

FRI0415

HUMAN SKELETAL MUSCLE XENOGRAFTS TO MODEL SPORADIC INCLUSION BODY MYOSITIS

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Background: Sporadic inclusion body myositis (IBM) is the most common acquired muscle disease in adults over the age of 50, yet the underlying cause of the disease is unknown, and there are no disease-modifying therapies¹. The robust endomysial inflammation in addition to an increased association of IBM with specific HLA haplotypes and other autoimmune diseases suggests that IBM is primarily an autoimmune disease². However, an association with ageing, a lack of response to immunotherapy, and presence of pathological features such as ubiquitinated protein aggregates seen in neurodegenerative diseases suggest the immune response may be secondary to myodegeneration. Thus, the relationship between inflammation, inclusions, and myodegeneration in IBM is poorly understood.

Objectives: Fundamental obstacles to therapeutic development for IBM include the limited understanding of disease pathogenesis as well as a lack of animal models. It is our objective to address these deficiencies by developing a novel mouse xenograft model of IBM.

Methods: In this xenograft model, human muscle biopsy specimens are transplanted into immunodeficient mice. The human myofibers cut during the biopsy procedure degenerate, but new muscle fibres regenerate from the patient's satellite cells. This newly regenerated muscle is revascularized and innervated by the mouse host². Xenografts are collected at various post-operative timepoints ranging from three to eleven months and cryosectioned to carry out histochemical and immunohistochemical analysis.

Results: Our preliminary data show that IBM xenografts develop pathologic features of the human disease.

At 4 months, collections of xenografts from a patient with healthy muscle, a dermatomyositis patient, and an IBM patient display successful regeneration. Regeneration appears less robust in IBM xenografts and is inversely associated with number of human CD3+ cells and sarcoplasmic MHC-I upregulation. A proportion of the CD8+T cells within the IBM xenografts are proliferative at 4 months, and this is significantly reduced at 6 months (Fisher exact test, $p < 0.0001$). In addition, at 8.5 months, the IBM xenograft shows rare fibres containing p62 positive puncta.

Conclusions: This xenograft model will allow us to investigate the interactions between human muscle and immune system in a mouse host. We are using this model for mechanistic studies and preclinical therapeutic testing in IBM.

REFERENCES:

- [1] Dalakas MC. The New England journal of medicine 2015;373:393–394. doi:10.1056/NEJMc1506827
- [2] Zhang Y, et al. Human molecular genetics 2014;23:3180–3188. doi:10.1093/hmg/ddu028
- [3] Benveniste O, et al. Acta Neuropathol 2015;129:611–624. doi:10.1007/s00401-015-1384-5
- [4] Schmidt J, Dalakas MC. Expert review of clinical immunology 2013;9:1125–1133. doi:10.1586/1744666X.2013.842467

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XIAP AS NOVEL INTEGRATOR OF TGF BETA SIGNALLING AND CANONICAL WNT SIGNALLING IN TISSUE FIBROSIS

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Background: Aberrant activation of profibrotic pathways is a key feature of systemic sclerosis (SSc). Extensive evidence characterises TGF β - and canonical WNT-signalling as key drivers of fibroblast activation. The crosstalk between those pathways, however, remains largely unknown. A better understanding of the interplay of different profibrotic pathways may be the key to the development of effective targeted therapies. XIAP (X-linked inhibitor of apoptosis protein) is an ubiquitously expressed member of the IAP protein family with important functions in tissue turnover. XIAP was recently described to be implicated in canonical Wnt signalling and TGF β signalling.

Objectives: The aim of this study is to characterise the role of XIAP in fibrotic disease.

Methods: XIAP-expression was analysed by qPCR, IF and Western blot. XIAP was targeted pharmacologically with Embelin. The activation of the canonical Wnt pathway was assessed by analyses of Wnt target genes and by TOPflash/FOP-flash luciferase reporter assay. *In vivo*, XIAP inhibition was analysed in two different models of fibrosis.

Results: The expression of XIAP is increased in the skin of SSc patients compared to matched healthy individuals with a particularly prominent expression in fibroblasts. The overexpression of XIAP is more pronounced in SSc patients with diffuse and active skin fibrosis compared to SSc patients with limited and inactive disease. The overexpression of XIAP is also reflected in several experimental fibrosis models: the model of sclerodermatous graft versus host disease, the model of bleomycin induced skin fibrosis and in Wnt10b transgenic mice. Stimulation with either recombinant Wnt1 or TGF β cytokine induces the expression of XIAP in cultured fibroblasts. Inhibition of XIAP reduced the Wnt1- and TGF β induced activation of fibroblasts with reduced collagen release and expression of myofibroblast markers. In addition, XIAP inhibition reverted the activated fibroblast phenotype in SSc fibroblasts with reduced expression of stress fibres and α SMA. The antifibrotic effects of XIAP inhibition occurred in non-toxic doses. Mechanistically, XIAP inhibition reduced the activation of canonical Wnt signalling as demonstrated by TOPflash reporter assays and by the analysis of canonical Wnt target genes. XIAP inhibition also reduced the expression of canonical Wnt target genes in Wnt10b transgenic mice. To analyse the effects of XIAP inhibition *in vivo*, two mouse models were used: the model of bleomycin-induced dermal fibrosis and Wnt10b-transgenic mice. In both models, the pharmacological inhibition of XIAP exerted anti-fibrotic effects with reduced dermal thickening, reduced myofibroblast counts and reduced hydroxyproline contents.

Conclusions: XIAP is upregulated in SSc fibroblasts in a TGF β -dependent manner and promotes fibroblast activation by fostering canonical WNT signalling. Our data suggest that XIAP mediates an amplification loop between TGF β and canonical Wnt signalling. Inhibition of XIAP may thus be a novel approach to target aberrant canonical WNT signalling in fibrotic diseases.

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CD86 EXPRESSION ON CULTURED SKIN FIBROBLASTS FROM SYSTEMIC SCLEROSIS PATIENTS: IN VITRO EFFECTS OF CTLA4-IG

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Background: Skin fibroblasts (SFs) are involved in the excessive production of extracellular matrix (ECM) proteins which characterises fibrosis in systemic sclerosis (SSc).¹ Myofibroblasts which are characterised by a higher expression of pro-fibrotic molecules (a-SMA: alpha-smooth muscle actin; S100A4: fibroblast-specific protein-1) as well as by the over-production of ECM proteins (FN: fibronectin; collagens type I and III), may originate from the activation and differentiation of resident fibroblasts after multiple profibrotic stimuli.²

CTLA4-Ig interacts with the cell surface costimulatory molecule CD86 and can downregulate the target cell activation.³

Objectives: To evaluate CD86 expression and the *in vitro* effects of CTLA4-Ig on skin fibroblasts (SFs).

Methods: Skin biopsies were obtained from 8 "limited" cutaneous SSc patients (treated only with vasodilators, mainly cyclic prostanooids) and 4 healthy subjects (HSs), after EC and patient informed consent. After 8 days (T8) of culture, SFs obtained from biopsies were treated for 24 and 48 hours, in the absence or in the presence of CTLA4-Ig (10, 50, 100 and 500 micrograms/ml). Evaluation of CD86 expression was performed by quantitative real-time polymerase chain reaction (qRT-PCR). In addition, also human macrophages obtained from PBMCs of SSc patients, were cultured. The statistical analysis was carried out by the non-parametric Mann-Whitney U test.

Results: Cultured SSc fibroblasts showed a very low gene expression level of CD86, compared to cultured macrophages of SSc patients, taken as positive control for CD86 expression (99% less). Therefore, cultured SSc fibroblasts treated for 24 hours and for 48 hours with CTLA4-Ig (10, 50, 100 and 500 micrograms/ml) did not show any significant modulation in the gene expression levels of CD86, compared to untreated fibroblasts (CNT). Interestingly, cultured HSs fibroblasts treated with CTLA4-Ig for 24 hours and for 48 hours showed a significant decrease in the gene expression of CD86, limited to the highest dose (500 micrograms/ml), compared to CNT (0.16% and 0.64% less, respectively) ($p < 0.05$).