LUNG TARGETED DELIVERY OF EVEROLIMUS AS A NEW TREATMENT OF SCLERODERMA-RELATED INTERSTITIAL LUNG DISEASE (SSC-ILD) DEVELOPED BY PSGL-1 KO MICE

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Background:Interstitial lung disease (ILD), the main cause of mortality in scleroderma (SSc) patients (1), has no treatment (2). P-selectin glycoprotein ligand 1 (PSGL-1), the main ligand for P-Selectin, is expressed on leukocytes and responsible for the initial steps of extravasation (3). The absence of PSGL-1 in mice spontaneously develops an autoimmune syndrome similar to human SSC with fibrosis, vascular damage, autoantibodies and pulmonary arterial hypertension in females, and almost 60% of animals older than 12 months develop ILD with aging (4). In this work, the therapeutic action of everolimus-loaded nanomedicine given by local administration as a treatment for ILD was evaluated. The intratracheal administration of everolimus loaded into liposomes decorated with hyaluronic acid (HA) is studied as an administration strategy to reach the inflammatory area and avoid the systemic effects and possible toxicity on epithelial cells

Objectives:
1) To study the effect of everolimus on bronchoalveolar lavage (BAL) cell populations and in lung pathology in SSC-ILD PSGL-1 KO mice
2) To analyze the intratracheal application of everolimus included in empty liposomes (Lip-Ev) vs. liposomes decorated with hyaluronic acid (Lip-HA+Ev) as an administration strategy to decrease drug toxicity and increase drug effectiveness

Methods:In an observational study, PSGL-1−/− C57BL/6 males older than 12 months (n=4) were treated intratracheally with 4 doses of Lip or Lip-HA (with or without everolimus included), once a week (Lip-Ev 295.67µg/mL; Lip-Ev 82.73µg/mL; Lip-HA-Ev 82.73µg/mL). Then, animals were euthanatized and BALs were obtained. BAL cells were stained for flow cytometry analysis. Lungs were embedded in paraffin blocks for blind histological analysis by a pathologist and evaluated for interstitial inflammation and fibrosis degree. Lip-HA was selected as the treatment of choice for a second experiment (n=8) following the same experimental design (86.22µg/mL)

Results: The observational study showed an increase in CD45+, alveolar macrophages (AM), eosinophils (Eos), granulocytes (Gr1+T) and T cells in the BAL of untreated PSGL-1−/− compared with WT mice. Everolimus reduced these populations to WT levels in all cases. Lip-HA-Ev administration was chosen for further experiments because a lower dose of the drug gave a better result than the dose in underdilated liposomes. Reduction of CD45+, AM, eosinophils, and CD45+ cells populations by Lip-HA-Ev confirmed. Lip-HA treatment increased the number of neutrophils and T cells, but this effect is controlled by the everolimus administration. Histological lung analysis showed an increase in interstitial inflammation and fibrosis in untreated PSGL-1−/− and empty Lip-HA experimental groups. Treatment with everolimus included in Lip-HA reduced the fibrotic and inflammatory interstitial lung lesions, reaching values similar to those observed in WT mice.

Conclusion:PSGL-1 KO mice present ILD associated with scleroderma (SSC-ILD) with an increase of CD45+, Gr1+, Eos, T cells and AM populations in the BAL. Intratracheal treatment with everolimus included in liposomes decorated with hyaluronic acid reduces immune cell infiltration and fibrosis once SSC-ILD is established

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ENDOTHELIAL ACTIVATION IN SYSTEMIC SCLEROSIS VASCULOPATHY: ROLE OF LONG NON-CODING RNA H19X

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Background: Long non-coding RNAs (IncRNA) are a class of non-coding transcripts which modulate many biological processes. Our previous studies showed that IncRNA H19X is pivotal in the regulation of TGFβ driven fibrosis in systemic sclerosis (SSc).

Objectives: We aimed to investigate whether H19X plays a functional role in the regulation of endothelial cell (EC) activation, which is crucial in SSc vasculopathy.

Methods: Single-cell RNA sequencing (scRNA-seq) data from 27 dCSSc and 10 healthy control (HC) skin biopsies, following 10X Genomics partitioning and cDNA preparation, were analyzed for H19X expression in skin ECs, using Seurat package in R. A total of 4,981 ECs, of which 1,583 cells originated from HC and 3,398 cells originated from SSc patients, ranging from 59 to 342 ECs per subject, characterized by enrichment of EC markers of CD31, VWF and PAR2.

Expression of H19X in Human Dermal Microvascular ECs (HDMEC) was analyzed by qPCR. HDMEC were stimulated with different proinflammatory cytokines including IFNβ, IFNγ, TGFβ, TNFα, IL-6, IL-1β and IL-4 at biologically relevant concentrations. In order to ascertain its effect in ECs, H19X was silenced in HDMECs using locked nucleic acid antiserine oligonucleotides (LNA GapmeRs).

Results: scRNA-seq data showed that H19X was significantly upregulated in SSc compared to healthy ECs (p=0.0095). Based on the differentially expressed gene profiles among subclusters, ECs were further annotated as arterial (SEMA3G, HEY1), capillary (CA4, RGCC), venous (ACKR1, VCAM1), lymphatic (PROX1, DX1) ECs, as well as two aberrant clusters, proliferating (TOP2A, MKX7) and injured (HSGP2, APLNR) ECs, which were dominated by the SSc ECs. Specifically, the highest expression of H19X was found in injured SSC ECs and capillary SSC ECs. Overall, 13% SSc EC, about 51 cells, expressed detectable levels of H19X. In HDMEC (n=3), H19X was consistently induced by IFNβ, IFNγ and IFNγ. Time curve analysis demonstrated that the strongest induction was observed at 48h (1.5±0.2, 1.6±0.4 and 2.1±0.3 fold increase respectively). The combination of different IFNs determined stronger H19X induction after 48h stimulation, with a 2.4±0.1 increase with the combination of all IFNs and a 2.4±0.1 increase after the combination of IFNβ+γ.

Importantly, H19X knockdown lead to consistent and significant decrease of mRNAs of several adhesion molecules, including VCAM1, E- Selectin and P-Selectin, both in untreated HDMEC and after IFN stimulation. A decrease of VCAM and P-Selectin could be also demonstrated with WB analysis. No change was seen in other EC activation markers, including endothelin-1 and angiogenesis markers including VEGF, VEGFRα, Tie2 and thrombospondin.

Conclusion: This is the first report analyzing a potential role of IncRNA H19X in SSc vasculopathy. Our results suggest that IncRNA H19X could act as a regulator of adhesion molecules expression in EC, possibly mediated by IFNs and be involved in EC activation.

REFERENCES:

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