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Systemic sclerosis, myositis and related syndromes — etiology, pathogenesis and animal models.

**FRI0396**

**THE ATM KINASE AND PTEN, DRIVE MYOFIBROBLASTS DIFFERENTIATION BY ACTIVATING THE TGFB AUTOCRINE LOOP**

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**Background:** Pulmonary fibrosis is a major cause of mortality in scleroderma (SSc) and Idiopathic Pulmonary Fibrosis (IPF). Fibrosis is driven by Inappropriate myofibroblast differentiation and persistence. Understanding this process, is vital for developing an effective treatment. Angiotensin II is implicated in fibroblast activation in the heart and kidney, through interactions with growth factors (e.g. EGF and TGFβ).

**Objectives:** We examined the role of Angiotensin II in myofibroblast activation in the lung.

**Methods:** Lung fibroblasts were isolated from SSc, IPF, or control patient lungs (6 each). Myofibroblasts were also cultured from PTEN null and wild-type mice. Protein expression after angiogenin II treatment (AngII) was investigated by western blotting. Myofibroblast differentiation and function was assayed through the contraction of 3D collagen gels and scratch migration assays. The signalling pathways involved were dissected using specific inhibitors: PI3-kinase/AKT (wortmannin, LY294002), TGFβ (1d11 neutralising antibody, SB431542 ALK5 inhibitor) Ataxia-Telangiectasia Mutated (ATM – Ku55933), AngII (Losartan).

**Results:** SSc and IPF lung fibroblasts showed increased AKT phosphorylation and suppressed PTEN expression (p<0.05). Myofibroblast-like, with higher αSMA expression (p<0.05), increased collagen gel contraction (control: 207±14 vs SSc; 92±15 vs IPF 91±21, p<0.05), and enhanced migratory capacity (p<0.05). PTEN-null fibroblasts showed a similar phenotype. AngII treatment activated AKT, suppressed PTEN and induced myofibroblast differentiation in normal lung fibroblasts. In both AngII-treated and PTEN null lung fibroblasts AKT activation required the ATM kinase. Inhibition of AKT either with PI3K or ATM inhibitor abrogated these effects. The increased expression of Myofibroblast-related genes after AngII treatment, was also blocked by inhibition of TGFβ. Ataxia-Telangiectasia Mutated (ATM – Ku55933), AngII (Losartan).

**Conclusions:** Our data demonstrate for the first time that AngII signals via the ATM kinase, which together with PTEN suppression are essential for the activation of AKT by AngII. AngII promotes myofibroblast differentiation, by stimulating the fibroblast TGFβ autocrine loop through AKT. Our data shows that activation of AKT through ATM and PTEN, may serve as the molecular link between pulmonary hypertensive and lung fibrosis in fibrotic diseases.

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**FRI0397**

**PECULIAR EXPRESSION OF AUTOPHAGY BIOMARKERS IN NECROTIZING AUTOIMMUNE MYOPATHY MUSCLE**

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**Background:** Immune mediated necrotizing myopathy (IMNM) is a recently recognised pathology within the spectrum of idiopathic inflammatory myopathies (IMMs). Specific autoantibodies and the response to immunosuppressants aid to make the diagnosis and suggest immune-mediated pathogenesis, although histopathological features are not specific for IMNM. Autophagy and ubiquitin-proteasome system are two interacting systems by which dysfunctional cellular components are degraded in the cell. Their dysregulation, in sporadic inclusion Body Myositis (sIBM), seems to be responsible for the protein aggregates. The autophagy dysfunction in IMNM was not widely investigated.

**Objectives:** To investigate autophagy marker expression, macrophages localization and accumulation of misfolded proteins in non-necrotic fibres of IMNM muscle in comparison with Dermatomyositis (DM), Polymyositis (PM) and sIBM.

**Methods:** Among 52 IMMs diagnosed from January 2015 to June 2017, we reviewed muscle biopsies and stored sera. Six subjects were included in the IMNM group, characterised by many necrotic muscle fibres, regenerating muscle fibres and no significant inflammation despite of numerous but scattered macrophages removing necrotic muscle fibres. Two patients had anti-signal recognition particle (SRP) autoantibodies, two patients anti-3-hydroxy-3-methylglutaryl-enzyme A reductase (HMGCR), the others tested negative for specific autoantibodies. All IMNM patients had a positive response to immunosuppressants. Muscle sections were immunolabelled with the following antigens: ubiquitin, autophagy markers LC3b, p62 (a receptor of autophagy), TOP-43 (a marker of ubiquitinated proteic inclusions), SM31 and SM310 (Phosphorylated Neurofilaments), CD31 (endothelial cell marker), C5b-9 (membrane attack complex), CD4 (T-helper lymphocytes), CD8 (suppressors lymphocytes), CD68 (macrophages), CD20 (B-lymphocytes), CD56 (NK lymphocytes and regenerating muscle fibres), MHC I, MHC II. Quantitative results were compared among IMNM (n=6), DM (n=4), sIBM (n=4), PM (n=5) and healthy controls (n=4).

**Results:** In IMNM, inflammation was mild compared with DM, PM, sIBM, and consisted in sporadic endomyosial and/or perivascular cells CD68+. Skeletal muscle fibres (SMFs) containing LC3b+puncta were significantly higher in IMMN and IBMs than in DM or PM. In all IMNM, the greater proportion of LC3b+puncta was localised in CD68+ fibres (figure 1), instead, sIBM showed a high number of LC3b+puncta in vacuolated SMFs with low expression of CD68+SMFs. As expected, P62 and SM31 aggregates were significantly higher in sIBM than in the other IMMs, even if, also in IMNM, there were moderate p62 accumulations and a little proportion of SMFs stained by SM31. Finally, in IMMN there was the highest number of ubiquitin +SMFs.

**Conclusions:** These findings suggest an involvement of cellular clearance systems in the pathophysiology of IMNM similarly to sIBM. Nevertheless, LC3b+puncta in regenerating fibres can be considered a peculiar biomarker in IMNM. Further studies of larger cohort of patients are needed to better define IMNM.

**Disclosure of Interest:** None declared

**FRI0398**

**SL-401, A NOVEL TARGETED THERAPY DIRECTED TO THE INTERLEUKIN-3 RECEPTOR (CD123), KILLS PLASMACYTOID DENDRITIC CELLS FROM SYSTEMIC SCLEROSIS PATIENTS**

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**Background:** SL-401 is a novel biologic targeted therapy directed to the interleukin-3 receptor (CD123), kills plasmacytid dendritic cells from systemic scleroderma patients.

**Objectives:** To investigate the potential of SL-401 in the treatment of systemic sclerosis.

**Methods:** Sl-401 is a novel biologic targeted therapy directed to the interleukin-3 receptor (CD123), kills plasmacytid dendritic cells from systemic scleroderma patients. Further studies of larger cohort of patients are needed to better define IMMN.

**Disclosure of Interest:** None declared