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Type I Interferon activation of monocytes in Systemic Sclerosis is cGAS-STING dependent and can be directly induced by dermal fibroblasts: a promising therapeutic target

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Background:

Type I IFN activation has been shown to correlate with disease activity and progression in Systemic Sclerosis¹ (SSc). In vitro studies have shown that SSc dermal fibroblasts induce pro-inflammatory activation of monocytes. Here we aimed to study the source of Type I IFN activation in SSc.

Objectives:

To explore the role of cGAS-STING-IRF3 pathway in type I IFN activation in SSc and assess the effect of its blockade in an ex-vivo SSc model.

Methods:

Dermal fibroblasts from SSc and healthy controls (HC) skin biopsies were co-cultured, in a ratio 1 to 5, for 48 hours with THP1 monocytes stably transfected with Lucia gene. Lucia is a secreted luciferase reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements (ISRE), that is activated by several pathways, including cGAS-STING-IRF3. After co-culture, luminescence-fiber assay was conducted on supernatants to quantify Lucia luciferase activity as an indirect measure of ISRE transcription. In ex vivo validation studies, human peripheral blood mononuclear cells (PBMCs) from SSc patients (n=12) were isolated and cultured in RPMI 1640 with 10% FBS and treated with DMSO as control, a cGAS, or a STING inhibitor for 16 hours. mRNA expression of IFN-related genes was assessed via real-time PCR. Protein phosphorylation was assessed by Western blot. Paired Wilcoxon test was used for statistical analysis.

Results:

SSc fibroblasts induced more than 2-fold transcription of ISRE in THP1 compared to healthy fibroblasts, as assessed by Luciferase activity (mean [SD] luminescence, 4234 [1927] vs 1852 [425], p=0.02). The activation of ISRE was associated with significant phosphorylation of IRF3. In turn, P-IRF3 levels were suppressed after cGAS or STING inhibition. Accordingly, targeting cGAS/STING pathway abrogated SSc fibroblasts-induced activation of ISRE elements by 91% (360 [96] vs 4234 [1927], p-value<.0001) and 89% (434 [95] vs 4234 [1927], p-value<.0001) for cGAS and STING inhibitors, respectively. Ex vivo, SSc PBMCs showed an average 70% increase in basal Type I IFN activation compared to HC PBMC, as assessed by rt-PCR of 5 Interferon inducible genes (namely, CXCL10, IFIT1, MX1, OAS, ISG15). Treatment of SSc PBMCs with cGAS and STING inhibitor suppressed the increased ISG expression by overall 21% and 40% (p-value=0.002), respectively, the latter bringing ISG to levels not significantly different from HC PBMCs.

Conclusion:

SSc fibroblasts induce Type I IFN activation in THP1 cells in a cGAS/STING dependent way. Ex-vivo, inhibition of cGAS/STING pathway in SSc PBMCs suppressed the upregulation of Interferon inducible genes. cGAS/STING pathway activation is a promising target to modulate Type I IFN activation in SSc.

References:

1 - Kakkar V, Assassi S, Allanore Y, Kuwana M, Denton CP, Khanna D, Del Galdo F. Type 1 interferon activation in systemic sclerosis: a biomarker, a target or the culprit. *Curr Opin Rheumatol*. 2022 Nov 1;34(6):357-364. doi: 10.1097/BOR.0000000000000907. Epub 2022 Sep 16. PMID: 36125916; PMCID: PMC9594133.

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