Visfatin down-regulates growth-promoting podoplanin regulates the migration of ASCs

**Objectives**
The aim of this study was to perform comparative basic characterisation of ASCs of AS patients (AS/ASCs) and ASCs line originating from healthy volunteers (hASCs).

**Methods**
AS/ASCs of 15 AS patients and commercially available hASCs lines were used. Phenotype and expression of adhesion molecules (ICAM-1, VCAM-1) on ASCs were evaluated by flow cytometry. Basal and cytokine (tumor necrosis factor + interferon-g or interleukin-23)-induced secretion of nine factors (TGFß, IL-6, galectin, LIF, IL-1Ra, PGE2, sHLA-G, TSG6 and kynurenines) known to mediate immunomodulatory activity of MSCs was measured by ELISAs. ASCs were co-cultured with either anti-CD3/CD28-stimulated CD4 T lymphocytes or mitogen-stimulated peripheral blood mononuclear cells (PBMCs) of allogeneic healthy volunteers. Expression of activation markers (HLA-DR, CD25, CD69) on T cells and these cells proliferation were evaluated by flow cytometry.

**Results**
There were no significant differences in the phenotype, expression of adhesion molecules and secretory potential between hASCs and AS/ASCs. Inhibition of T cell proliferation was found in both co-culture systems, while modulation of activation markers expression (CD25 down-regulation, CD69 up-regulation) on CD4+ T and CD8+ T cell subsets was stated in ASCs-PBMCs co-cultures only and hASCs and AS/ASCs exerted similar effects.

**Conclusions**
AS/ASCs have phenotype and basic biological features (i.e. secretory potential, some attributes related to immunomodulatory activities) comparable to hASCs.

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Podoplanin regulates the migration of mesenchymal stromal cells and their interaction with platelets

**Objectives**
To investigate the influence of visfatin on H19 expression during osteogenesis.

**Methods**
Human MSCs from healthy donors (hMSC) and primary human MSCs from osteoarthritic patients (phMSC) after knee replacement surgery were treated with differentiation medium to induce osteogenic differentiation (OD). Matrix mineralization (MM) was quantified after 21 days by Alizarin Red. H19 expression by realtime PCR and IL-6 production by ELISA were measured.

**Results**
IncrNA H19 was up-regulated during OD. Although the H19 upregulation was not altered by co-stimulation with resistin, leptin or TNF, visfatin co-stimulation during OD down-regulated H19 expression up to 10-fold as compared to unstimulated MSCs. The effect was significant in phMSCs at two of three measured time points (day 7 p=0.03; day 14 p=0.002, n=3) and in hMSCs at day 14 (p=0.0003, n=4). Visfatin co-stimulation of MSCs in OD increased MM, as well as IL-6 levels. However, TNF did not alter H19 expression or increase MM.

**Conclusions**
Visfatin co-stimulation during osteogenesis down-regulated IncrNA H19 expression, indicating a loss of the growthpromoting effects of IncrNA H19 in affected areas of destructive bone disease. This regulatory effect was specific to visfatin and did not occur upon co-stimulation with other adipoikines or inflammatory stimuli such as TNF supporting a TNF-independent effect of visfatin.

**REFERENCES**

Disclosure of Interest None declared.

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Career situation of first and presenting author Young investigator.

**Introduction**
Within the rheumatoid joint, mesenchymal stromal cells (MSC) up-regulate podoplanin with unknown consequences for disease pathogenesis. The function of podoplanin has been linked to enhanced migratory potential and interactions with platelets. However, it is unclear how these two cell types interact with one another, given that MSC and platelets are usually located in different anatomical compartments (tissue vs blood respectively) separated by the blood vascular endothelial cells (EC).

**Objectives**
Here, we examined the functional consequences of podoplanin expression on the migratory potential of MSC and their interactions with circulating platelets.
Methods Human MSC were isolated from healthy controls. Comparisons were made between podoplanin positive and negative MSC. MSC migration across 8 um pore filters following treatment with anti-siRNA podoplanin or Rho GTPases inhibitors was assessed. MSC-platelet interactions were assessed by cultivating MSC on the basal surface of 3 um pore filters and perfusing fluorescently labelled platelets in whole blood over the apical surface. In some cases, the apical surface of the filter was pre-coated with EC, forming an EC-MSC coculture, prior to platelet perfusion.

Results Expression of podoplanin significantly enhanced the migration of MSC compared to MSC lacking podoplanin. Rac-1 inhibition altered the membrane localisation of podoplanin and in turn significantly reduced MSC migration. Blocking Rac-1 activity had no effect on the migration of MSC lacking podoplanin, indicating that it was responsible for regulation of migration through podoplanin. When podoplanin-expressing MSC were seeded on the basal surface of a porous filter, they were able to capture platelets perfused over the uncoated apical surface and induce platelet aggregation. Similar microthrombi were observed when EC were co-cultured on the apical surface. Confocal imaging shows podoplanin-expressing MSC extending processes into the EC layer, which could interact with circulating platelets. In both models, platelet aggregation induced by podoplanin-expressing MSC was inhibited by recombinant soluble CLEC-2.

Conclusions Podoplanin enhances the migratory capacity of tissue-resident MSC enabling them to move more rapidly within the rheumatoid joint. Moreover, podoplanin allows MSC to interact with both circulating and tissue platelets to elicit either protective or pathogenic responses.

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Disclosure of Interest None declared.

P119 EPIGENETIC REGULATION OF WNT SIGNALING IN SYSTEMIC SCLEROSIS VIA SFRP1

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Objectives To examine the role of IL-23R signaling during physiological bone remodeling and radial bone growth through regulation of osteoblast differentiation.

Methods Femurs of naïve 7-, 12- and 26-week-old IL-23R GFP/ GFP (IL-23R) and IL-23R+/+ (WT) littermate mice were used for micro-CT analysis of the bone and a three-point bending test for bone strength. Bone marrow (BM) cells were either cultured towards osteoclasts with M-CSF and RANKL or were cultured towards osteoblasts with β-glycerophosphate and vitamin C. Osteoclast differentiation and activity were assessed Wnt inhibitor that we have previously demonstrated to be involved in systemic sclerosis.

Methods Diffuse systemic sclerosis and healthy control serum was collected from 10 patients and controls. sFRP1 was measured with a standard ELISA method using a commercial ELISA. qPCR was performed with Ss fibroblasts or control fibroblasts using Taqman specific kits for miR27a3p and RNU44. Data was normalised to RNU44 and shown as fold change to controls. Transfection of microRNA mimics was performed using 100 nM of miR27 mimics or matched concentration of scramble after 48 hours post transfection media was collected and the cells lysed in RIPA buffer and lysates subjected to western blotting.

Results We found reduced levels of sFRP1 in systemic sclerosis sera compared to healthy controls (n=10). Using taqman PCR we also found elevated levels of microRNA27a3p in systemic sclerosis fibroblasts. Using software to predict targets it was identified that sFRP1 is a direct target of microRNA27a3p. We could demonstrate after transfection of microRNA27a3p into healthy dermal fibroblasts compared to scramble controls that the levels of the target sFRP1 was reduced and elevated levels of collagen1 and beta catenin was present. This suggest that sFRP1 is a direct target leading to upregulation of Wnt signaling. We also found reuced levels of anti-fibrotic PPAR-gamma also after transfection. siRNA knockdown of sFRP1 using small interfering RNA leads to upregulation of collagen and Axin2 and lactate elevation.

Conclusions sFRP1 is regulated by miR27a2p in systemic sclerosis. MiR27a3p can also regulate PPAR gamma. Enhanced Wnt signaling is associated with metabolic alterations.

Disclosure of Interest None declared.