SAT0313 **ROLE OF CD248 MOLECULE AS POTENTIAL REGULATOR OF TRANS-DIFFERENTIATION TOWARD MYOFIBROBLASTS OF PERIVASCULAR STROMAL CELLS IN SYSTEMIC SCLEROSIS PATIENTS**

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Background: The microvascular damage is a pivotal event in the pathogenesis of Systemic Sclerosis (SSc) and, after injury, both endothelial cells (ECs) and pericytes might trans-differentiate toward myofibroblast, responsible of fibrosis. Platelet-derived growth factor B (PDGFB) and transforming growth factorβ (TGFβ) play a key role in SSc pathogenesis. PDGFB is a potent mitogen for myofibroblasts, while TGFβ stimulate myofibroblast activation, including alpha smooth muscle actin (αSMA) expression. A key regulator of PDGFB and TGFβ signaling may be the CD248, a trans-membrane receptor required for proliferation and migration of pericytes and fibroblasts. It has been showed that, in an animal model of kidney fibrosis, the genetic deletion of CD248 modulates the response of renal pericytes to injury, by reducing the differentiation of myofibroblasts. The expression of CD248 is required for TGFβ-induced αSMA expression in pericytes and CD248 enhances the PDGFB pathway, mediating the proliferation and migration of perivascular cells.
Objectives: The aim of this work was to evaluate the expression of CD248, in SSc skin biopsies and its possible role in perivascular stromal cells proliferation, responsible to myofibroblast trans-differentiation, during SSc.
Methods: After ethical approval, skin biopsies and bone marrow mesenchymal stem cells (MSCs) were collected from 20 diffuse SSc patients and 10 healthy control (HC). CD248 expression was investigated in the skin, and in isolated MSCs treated with TGFβ or PDGFB, by immunofluorescence, qRT-PCR and western. Furthermore, we silenced CD248 in SSc-MSCs, to confirm the role of this molecule in TGFβ- or PDGFB-signaling modulation.
Results: CD248 expression in SSc skin was significantly higher when compared with HC skin. In particular, an increased expression of CD248 was found in ECs, stromal fibroblast and perivascular like stromal cells, co-expressing CD90, a marker of un-differentiated MSCs. Furthermore, in both, HC- and SSc-MSCs, TGFβ treatment induced a significant reduction of CD248 mRNA expression, in parallel with a significant increase of αSMA and a decrease of proliferation (ki67), when compared with untreated- (UT-) cells. Interestingly, the ability of TGFβ to inhibit CD248 expression in HC-MSCs was significantly higher than SSc-MSCs, suggesting that local environment in SSc patients affect TGFβ ability to suppress CD248 expression in SSc-MSCs. After treatment with PDGFB in both SSc- and HC-MSCs, CD248 expression was not affected, while significant reduction of αSMA and an increased expression of Ki67 was observed compared with UT-cells. After silencing of CD248 in SSc-MSCs, both TGFβ and PDGFB signaling were inhibited.
Conclusions: CD248 over-expression may play an important role in tissue fibrosis by modulating the pericytes to myofibroblast trans-differentiation, via regulation of both PDGFB and TGFβ signaling, during SSc.
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SAT0314 **A NOVEL HIGHLY SELECTIVE 5-HYDROXYTRYPTAMINE 2B (5-HT2B) RECEPTOR ANTAGONIST AMELIORATING FIBROSIS IN PRECLINICAL MODELS OF SYSTEMIC SCLEROSIS**

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Background: Microvascular injury is one of the first pathological events in systemic sclerosis, and precedes the fibrosis. A consequence of vascular damage is the exposure of subendothelial connective tissue that causes activation of platelets and local serotonin (5-hydroxytryptamine, 5-HT) release. Binding of 5-HT to 5-HT_{2B} receptors on *e.g.* fibroblasts results in increased myofibroblast differentiation and release of excessive amounts of matrix proteins subsequently leading to fibrosis. Thus, pharmacologic inhibition of 5-HT_{2B} receptor signalling may represent a new treatment opportunity for systemic sclerosis.
Objectives: The objective of the present study was to evaluate a novel highly selective 5-HT_{2B} receptor antagonist for its ability to reduce the production of matrix proteins in human dermal fibroblasts and to ameliorate fibrosis in the tight-skin-1 model of systemic sclerosis.
Methods: Dermal fibroblasts isolated from patients with systemic sclerosis were cultured with different concentrations of the 5-HT_{2B} receptor antagonist AM1125 with or without 1 μM 5-HT. Anti-fibrotic effects were evaluated by measuring matrix production, myofibroblast differentiation and TGF-β production. The tight-skin-1 model was used to evaluate anti-fibrotic effects *in vivo* using a therapeutic treatment approach. AM1125 was administered at 10 and 50 mg/kg orally, b.i.d. from week 5 to week 10. Hypodermal thickening, myofibroblast counts and collagen production (hydroxyproline) were evaluated at the end of the treatment period.
Results: *In vitro* the 5-HT_{2B} receptor antagonist AM1125 dose-dependently reduced TGF-β, PAI, nuclear SMAD2/3 and collagens in human dermal fibroblasts. In addition, stress fiber formation and α-SMA mRNA were reduced indicating decreased myofibroblast differentiation. Therapeutic treatment with AM1125 at 50 mg/kg reduced hypodermal thickness ($p<0.001$), myofibroblast counts ($p<0.05$) and hydroxyproline content ($p<0.01$) in the tight-skin-1 model. In addition, the lower dose (10 mg/kg) reduced hypodermal thickness ($p<0.001$).
Conclusions: The results demonstrate that the 5-HT_{2B} receptor antagonist AM1125 prevents pro-fibrotic events in human dermal fibroblasts and attenuates dermal fibrosis using a therapeutic treatment approach in the tight-skin-1 model. Thus, 5-HT_{2B} receptor antagonists, exemplified by the novel compound AM1125, could represent a new treatment opportunity for systemic sclerosis.
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SAT0315 **IMATINIB-LOADED TARGETED GOLD NANOPARTICLES AMELIORATE EXPERIMENTAL LUNG FIBROSIS INDUCED BY BLEOMYCIN**

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Background: Systemic Sclerosis (SSc) is an autoimmune disorder frequently affected by an interstitial lung involvement (ILD) that significantly deteriorates long term outcomes. In previous experiments we proved that specifically engineered gold-nanoparticles (GNP) loaded with imatinib and targeted with an anti CD44 Ab (GNP-HCim) significantly inhibited proliferation and induced apoptosis of fibroblast-like cells derived from ILD-SSc patients [1]. *In vitro*, GNP-HCim showed higher efficacy compared to the drug alone.
Objectives: To demonstrate in vivo the efficacy of GNP-HCim in ameliorating bleomycin-induced lung fibrosis
Methods: Eight-week-old C57BL6 male mice (n=8/group) were assigned to either: (1) controls receiving intratracheal aereosolization of saline solution and unloaded functionalised GNP (GNP-HC); (2) mice treated with intratracheal instillation of bleomycin (50 uL) on day 0 and GNP-HC; (3) mice treated with bleomycin on day 0 plus GNP-HCim; (4) mice treated with bleomycin plus intraperitoneal (i.p.) Imatinib (50 mg/kg, once daily). GNP-HC or GNP-HCim were administered by intratracheal instillation on day 10–15–20–25 and 3 h before culling. All mice were sacrificed on day 28. Lung specimens were analysed by electron microscopy, immunohistochemistry and immunofluorescence (IF). Data were evaluated by 2 blind observers and analysed with GraphPrism software for statistics.
Results: The administration of imatinib i.p or via GNP-HC reduced pathologic changes of the lungs as evaluated by the Lung Injury score and the Ashcroft score ($p<0.05$ for both). Collagen quantification by Picro Sirius Red revealed a significantly reduced staining only in the GNP-HCim group (p=0.0135 vs controls). IF revealed a significant reduction in αSMA+ myofibroblast counts when mice were inhaled with GNP-HCim (14.36±1.69/hpf) or injected i.p with imatinib (7.64±1.17/hpf) as compared to the controls (24.01±3.58/hpf, p=0.003 and p=0.0013 respectively). IF also showed significantly reduced counts of CD45+ (15.21±1.87/hpf vs 29±2.247/hpf in controls, p=0.0006) and CD44+ cells in groups treated with GNP-HCim (18.61±1.495) or imatinib i.p. (13.57±0.864), versus controls (28.56±2.854, p=0.0111 and p=0.0004 respectively). In imatinib i.p. and GNP-HCim-treated groups there was a significant reduction of phosphorylated c-Abl and PDGF-R, two downstream targets of imatinib, to levels comparable to group 1 controls with respect to the bleomycin group ($p<0.05$ for both). Finally, electron microscopy revealed accumulation of GNP-HCim and GNP in alveolar macrophages.
Conclusions: In the experimental model of bleomycin-induced lung fibrosis imatinib delivered to lungs through inhalation of anti CD44 targeted GNP was as effective as imatinib administered by i.p. route. These data favour the use of GNP in the development of new therapeutic approaches to SSc-ILD, which might be associated to a lower toxicity and side effects of systemic treatment.
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