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POSO424

DETERMINING CIRCULATING ENDOTHELIAL CELLS USING CELLSEARCH SYSTEM IN SYSTEMIC SCLEROSIS PATIENTS

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Background: Endothelial damage and fibroproliferative vasculopathy of small vessels are pathological hallmarks of Systemic Sclerosis (SSc). Detection and analysis of circulating endothelial cells (CECs) derived from affected blood vessels may be an informative tool to study vascular dysfunction and could be considered a novel biomarker of scleroderma vasculopathy. Our group first showed the presence of CECs in SSc by fluorescence-activated cell sorting (FACS), demonstrating that a raised counts of active CECs may represent direct evidence of active vascular disease in SSc. Despite these interesting data, issues related to difficulties in CECs analysis and separation from other circulating cell populations have been raised. With this study, we set out to determine if HOTAIR expressed in SSc dermal fibroblasts was a contributing factor to the high levels of C9K found in SSc patient skin

Objectives: To assess the counts of CECs determined by the CS in SSc patients and to evaluate their clinical implication and potential as vascular biomarker in SSc.

Methods: 10ml of blood samples were collected from 29 subjects (19 SSc patients and 10 healthy donors - HDs) and stored in tubes containing a specific preservative, to allow the analysis of 4ml of blood within 72 hours, according to manufacturer instructions. Out of 19 SSc patients, 18 were female, 10 had the disease in SSc. Despite these interesting data, issues related to difficulties in CECs analysis and separation from other circulating cell populations have been raised. With this study, we set out to determine if HOTAIR expressed in SSc dermal fibroblasts was a contributing factor to the high levels of C9K found in SSc patient skin

Methods: When analyzed according to disease subset, both iCSCs and dCSCs showed significantly increased levels of CECs in HDs with CD34+ and CD45+ in a similar fashion observed in the number of CECs in patients with iCSCs compared to those with dCSCs. Regarding vascular involvement, the CECs counts strictly correlated with the presence of digital ulcers (DU) (P=0.0001) showing a median of 863 cells/4ml for the SSc patients with DU versus a median of 276.2/4ml for the HDs without DU. No statistical correlation was found between CECs and serological autoantibody pattern, skin parameters, or joint and muscle involvement. Patients with active disease and the EULAR Activity Index, showed a higher CECs value than those with inactive disease (P=0.0012).

Conclusion: The method of CECs detection in peripheral blood has been recently proposed as a marker of endothelial damage in different vascular diseases, including SSc. However, currently no standardized method is available to determine CEC counts, which makes reported data on CECs reliable and suitable. The CS system is a commercially available semi-automated system that enables standardized determination of CECs. Thus, we examined clinical utility of CECs count by this system and could be a promising tool for monitoring active disease and evaluating therapeutic responses to vascular and immunosuppressive treatments.

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POSO425

INCREASED KERATIN 9 EXPRESSION IN SYSTEMIC SCLEROSIS SKIN IS DRIVEN BY THE LINCNRA HOTAIR FROM FIBROBLAST DERIVED EXOSOMES

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Background: Skin fibrosis is the hallmark fibrotic manifestation of systemic sclerosis (SSc). Despite a key role of tissue fibroblasts, skin changes extend to the keratinocyte layer, which contribute to the loss of skin function. RNA seq. analysis of SSc patient forearm skin showed that palmpplanctor specific Keratin 9 (K9) was highly expressed (1). SSc affected skin shares several features with palmpplanctor skin including increased keratinocyte layer thickness and lack of hair. Seminal work of last decade has shown that long noncoding RNA in the HOX loci play a crucial role in skin keratinocyte differentiation (2), the incRNA HOTAIR being one of the HOX IncRNAs mostly expressed in the palmpplanctor region.

Objectives: Following recent data suggesting a role of HOTAIR in the profibrotic phenotype of dermal fibroblasts in SSc, here we set out to determine if HOTAIR expressed in SSc dermal fibroblasts was a contributing factor to the high levels of C9K found in SSc patient skin

Methods: Full-thickness skin biopsies were surgically obtained from the forearms of patients with SSc of recent onset. Fibroblasts were isolated and cultured in monolayers. HOX transcript antisense RNA (HOTAIR) was expressed in healthy dermal fibroblasts by lentiviral induction employing a vector carrying the specific sequence. Exosomes were isolated from dermal fibroblast media using the Total exosome isolation reagent (Thermo Fisher). Enhancer of zeste 2 (EZH2) was blocked with GSK126 inhibitor. Skin equivalents were created using scramble and HOTAIR expressing fibroblasts with primary keratinocytes

Results: Media from both SSc patient fibroblasts and HOTAIR expressing fibroblasts induced C9K expression in healthy keratinocytes in vitro, in addition HOTAIR expressing fibroblasts induces C9K expression in keratinocytes in 3D skin equivalent models. Media fractionation studies indicated that HOTAIR was present in fibroblasts exosomes and found at a higher concentration (2.7 fold p=0.01) in exosomes from SSc fibroblasts. Importantly, transfection of Exosomal RNAs from SSc fibroblasts could reproduce the increase in C9K in keratinocytes. Mechanistically, C9K induction was mediated by changes to the histone methyl-profile in the keratinocytes through EZH2.

Conclusion: Pro-fibrotic dermal fibroblasts in systemic sclerosis contribute to the overall skin loss of function by inducing C9K in adjacent keratinocytes through transfer of the long non-coding RNA HOTAIR. Unraveling the crossstalk of activated fibroblasts with adjacent cells may lead to identify therapeutic targets to re-establish tissue homeostasis and function during fibrosis.

REFERENCES:


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POSO426

CIRCULATING MICRORNA PROFILING IN PATIENTS WITH ANTI-SYNTHESE SYNDROME AND INTERSTITIAL LUNG DISEASE

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Background: Anti-synthetase syndrome (ASSD) is an autoimmune disease characterized by autoantibodies against one of many aminocacyl transfer RNA (tRNA) synthetases. Interstitial Lung Disease (ILD) in ASSD patients is frequent, often severe and rapidly progressive, causing much of the increased morbidity and mortality associated with ASSD as compared to other idiopathic inflammatory myopathies [1].
OBJECTIVES: In this study, we hypothesized that immune-related miRNAs may be associated with presence/absence of lung involvement in patients with ASSD and help predict disease course.

METHODS: A total of 15 ASSD patients were enrolled: 11 with ILD and 4 without ILD. Differentially expressed miRNAs were identified in plasma derived exosome, using miRNA PCR array (MIHS-111ZG, Qiagen) including 84 miRNAs involved in activation and differentiation of T and B cells.

RESULTS: Among all miRNAs analyzed we found that miR-15b-5p, miR-23a-3p, miR-25-3p, miR-30a-5p and miR29c-3p were up-regulated in ASSD-ILD patients (p<0.05) as compared to patients without lung involvement (Figure 1). To evaluate the effectiveness of the five miRNAs for predicting ILD among ASSD patients, ROC curves were constructed. The AUCs of miR-15b-5p, miR-25-3p, miR-30a-5p and miR29c-3p were 0.83, 0.87, 0.86 and 0.89, respectively (p=0.05 for miR-25-3p and p<0.05 for all other curves). The prediction of the biologic targets and pathways as well as cellular processes by DIANA-miPath analysis showed that all miRNAs associated with ILD presence are involved in PI3K-Akt signaling pathway.

CONCLUSION: Our study shows that, in ASSD patients with ILD, miR-15b-5p, miR-23a-3p, miR-25-3p, miR-30a-5p and miR29c-3p were up-regulated compared to patients without evidence of ILD. A clear involvement in immune and inflammatory diseases was documented for the miRNAs identified [2] and, for many of these, studies in the literature indicate a possible role in pulmonary fibrosis [3]. It is notable that these miRNAs were related to PI3K-Akt signaling pathway that regulate cell proliferation, differentiation and apoptosis [4]. It has also been demonstrated that in lung fibroblast the PI3K–Akt signals can be aberrantly activated [5]. The identification of markers could be important in the early identification of the disease and for its treatment.

Figure 1. Comparison of relative levels of five miRNAs among patients with and without lung involvement were expressed as log, transformed values. **p<0.05, ***p<0.01

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POSO427

CLINICAL CHARACTERISTICS OF PATIENTS WITH SYSTEMIC SCLEROSIS AND GASTRIC ANTRAL VASCULAR ECTASIA (GAVE)

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BACKGROUND: Gastric antral vascular ectasia (GAVE) is one of the gastrointestinal (GI) manifestations related to systemic sclerosis (SSc). It can be presented as iron deficiency anemia or even upper gastrointestinal bleeding. GAVE is diagnosed by endoscopy observing an image of confluent vascular ectasias that is oriented longitudinally on the folds of the antrum in the appearance of “watermelon.” The definitive treatment for this manifestation consists in endoscopy-guided fulguration when the clinical situation allows it.

OBJECTIVES: The objective was to study a cohort of SSc patients at their first endoscopy. The clinical characteristics, laboratory tests and treatments received from SSc patients with GAVE were compared to those without this GI manifestation.

METHODS: From the cohort of patients with SSc in Hospital Universitari Vall d’Hebron, a total of 269 patients who had undergone at least one endoscopy during follow-up were selected. Twenty seven were diagnosed with GAVE. We compared the clinical, analytical and treatment characteristics of these patients with the remaining 242 who did not present GAVE. The statistical study was carried out using the SPSS 20.0 package (Chicago, IL), a p <0.05 was considered as statistical significance.

RESULTS: The prevalence of GAVE in SSc patients was 10.0%. Patients with GAVE had a higher median age SSc onset taking into account the first non-Raynaud’s phenomenon (RP) symptom attributable to the disease (56.6 vs 48.0 years, p = 0.001). The median age at first endoscopy was 56.5 years in GAVE group compared with 61.7 in the group without GAVE.

CONCLUSION: Our findings demonstrated a new aspect of pro-inflammatory type IFN signaling in SSc. We described a novel regulation feedback loop in monocytes, in which CD52 suppresses IFN signature, while its expression is inhibited by IFN-induced activity (mainly HSC class IIa). Therefore, targeting the CD52-IFN-HDAC axis might serve as a novel therapeutic strategy in SSc.

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POSO428

REGULATION OF IFN SIGNATURE BY HDAC CLASS II-CD52 AXIS IN SYSTEMIC SCLEROSIS

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BACKGROUND: Systemic sclerosis (SSc) is associated with an interferon (IFN) signature, which is defined by a higher expression of IFN-stimulated genes (mainly in response to IFNα). Histone deacetylases (HDACs) are a family of epigenetic modifiers mediating immune function. HDACs function via diverse molecular mechanisms, including direct inhibition of gene transcription or indirectly through modulation of nuclear transcription factors such as NF-kB and STATs. CD52 protein regulates T cell receptor and NF-kB signalling. Previously, we showed downregulation of CD52 in SSc monocytes, however, the influence of CD52 on IFN signature has not been studied yet.

OBJECTIVES: We investigated the role of CD52 in the regulation of IFN response in monocytes. Moreover, we explored the regulatory mechanisms of CD52 expression to identify the involvement of HDACs in that process.

METHODS: RNAseq of CD14+ monocytes isolated from peripheral blood of lcSSc (n=5, age=54.4±6.7), dcSSc patients (n=5, age=51 .8±7 .2) and age- and sex-matched healthy controls (HC) (n=5, age=50.8±9.7) was performed using Illumina HiSeq 4000 platform. Differentially expressed genes were computed using DESeq2 algorithm. Gene ontology and pathway analysis were performed using Metacore software and ShinyApp. CD25 activity in monocytes was blocked by monoclonal antibody Alemtuzumab [10ug/ml] and IFN signature genes expression was assessed upon IFNα stimulation [1ng/ml] by qPCR and ELISA. HDAC-dependent regulation of CD52 expression in CD14+ monocytes from HC was analysed on mRNA and protein levels after treatment with pan-HDAC inhibitor valproic acid and HDAC class IIa inhibitor TBP269 (both 2.5μM).

RESULTS: Pathway analysis revealed significant alterations in interferon signalling in SSc monocytes. Monocytes treated with Alemtuzumab and IFNα showed induced expression of STAT1 (p=0.023, N=4), CXCL9 (p=0.062, N=4) and CXCL10 (p=0.005, N=4). Treatment with Alemtuzumab revealed a lower proportion of anti-topoisomerase I (16.6% vs. 18.6%, p = 0.052). No difference was found in prevalence of anti-RNA polymerase III antibodies between groups. Patients with GAVE were treated less frequently with non-glucocorticoid immunosuppressants prior to diagnostic endoscopy (0% vs 20.2%, p = 0.010). The 33.3% of patients with GAVE were treated with endoscopic fulguration, and 66.7% of them received supplementary treatment with oral iron.

Conclusion: SSc patients with GAVE had higher age at SSc onset, more frequency of Barrett's esophagus and intestinal involvement, prevalence of anti-centromere antibodies, early or active Cutolo capillaroscopy and lower prior non-glucocorticoids treatment.

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