Background and objectives The key cells in the pathogenesis of tissue fibrosis are myofibroblasts. It is widely accepted that myofibroblasts are increased in SSc skin and their number correlates with the skin fibrosis. The mechanisms underlying their number and variability are unknown. The heterogeneity in the myofibroblasts number may explain the variability of some in vitro studies published on systemic sclerosis (SSc) fibroblast biology and may mirror the clinical heterogeneity of SSc patients both in natural history and severity of skin fibrosis. Our purpose was to unravel the specific myofibroblasts transcriptome of SSc skin biopsies.

Materials and methods Four patients with early diffuse SSc, before any immunosuppression, were enrolled in the study. Skin biopsy on forearm was performed and the fibroblasts subcultured for three passages. 250 acetone fixed α -SMA positive cells were isolated by laser capture microdissection (LCM) for mRNA analysis by Affymetrix Gene array and qRT-PCR validation. Pathway analysis was performed according to David-NIH software. Immunofluorescence (IF), followed by confocal laser scanning microscopy (CLSM), was conducted. Normal dermal fibroblasts were utilised to evaluate the effects of TGF- β stimulation both at mRNA and protein level.

Results qRT-PCR for α -SMA showed a 3.7 fold increased expression of α -SMA in the LCM captured cells. Microarray analysis identified 269 genes upregulated more than two fold in the myofibroblasts: 24 were clearly reconducible to profibrotic activation; 16 were ribosomal genes; 14 were genes involved in oxidative phosphorylation, 28 in cell to cell adhesion and 7 in antigen processing and presentation. The remaining genes were not classifiable in any specific functional pathway and comprised tropomyosin, reticulocalbin 1, caldesmon 1 and neuroblastome breakpoint family (NBPF). IF studies followed by CLSM confirmed the expression, never shown before, of NBPF in dermal fibroblasts. Functional studies on normal dermal fibroblasts indicated that NBPF was not inducible by 24 or 48 h stimulation with TGF- β neither at mRNA or protein level. IF followed by CLSM of α -SMA positive versus negative cells showed a specific expression profile for procollagen-1, caveolin-1, β -catenin and phospho-RB whereas SMAD3, SMAD1, SMAD5 and NF- κ B did not differ between α -SMA positive or negative cells.

Conclusions Myofibroblast transcriptome displayed, besides predictable genes involved in the increased ECM production and TGF- β pathway activation, genes involved in several pathways not known to be specific of myofibroblasts or inducible by TGF- β . The specific expression of these genes may reflect either a specific metabolic status or a specific differentiation lineage of myofibroblasts.

A73 MYOFIBROBLAST SPECIFIC GENE EXPRESSION SIGNATURE IN SYSTEMIC SCLEROSIS, MORE THAN TGF- β INDUCED ACTIVATION

Giuseppina Abignano,^{1,2} Heidi Hermes,³ Justin Gillespie,¹ Sergio A Jimenez,³ Paul Emery,¹ Francesco Del Galdo¹ ¹Scleroderma Research Centre, Section of Musculoskeletal Disease, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK; ²Rheumatology Unit, Second University of Naples, Naples, Italy; ³Scleroderma Centre, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

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