HAQ

Convergent validity

AHFI	0.61	<0.001
HFI	0.51	<0.001
Kapandji	-0.46	<0.001

Divergent validity

Anxiety (HADa)	0.57	<0.001
Depression (HADd)	0.47	<0.001
Disease duration	0.10	0.320
Age	0.28	0.005

Health Assessment Questionnaire; SSc : systemic sclerosis; AHFI: Arab hand functional index; HFI: Hand Functional Index.

Table 2. Factor analysis of the AHFI and HAQ

	Eigenvalue	Variance. %	Cumulative %
AHFI			
Factor 1	5.411	54.114	54.114
Factor 2	1.034	10.338	64.453
HAQ			
Factor 1	4.051	50.638	50.638

AHFI: Arab hand functional index; HAQ: Health Assessment Questionnaire.

AB0200

INHIBITION OF DOT1L ACTIVITY IN HUMAN SKIN FIBROBLASTS: A TRANSCRIPTOME ANALYSIS

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Background: The pathophysiology of fibrosis, a hallmark of diseases like Systemic Sclerosis, is still not well understood. The role of epigenetic factors is increasingly explored. DOT1L, the unique H3K79-methyltransferase, methylates histone 3 at the Lysine residue at position 79, thereby regulating gene expression programs. Inhibition of DOT1L in cartilage and bone has cell-type specific effects on Wnt signaling, a pathway suggested to play an important role in fibrotic disease.

Objectives: To study the changes in the transcriptome of dermal fibroblasts after DOT1L inhibition.

Methods: Primary human dermal fibroblasts isolated from abdominal skin were treated with DOT1L-inhibitor EPZ-5676 or vehicle and stimulated or not with TGF- β or CHIR99021 (activating Wnt signaling). RNAseq was performed using the NextSeq500 as a platform and TruSeq as library prep kit. The preprocessed reads were aligned to the reference genome of Homo-sapiens (Ensembl.GRCh38.88(GRCh38)). Statistical comparative analysis for differential expressed genes (DEG) was done using the edge package in R version 3.20.9. The resulting p-values were corrected for multiple testing with Benjamini-Hochberg to control for false discovery rate (FDR). DEG were selected based on FDR-value less than 0.05 and a fold change of an absolute log₂-ratio larger than 1. Pathway analysis was done using PANTHER14.0. Western Blot for di-methylated H3K79 was performed to confirm successful DOT1L inhibition.

Results: The DOT1L-inhibitor EPZ-5676 effectively reduced H3K79 di-methylation in the treated samples. RNAseq analysis revealed 663 differentially expressed genes in dermal fibroblasts exposed to the DOT1L inhibitor, based on group analysis: 77 genes were downregulated and 556 upregulated. There was no significant interaction between inhibition of DOT1L and stimulation of the cells with TGF- β or CHIR99021. Pathway analysis showed involvement of primarily the integrin signaling, the cadherin pathway and Wnt signaling.

Conclusion: More than 600 differentially expressed genes were discovered by RNA sequencing in human dermal fibroblasts exposed to DOT1L inhibition (independent of TGF- β or CHIR 99021 stimulation). These gene lists and their networks indicate that DOT1L activity affects integrin signaling, the cadherin and Wnt pathway, suggesting a potential impact on the fibrotic process that is leading to pathology in SSc.

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AB0201

THE PATHOGENIC EFFECTS OF IMMUNE COMPLEXES CONTAINING SCLERODERMA-SPECIFIC AUTOANTIBODIES IN ENDOTHELIAL CELLS

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Background: In systemic sclerosis (SSc), autoantibodies provide the most reliable tool to predict disease subset and the pattern of organ involvement.

Objectives: Consistently with the diagnostic and prognostic role of these autoantibodies, our group has previously demonstrated that immune complexes (ICs) containing scleroderma specific autoantibodies elicit a pro-inflammatory and pro-fibrotic signalling cascade in healthy skin fibroblasts, thus contributing to SSc aetiopathogenesis. In addition, we showed that SSc-ICs upregulate Toll-like Receptors (TLRs) and that pre-treatment with DNase and RNase prevent SSc-IC-mediated effects in fibroblasts, thus suggesting that SSc-ICs might engage TLRs via their nucleic acid components. The objective of the present study is to investigate the pathogenicity of SSc-ICs in endothelial cells (ECs).

Methods: Human umbilical vein ECs (HUVECs) have been isolated from umbilical vein and cultured in adequate conditions. ICs have been purified from sera of SSc patients bearing different autoantibody specificities (anti-bodies against centromeric proteins, DNA topoisomerase I, RNA polymerase and Th/To), patients with systemic lupus erythematosus (SLE), primary anti-phospholipid syndrome (PAPS) or healthy controls using poly-ethylen glycol precipitation. Cell cultures have been incubated with pathologic and control ICs and TLR3 [Poly(I:C)] and TLR4 (LPS) agonists. Several parameters have been assessed: mRNA levels of endothelin-1 (ET-1) and type I interferons (IFN- α and IFN- β) have been investigated by real-Time PCR; ICAM-1 expression has been evaluated by cell-ELISA and the secretion of IL-6, IL-8, tumour growth factor (TGF)- β 1 and Pro-Collagen α 1 in culture supernatants has been measured by commercial ELISA kits. The involvement of intracellular signalling pathways culminating with the activation of p38MAPK has been assessed. Furthermore, skin fibroblasts from healthy controls were stimulated with supernatants from HUVEC incubated with scleroderma and control ICs. The mRNA levels of collagen (col) α 1 and matrix metalloproteinases (MMP)-1 and the secretion of TGF- β 1 were evaluated in fibroblasts. Statistical analysis was performed comparing ICs from SSc and disease controls to NHS-ICs.

Results: Stimulation of HUVEC with scleroderma and control ICs resulted in the modulation of study mediators, as detailed in Table 1. When skin fibroblasts from healthy controls were stimulated with supernatants from HUVEC incubated with scleroderma and control ICs, we observed a significant modulation of mediators (Table 2).

Conclusion: These data provide the first demonstration of the pathogenicity of ICs isolated from scleroderma patients with different autoantibody specificities in ECs. According to our preliminary data, the activation of ECs induced by SSc-ICs might ultimately lead to a pro-fibrotic phenotype in healthy skin fibroblasts. SSc-ICs might thus be at the crossroad of scleroderma pathogenesis, contributing to the recruitment of the many cells involved in disease onset.

Table 1. Modulation of study mediators in HUVEC.

	ICAM-1	IL-6	IL-8	TGF- β 1	Coll α 1	ET-1	IFN- α	IFN- β	P38MAPK
Poly(I:C)	***	***	***	NA		NA			***
LPS	***	***	***	NA		NA			***
ATA-IC		*	***	*		**			***
ACA-IC	**	*	***	*					***
ARA-IC		**	***	*					***
anti-Th/To-IC	***	**		*		**			
PAPS-IC	**			NA		***			
SLE-IC			**	NA					**
NHS-IC									

*= p<0.05; **= p<0.001, ***=p<0.0001

Table 2. Modulation of study mediators in fibroblasts.

	TGF- β 1	Coll α 1	MMP-1
TGF- β	NA	***	
ATA-IC	*	**	
ACA-IC	***	**	*
ARA-IC			*
anti-Th/To-IC	***	***	*
NHS-IC			

*= p<0.05; **= p<0.001; ***=p<0.0001