INVESTIGATING THE ROLE OF THE SCF/c-Kit PATHWAY IN SYSTEMIC SCLEROSIS CLINICAL SUBSETS: IMPLICATIONS FOR PATHOGENESIS AND THERAPY

Project grant from the Raynaud's and Scleroderma Association

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Background to the project c-Kit is a receptor tyrosine kinase present on the surface of progenitor cells, as well as mast cells, and melanocytes. The ligand for c-Kit, SCF, is increased in environmental stress damaged cells, leading to recruitment of c-Kit positive cells into sites of injury. Because of this background c-Kit was felt to be of potential importance in scleroderma, since it is believed that fibroblast precursors, and mast cells contribute to the fibrosis, pruritus, and inflammation experienced by patients. Also the frequent and severe pigment changes in the skin of patients are potentially due to abnormal SCF/c-Kit activation.

In this project scleroderma skin and lung fibroblasts, tissue fluid, plasma and media from cultured cells were studied for activity of SCF/c-Kit. The role of c-Kit in migration of fibroblasts was analysed using novel aligned collagen tissue culture chips, designed to model invasion of the fibrotic process through skin and organs. Potential therapies targeting c-Kit were studied in this system for their ability to inhibit, thereby preventing spread of the scleroderma disease process.

What was shown Our data indicated that c-Kit is present on activated fibroblasts cultured from patients, and that SCF acting through c-Kit has important effects on the fibroblasts, leading to migration and proliferation. We, and others, have found increased SCF levels in scleroderma cells and a general finding of this project was that the full length SCF is increased on the surface of scleroderma fibroblasts. c-Kit was present at equal levels in both healthy and scleroderma fibroblasts. However, blocking c-Kit efficiently inhibited migration and proliferation of scleroderma cells.

In these studies lung fibroblasts gave the most consistent results, and migration on the aligned collagen substrates was used to model spreading and invasion of the fibrotic process in the lungs of scleroderma patients. Reproducible and consistent results were obtained with the novel aligned collagen system. c-Kit was amongst the maximally increased phosphoprotein in migrating cells. Neutralising anti-c-Kit blocked the aligned migration. Imatinib, an inhibitor of the c-Kit tyrosine kinase, blocked migration also. Synergy was seen when heparin, which binds and neutralises SCF, was added to the media of cells using this system. Heparin in combination with imatinib profoundly inhibited lung fibroblast migration.

One important question is whether c-Kit is present at a low level on all lung fibroblasts studied or whether a subpopulation of stem cells present in the cultures is responsible for the c-Kit expression seen. By FACS analysis it was shown that c-Kit

expression is characteristic of a small subpopulation of cells present in the lung fibroblast cultures. Using magnabeads to isolate c-Kit positive cells, this subpopulation was profiled further by qPCR for stem cell markers (Oct, Nanog, SOX2), which were enriched in the c-Kit positive scleroderma cells.
In addition an attempt was made to understand the factors regulating the SCF/c-Kit pathway in scleroderma fibroblasts. Adding inflammatory factors (IL-6, TNFα, IL-31) did not alter SCF/c-Kit. However adding growth factors, such as TGFβ or PDGF, led to greatly decreased c-Kit. It is possible that the subpopulation of cells expressing c-Kit, are a population of stem cells which commit to differentiation when exposed to growth factors such as TGFβ.

Implications of the findings for scleroderma as a whole Based on the results of this project it is proposed that in scleroderma patients induction of SCF leads to recruitment and maintenance of activated c-Kit positive fibroblast precursors, creating a pro-fibrotic niche in the involved tissues. Targeting the SCF/c-Kit pathway could be used to block fibroblast precursor recruitment and activation in scleroderma patients. Existing therapies such as heparin and tyrosine kinase inhibitors, could be used in combination to arrest progression and spreading of fibrosis, with particular relevance to patients with progressing lung involvement.

Publication and dissemination of results Based on the work funded by the grant award from RSA, this project has led to the following presentations at national and international meetings

August 2015, **14th Scleroderma Research Workshop**: Poster presentation: Investigating the role of c-kit positive subpopulation in Scleroderma lung fibroblasts.

November 2014, **ACR Boston**: Investigating the SCF/c-kit in Scleroderma Fibrosis.

May 2014, **BSR Liverpool**: The role of SCF/c-kit in Patients with Systemic Sclerosis.

In addition a full research article has been prepared and is under final review by the authors “USE OF NOVEL ALIGNED COLLAGEN TISSUE CULTURE MATRICES INDICATES A ROLE FOR AUTOCRINE SCF/c-KIT PATHWAY IN PROMOTING SCLERODERMA LUNG FIBROBLAST MIGRATION” Bahja Abdi Ahmed, Henry Lope*, Sarah Karrar, Justin Hsuan, George Martin, Xu Shiwen, Christopher P Denton, David Abraham, Richard Stratton