiratory Medicine) for future clinical input into this endeavour. MT has mouse models of IBD [14] and KP-H has mouse models of organ fibrosis [15]. APM and TJ have expertise in SPR of integrins [16]. MH will provide recombinant human integrins for further screening of affimers.

Summary budget of project costs

Contribution to screening costs (Leeds): £2,000. Recombinant integrins: £1,600. SPR testing and *in-vitro* screening: £600. Testing effects of affimers on TGF-β activation in cell-based assays: £500. Mouse purchase and housing: £350. Histology costs: £150. Collaboration travel costs: £100. Consumables (including SPR chips):£600. Total cost £5,900.

Projected Outcomes

- Publication of our work concerning the generation of affimers against αVβ8 and their characterisation *in vitro*.
- The affimers generated here should allow us to detect, for the first time, mouse αVβ8 at sites of inflammation and fibrosis *in vivo.*
- Some of the affimers developed here could cross-react with human αVβ8 and therefore may have uses in the clinic (e.g. as a biomarker in diagnostics/pathology). Our studies will also provide proof-of-principle for future development of therapeutic agents directed against the human receptor.
- In the immediate term, we would apply for external funding to use the affimers to carry out studies of the role of αVβ8 in mouse disease models, and to develop affimers targeting the human integrin.
- In the longer term, development of these reagents should also result in a greater understanding of the mechanisms of normal tissue ageing, leading to a better appreciation of how to slow down or reverse tissue degeneration.

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