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Original Research Article

miRNA-133 and lncRNA-H19 expressions and their relation to serum levels of PKM2 and TGF-β in patients with systemic sclerosis☆

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ABSTRACT

Background and aims: Systemic sclerosis (SSc) is a common autoimmune disorder involving the skin, blood vessels, and internal organs with an elusive pathophysiology. SSc is believed to be a genetically prone T-cell-mediated autoimmune disease. miRNAs and lncRNAs were thought to be involved in the etiology of several immunological diseases including SSc. This work aimed to assess the expression of miRNA-133, lncRNA-H19, PKM2, and TGF-β levels in SSc in comparison to controls and their relationship to the clinical course and severity of disease.

Patients and methods: Fifty patients with SSc and 40 healthy age and sex-matched controls were included in this study. miRNA-133 and H19 expression levels were detected using quantitative RT-PCR while serum levels of PKM2 and TGF-β were measured using ELISA techniques. Patients' clinical data and treatments received were extracted and correlated with proteins investigated.

Results: Our results showed that miRNA-133 was significantly downregulated in SSc patients in comparison to controls (Mean + SD of SSc = 0.61 ± 0.22, Mean ± SD of HC = 0.97 ± 0.007, p = 0.003). However, there was significant upregulation of the serum expressions of all other tested biomarkers in SSc patients in comparison to controls; H19 (Mean + SD of SSc = 10.37 ± 3.13, Mean ± SD of HC = 1.01 ± 0.01, p = 0.0001), PKM2 (Mean + SD of SSc = 28.0 ± 4.84, Mean ± SD of HC = 16.19 ± 1.32, p = 0.005) and TGF-β (Mean + SD of SSc = 150.8 ± 6.36, Mean ± SD of HC = 23.83 ± 0.93, p = 0.0001). We also detected several correlations between serum levels of the investigated proteins in patients with SSc.

Conclusion: Along with TGF-β, our results show that miRNA-133, H19, and PKM2 seem to be potential contributors to SSc pathogenesis and could be promising biomarkers in the diagnosis of SSc patients. The lncRNA-H19 correlations with TGF- β, miRNA-133, and PKM2 suggest a possible influential effect of this RNA molecule on the pathogenesis of SSc.

1. Introduction

Systemic sclerosis (SSc), also known as scleroderma, is an autoimmune disease that affects the skin, blood vessels, and internal organs, ending in vasculopathy and fibrosis. Along with the characteristic skin involvement, other internal organs that might be compromised include the lungs, digestive system, heart, and kidneys [1–3]. The precise etiology of SSc is still unknown [4], however, an autoimmune profile involving both the cellular and humoral immune systems with the development of autoantibodies, vascular alterations, and fibrosis are characteristics of SSc [5,6].

In SSc, endothelial cell damage caused by nonspecific serum toxic substances or T-cell derived proteolytic enzymes, endothelial cell-directed autoantibodies, vasculotropic viruses, inflammatory cytokines, oxidative stress, and environmental stress is the initial vascular insult. Endothelial cell dysfunction results in increased expression of endothelial adhesion molecules, altered production of vasoactive mediators, platelet activation, and fibrinolytic pathways. Platelets that

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have been activated emit thromboxane A2, platelet-derived growth factor (PDGF), and TGF-β, which promote vasoconstriction and contribute to fibroblast activation and myofibroblast *trans*-differentiation promoting fibrosis and deposition of extracellular matrix (ECM) components such as collagen and proteoglycans [7,8].

TGF-β stimulates fibroblast terminal differentiation into myofibroblasts, which release more ECM components and TGF-β, resulting in tissue contraction [9]. In mesenchymal cells, TGF-β is a potent inducer of glycolysis with the synthesis of pyruvate from glucose. A range of cellular enzymes is involved in this process, the most significant of which is pyruvate kinase (PK) [10,11].

The PK muscle (PKM) gene is alternatively spliced to create the PKM1 or PKM2 isoforms [12,13]. The PKM isoform expression is regulated by three heterogeneous nuclear ribonucleoproteins (hnRNPs), polypyrimidine tract-binding protein 1 (PTBP1); also known as hnRNPI, hnRNPA1, and hnRNPA2 [13]. Earlier research on fibroblasts and other cell types has linked hnRNPs as miRNA downstream targets [14].

At least 30 % of protein-coding genes are synchronized by microRNAs (miRNAs) [15]. miRNAs have been shown to target PTB1 with members like miRNA-133 that are muscle-specific [16]. Downregulation of miRNA-133 is responsible for the overexpression of TGF-β, which led to an increase in TGF-β signaling in cardiac fibroblasts [17]. These findings imply that miRNA-133 regulation may be involved in the pathophysiology of SSc.

Long non-coding RNAs (lncRNAs), RNA transcripts that do not code for proteins, are longer than 200 nucleotides. LncRNAs have been linked to a variety of disorders, including cancer, Alzheimer's disease, cardiovascular disease, diabetes mellitus, RA, and SLE [18,19]. lncRNA-H19, which is found on chromosome 7 in mice and chromosome 11 in humans, is an imprinted gene that is expressed solely from the maternal allele [20,21]. H19 has been shown to interact with a range of proteins and miRNAs via decoy, scaffold, and guide modes of action to control genes involved in cell proliferation, migration, differentiation, and tumorigenesis [22]. Previous research on cancer patients revealed that H19 stimulates and activates tumor-specific PKM2, which is required for the miRNA-675-mediated enhancement of liver cancer cell proliferation and gene expression during carcinogenesis [23]. This shows that the modulation of H19 expression levels is critical in the regulation of PKM2.

On account of the intertwined relationship between miRNA-133, lncRNA-H19, PKM2, and TGF-β and their involvement in various immune and fibrotic processes, we aimed to investigate the expression and correlation between these biomarkers in patients with SSc and their relationship with the clinical course and diversity of the disease.

2. Subjects and methods

2.1. Study design and participants

This case-control study incorporated 90 subjects; 50 SSc patients diagnosed according to the criteria of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [24] and 40 age- and sex-matched healthy controls. All patients were recruited from the Dermatology outpatient clinic, Cairo University Hospital. A written informed consent was obtained from all included subjects before taking part in the study. Ethical committee approval was obtained from the local ethical committee, Cairo hospitals, Cairo University "Ethical Code MD-203-2021". Any patient with associated other connective tissue disorders was excluded from this study. Patients with pregnancy, lactation, hormonal therapy, and any history of malignancy or concurrent infections were also excluded.

2.2. Patients assessment

Dermatological examination and evaluation of the extent and severity of SSc using "Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee (1980)" [25] were done for all patients. The skin thickness of our patient group was assessed via mRSS based on palpation at 17 body sites. A score of 0 indicates normal skin thickness, 1 mild skin thickness, 2 moderate skin thickness, and 3 severe skin thickness. The score is calculated by summing the rating from all 17 areas (range 0–51). We hypothesized 3 grades for the mRSS; low grade (0–5), moderate (6-15), and high (16–51) with an inability to make skin folds between two fingers.

2.3. Blood sample collection

A 5 ml venous blood sample from each participant was collected in tubes. Samples were permitted to clot for 15 min and then centrifuged at $3000\times g$ for 10 min. The serum samples were separated and stored at −20 °C until the time of use for Molecular and ELISA techniques.

2.4. RNA extraction and complementary DNAs (cDNAs) synthesis

Total RNA was extracted by miRNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Reverse transcription was carried out on extracted RNA using miScript® II RT kit (Qiagen, Germany. Cat. No. 218161) according to the manufacturer's instructions.

2.5. Serum miRNA-133 and H19 assay

Real-time PCR was done using miRCURY LNA SYBR® Green PCR Kits to assess the relative expression levels of miRNA-133 (Qiagen, Germany. YP00204788) and H19 (Qiagen, Germany. Cat. No. 330701).

Primers for miRNA-133 (miRBase accession number is MIMAT0000427), H19, and both housekeeping genes Hs_SNORD68 (Cat. No. 00033712) and GAPDH received from Qiagen in Valencia, California, USA.

The real-time thermocycler Rotor gene Q System (Qiagen, USA) was programmed as follows: heating at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15s then annealing and extension at 60 °C for 60s.

The relative gene expression to internal controls (SNORD-68 and GAPDH) was calculated using the ΔCt method. Relative expression for both miRNA-133 and H19 was calculated using the 2-ΔΔCt method.

2.6. Serum PKM2 and TGF-β assay

Quantitative determination of PKM2 concentrations in serum was done with a Human Tumour Type M2 Pyruvate Kinase ELISA kit (Cat. No E2125Hu) provided by Bioassay Technology Laboratory, China, and according to the manufacturer's instructions.

Quantitative determination of TGF-β concentrations in serum was done with an Invitrogen Multispecies TGF-β kit (Cat. No. KACL1688/KAC1689) provided by Biosource, California, and according to the manufacturer's instructions.

2.7. Statistical analysis

The statistical data was analyzed using the statistical package of social science (SPSS) version 25 on Windows 8.1. All statistical data are displayed as means ± standard deviation (SD). The post-hoc comparison test was used for pairwise comparisons. Pearson's correlation was used to evaluate the relationships between the variables under study. Receiver Operating Characteristic (ROC) curves were employed to assess the miRNA-133, H19, PKM2, and TGF-β diagnostic performances. For interpretation of results, significance was adopted at P-value ≤0.05.

3. Results

3.1. Demographic and clinical data of the study subjects

Table 1 provides the demographic characteristics of the study participants. There were no significant differences between SSc patients and the control group regarding age and sex ($p = 0.138$, 039 respectively).

The clinical characteristics of SSc patients are listed in Table 2.

3.2. Expression levels of studied parameters and their relation to clinical and demographic data

In comparison to controls, SSc patients had significantly lower miRNA-133 expression levels compared to the control group (Mean ± SD of SSc = 0.61 ± 0.22, Mean ± SD of HC = 0.97 ± 0.007, $p = 0.003$). However, the SSc patients had statistically significant higher expression levels of all other biomarkers compared to the control group; H19 (Mean ± SD of SSc = 10.37 ± 3.13, Mean ± SD of HC = 1.01 ± 0.01, $p = 0.0001$), PKM2 (Mean ± SD of SSc = 28.0 ± 4.84, Mean ± SD of HC = 16.19 ± 1.32, $p = 0.005$), and TGF-β (Mean ± SD of SSc = 150.8 ± 6.36, Mean ± SD of HC = 23.83 ± 0.93, $p = 0.0001$) (Table 3 and Fig. 1).

3.3. The relation of expression levels of studied parameters to the clinical and demographic data

The mean serum levels of TGF-β, miRNA-133, H19, and PKM2 in SSc patients concerning the demographic data and clinical characteristics are listed in Table S1.

The mean serum TGF-β was significantly higher in patients above 40 years of age (Mean ± SD = 166.04 ± 7.80, $p = 0.001$) and those with later onset SSc (Mean ± SD = 164.76 ± 9.02, $p = 0.046$), while the mean serum H19 was significantly higher in patients below 40 years of age (Mean ± SD = 15.05 ± 5.40, $p = 0.002$) and those with earlier onset SSc (Mean ± SD = 13.26 ± 3.91, $p = 0.018$). Moreover, TGF-β and PKM2 were significantly higher in female SSc patients (Mean ± SD = 156.55 ± 7.50, 29.44 ± 5.21 and $p = 0.027$, 0.014 respectively) in comparison to males (Table S1).

Regarding the cutaneous manifestations of SSc, the expression level of miRNA-133 was significantly more downregulated in SSc patients with pitting scars (Mean ± SD = 0.45 ± 0.17, $p = 0.009$) than those without pitting scars. The expression levels of both TGF-β and H19 were significantly more upregulated in SSc patients with digital ulcers (Mean ± SD = 156.75 ± 11.86, 15.24 ± 6.99 and $p = 0.035$, 0.010 respectively) and calcinosis (Mean ± SD = 172.71 ± 10.02, 26.51 ± 14.02 and $p = 0.031$, 0.007 respectively) Vs. those without. Moreover, the expression level of H19 was significantly more upregulated in SSc patients with telangiectasia (Mean ± SD = 23.84 ± 12.02, $p = 0.010$) Vs. those without (Table S1).

Regarding the systemic manifestations of SSc, the expression level of TGF-β was significantly more upregulated in SSc patients with DM (Mean ± SD = 168.0 ± 2.56, $p = 0.002$), HTN (Mean ± SD = 161.93 ± 8.93, $p = 0.028$), ILD (Mean ± SD = 178.12 ± 4.58, $p = 0.0001$), arthritis (Mean ± SD = 161.47 ± 8.53, $p = 0.01$), dry eye (Mean ± SD = 166.10 ± 9.23, $p = 0.017$), dry mouth (Mean ± SD = 165.33 ± 10.51, $p = 0.035$), and vasculitis (Mean ± SD = 177.75 ± 4.49, $p = 0.002$) compared with patients without these manifestations. Conversely, the expression level of miRNA-133 was significantly more downregulated in SSc patients with DM (Mean ± SD = 0.132 ± 0.09, $p = 0.0001$), arrhythmia (Mean ± SD = 0.21 ± 0.11, $p = 0.023$), and vasculitis (Mean ± SD = 0.32 ± 0.13, $p = 0.02$) Vs. those without. Moreover, the expression level of H19 was significantly more downregulated in SSc patients with ILD (Mean ± SD = 8.62 ± 3.61, $p = 0.05$) and arthritis

Table (1)
Demographic data (age and sex) of SSc patients and control group.

Clinical Data		SSc patients (n = 50)	Control (n = 40)	p-value
Age (years) Mean ± standard deviation (SD)		42.60 ± 10.83	40.0 ± 2.0	0.138
Sex	**Female**	39 (78.0 %)	28 (70.0 %)	0.39
	Male	11 (22.0 %)	12 (30.0 %)	

Abbreviations: **SSc,** systemic sclerosis.
By using T-test for equality of Means of the age of the patients and chi-square test for sex.
p-value >0.05 Not Significant (NS).
p-value <0.05 Significant (S).
p-value ≤0.001 Highly Significant (HS).

Table 2
Clinical characteristics of SSc patients included in the study.

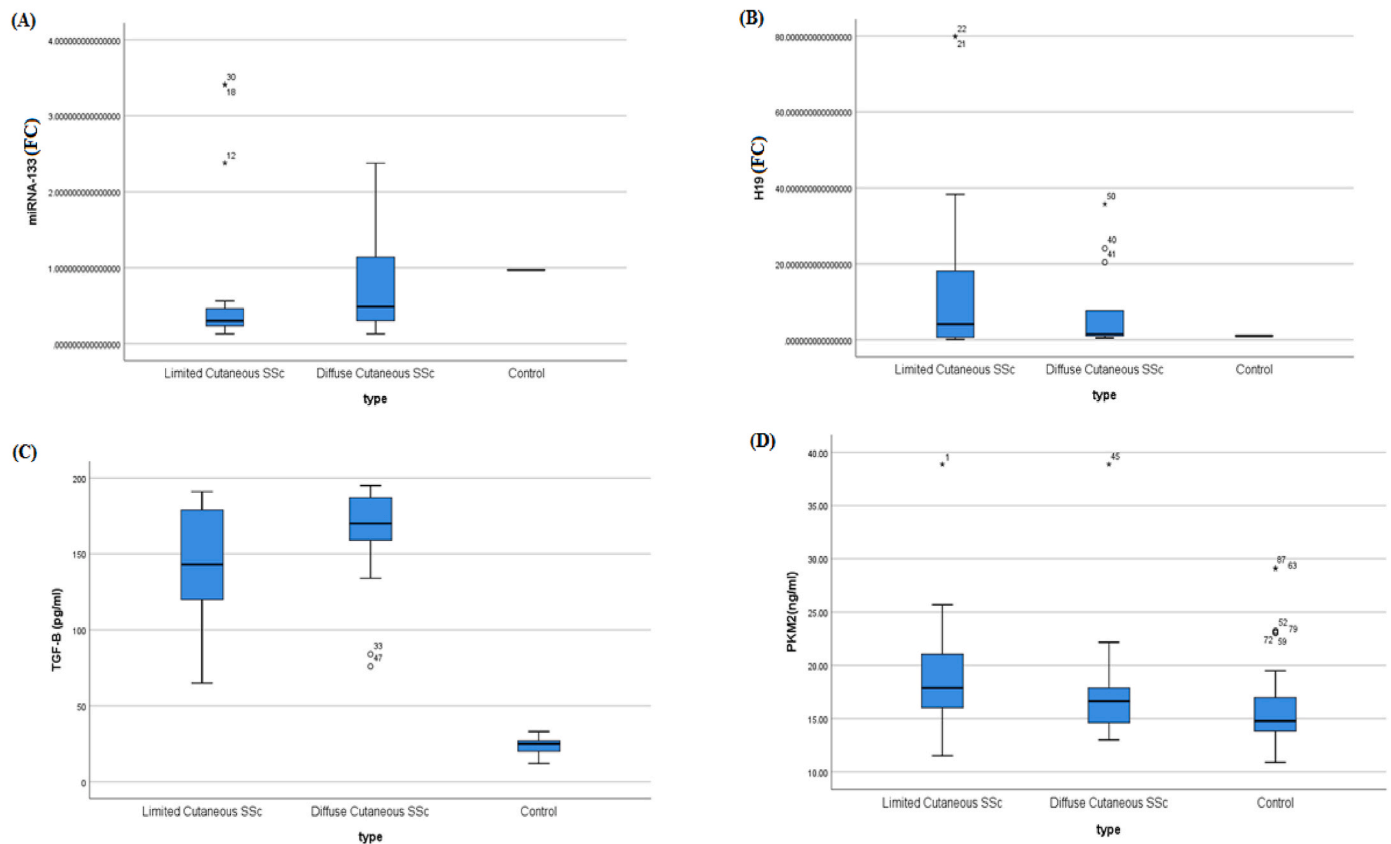
Variables		SSc patients (N = 50)			
		DcSSc (N = 17) 34 %		LcSSc (N = 33) 66 %	
		Count	%	Count	%
Skin tightness		17	100 %	33	100 %
Sclerodactyly		17	100 %	33	100 %
Pitting Scars		6	35 %	17	52 %
Digital Ulcers		8	47 %	26	79 %
Calcinosis		14	82 %	29	88 %
Telangiectasia		14	82 %	27	82 %
Diabetes Mellitus		14	82 %	32	97 %
Hypertension		10	59 %	25	76 %
Raynaud's Phenomenon		17	100 %	33	100 %
Dysphagia		17	100 %	33	100 %
Interstitial Lung Disease		4	24 %	20	61 %
Bronchiectasis		12	71 %	24	73 %
Pericardial Effusion		12	71 %	30	91 %
Arrhythmias		17	100 %	31	94 %
Arthritis		12	71 %	19	58 %
Neurological manifestations		17	100 %	33	100 %
Myositis		15	88 %	27	82 %
Dry eyes		10	59 %	30	91 %
Dry mouth		10	59 %	31	94 %
Vasculitis		12	71 %	30	91 %
Renal Disease		13	76 %	27	82 %
Treatment	**Azathioprine (AZA)**	13	76 %	18	55 %
	Methotrexate	3	18 %	8	24 %
	Mycophenolate mofetil (MMF)	4	24 %	1	3 %
	Vasodilators	13	76 %	21	64 %
	Anti-coagulants	6	35 %	5	15 %

Abbreviations: **SSc,** systemic sclerosis; **DcSSc,** diffuse cutaneous systemic sclerosis; **LcSSc,** limited cutaneous systemic sclerosis.

Table 3
Serum levels of studied parameters in patients and controls.

Biomarkers	Scleroderma (N = 50)	Control (N = 40)	p-value
	Mean ± SD	Mean ± SD	
miRNA-133 (FC)	0.61 ± 0.22	0.97 ± 0.007	**0.003***
H19 (FC)	10.37 ± 3.13	1.01 ± 0.01	**0.0001***
PKM2 (ng/ml)	28.0 ± 4.84	16.19 ± 1.32	**0.005***
TGF-β (pg/ml)	150.8 ± 6.36	23.83 ± 0.93	**0.0001***

Abbreviations: **SD,** standard deviation; **FC,** fold change; **miRNA-133,** microRNA-133; **PKM2,** pyruvate kinase muscle 2; **TGF-β,** transforming growth factor-beta; **ng/ml,** nanogram/milliliter; **pg/ml,** picogram/milliliter.
By using T-test for equality of Means * are significant.
p-value >0.05 Not Significant (NS).
p-value <0.05 Significant (S).
p-value ≤0.001 Highly Significant (HS).



ig. 1. Boxplot diagram for (A) miRNA-133, (B) H19, (C) PKM2, and (D) TGF-β in SSc patients and control.

(Mean ± SD = 3.72 ± 1.41, p = 0.001) Vs. those without. However, the expression level of H19 was significantly more upregulated in SSc patients with bronchiectasis (Mean ± SD = 19.04 ± 8.54, p = 0.028) Vs. those without. In the same context, the expression level of PKM2 was significantly more downregulated in SSc patients with bronchiectasis (Mean ± SD = 16.09 ± 1.16, p = 0.001), pericardial effusion (Mean ± SD = 15.86 ± 0.79, p = 0.016), and arrhythmia (Mean ± SD = 14.20 ± 3.48, p = 0.016) Vs. those without. Meanwhile, its expression level was significantly more upregulated in SSc patients with dry eyes (Mean ± SD = 35.26 ± 15.07, p = 0.020) Vs. those without (Table S1).

Regarding the treatment modalities of SSc, the expression level of miRNA-133 was significantly less downregulated in SSc patients treated with MMF (Mean ± SD = 1.56 ± 0.56, p = 0.002) Vs. those used another drug. Similarly, the expression level of H19 was significantly less upregulated in SSc patients treated with methotrexate (Mean ± SD = 3.49 ± 1.61, p = 0.016) and anticoagulants (Mean ± SD = 5.77 ± 2.94, p = 0.05) compared with patients not using them. Moreover, the expression levels of TGF-β (Mean ± SD = 159.84 ± 6.96, p = 0.038), H19 (Mean ± SD = 13.73 ± 4.71, p = 0.002), and PKM2 (Mean ± SD = 31.06 ± 7.02, p = 0.015) were significantly more upregulated in SSc patients treated with AZA Vs. those used other drugs (Table S1).

Regarding the modified Rodnan skin score (mRSS) of SSc patients, there were no significant differences between the grades of mRSS and the studied variables (p > 0.05) (Table S1).

3.4. Correlation between studied parameters

We detected a significant positive correlation between H19 and both miRNA-133 (r = 0.574, p = 0.003) and PKM2 (r = 0.429, p = 0.032) while with the TGF-β, it possesses a significant negative correlation with it (r = −0.502, p = 0.011). We studied the correlation between the mRSS and the studied parameters with no significant correlation (Table 4).

Table 4
Correlation between mRSS and the serum levels of miRNA-133, H19, PKM2, and TGF-β in SSc patients.

Biomarkers		miRNA-133 (FC)	H19 (FC)	PKM2 (ng/ml)	TGF-β (pg/ml)	mRSS (0-51)
miRNA-133 (FC)	**Pearson Correlation**	**1**	0.574	0.191	−0.127	0.012
	p-value		**0.003***	0.359	0.544	0.933
H19 (FC)	**Pearson Correlation**	0.574	**1**	0.429	−0.502	0.018
	p-value	**0.003***		**0.032***	**0.011***	0.903
PKM2 (ng/ml)	**Pearson Correlation**	0.191	0.429	**1**	−0.096	−0.123
	p-value	0.359	**0.032***		0.647	0.393
TGF-β (pg/ml)	**Pearson Correlation**	−0.127	−0.502	−0.096	**1**	0.187
	p-value	0.544	**0.011***	0.647		0.194
mRSS (0-51)	**Pearson Correlation**	0.012	0.018	−0.123	0.187	**1**
	p-value	0.933	0.903	0.393	0.194	

Abbreviations: **FC,** fold change; **miRNA-133,** microRNA-133; **PKM2,** pyruvate kinase muscle 2; **TGF-β,** transforming growth factor-beta; **ng/ml,** nanogram/milliliter; **pg/ml,** picogram/milliliter; **mRSS,** modified Rodnan skin score.
By using the Pearson correlation test, the correlation is significant at the 0.05 level (2-tailed).

3.5. Assessment of reliability of sensitivity and specificity of miRNA-133, H19, PKM2, and TGF-β by ROC curve analysis

Table 5 and Fig. 2 demonstrate the sensitivity and specificity analyses of our parameters using the ROC curve showing their diagnostic value. The ROC curve of miRNA-133 has an 84 % sensitivity and a 98 % specificity. The cut-off value of miRNA-133 is 0.56 with area under

Table (5)
Diagnostic and prognostic performances for miRNA-133, H19, PKM2, and TGF-β in SSc patients.

Biomarkers	AUC	Cut-off value	Sensitivity %	Specificity %	95 % C.I [(a)]	Accuracy	p-value
MiRNA-133	0.840	0.56	84 %	98 %	0.7200 to 0.9271	91 %	**<0.0001***
H19	0.652	7.67	64 %	97.5 %	0.4400 to 0.7200	80.75 %	**0.0224***
PKM2	0.942	25.69	99 %	85 %	16.44 to 19.48	92 %	**<0.0001***
TGF-β	0.946	156	100 %	89.1 %	77.25 to 94.55	94.5 %	**<0.0001***

Abbreviations: **AUC,** area under curve; **C.I,** confidence interval; **(a),** Youden index; **miRNA-133,** microRNA-133; **PKM2,** pyruvate kinase muscle 2; **TGF-β,** transforming growth factor-beta.

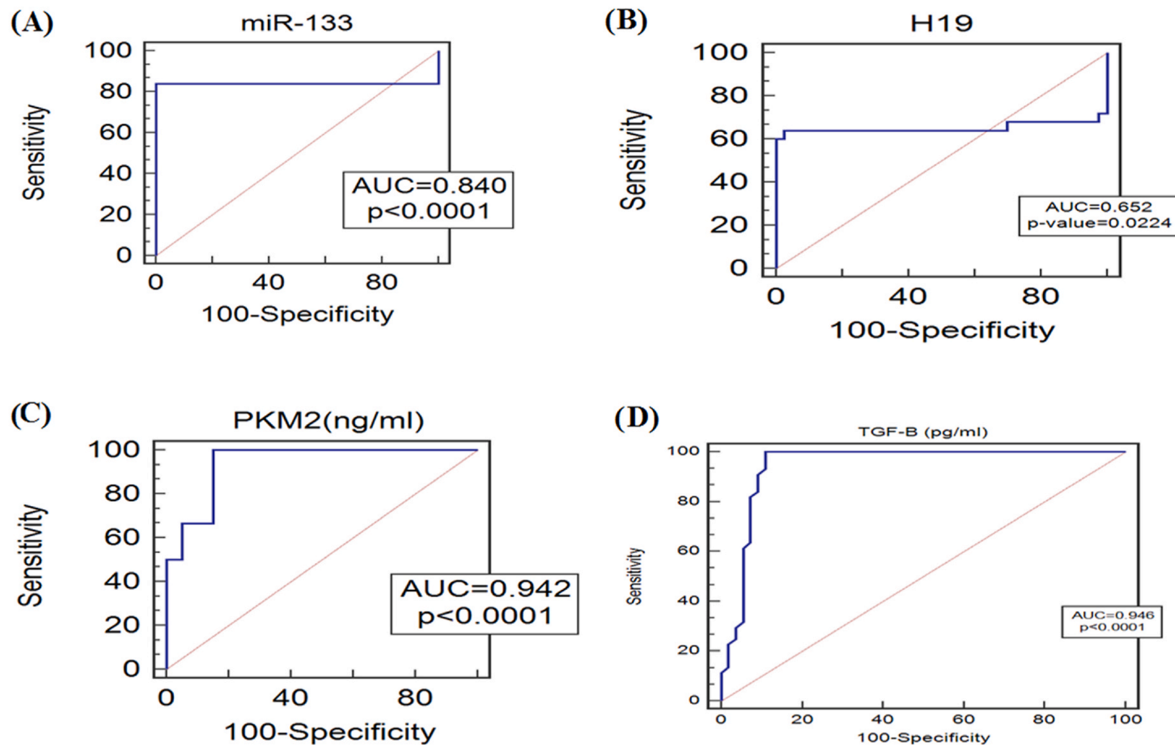


Fig. 2. ROC Curve for (A) miRNA-133, (B) H19, (C) PKM2, and (D) TGF-β biomarkers for SSc diagnosis.

curve of 0.84. While the cut-off value of H19 is 0.652 and area under curve 7.67 and sensitivity/specificity of 64 % and 97.5 % respectively. Moreover, the cut-off value of PKM2 is 25.69 ng/ml with sensitivity/specificity of 99 % and 85 % respectively. The cut-off value of TGF-β is 156 pg/ml with a sensitivity of 100 % and specificity of 89.1 %.

4. Discussion

Systemic sclerosis (SSc) is a heterogeneous systemic autoimmune fibrotic disease that is likely to entail the influence of environmental variables on genetically primed individuals [4,26–28] with epigenetic factors believed to be potential contributors to the disease's vast range of manifestations [4]. In the current work, we report a disturbance in the expression of 2 important epigenetic regulators of fibrosis and immune functions, namely miRNA-133 and lncRNA-H19 as well as significant positive correlations between both biomarkers in sera of patients with SSc in comparison to controls ($r = 0.574$, $p = 0.003$). We also detected abnormal associations of lncRNA-H19 with TGF-β and PKM2, which are both crucial proteins involved in the fibrotic process seen in SSc.

TGF-β is a major activator and propagator of myofibroblasts and other mesenchymal cell types and is believed to have a vital pathogenic consideration in fibrogenesis [29]. TGF-β's target genes include miRNAs, among which, miRNA-133 has been shown to be differentially expressed in SSc skin samples [27,30]. MiRNA-133 modulates the expression of inflammatory cells and several known fibrogenic cytokines, including TGF-β, IL-4, and IL-6 [31]. Although miRNA-133 inhibited TGF-β in cardiac tissue [32], we could not find a significant correlation between them in the sera of SSc patients ($r = -0.127$, $p = 0.544$), nonetheless, a larger sample size may be needed to verify this correlation.

In accordance with previous research [33–37], the expression of miRNA-133 was significantly downregulated ($p = 0.003$), while that of TGF-β was upregulated ($p = 0.0001$) among our SSc patients in comparison to controls.

Taking into consideration the major role of TGF-β in SSc development, its upregulation with advancing age in our patients' group; but not among controls, may relate to the accelerated onset of internal organ involvement among elderly SSc patients [38]. In agreement with previous investigations, female SSc patients had significantly higher serum levels of TGF-β compared to male patients ($p = 0.027$) which may be attributed to estradiol's upregulation of TGF-β production [39,40].

The fact that the digital scleroderma ulcerations respond well to therapy with divalproex sodium; valproate, that blocks TGF-β [41] can be related to the significantly higher serum expression levels of TGF-β in patients with such manifestations Versus those without as we observed in our patients suffering from digital ulcers and calcinosis.

As reported previously [42–44], the present study also demonstrates that SSc patients with DM, HTN, ILD, arthritis, dry eye, dry mouth, and vasculitis have significantly higher serum expression levels of TGF-β compared to patients without these associations. Such co-morbidities can be related to the diverse roles of TGF-β in the development of

insulin resistance and obesity; its stimulatory effect on the expression of ET-1 and vascular stiffness from collagen; its inhibitory role on the production of nitric oxide; its role in joint infiltration with polymorphonuclear leukocytes, and lymphocytes [45–48]. TGF-β can also stimulate the differentiation of Tregs towards IL-17-producing cells, which may contribute to the ANCA-associated vasculitis (AAV) of SSc [49].

On the other hand, miRNA-133 diminishes the expression of collagen I and collagen III; inhibits TGF-β; and suppresses apoptosis, fibrosis, and inflammation in cardiac tissue [32,50]. As all these processes are also involved in the pathogenesis of SSc, miRNA-133 may possibly play an imperative role in controlling the inflammatory process and collagen formation in patients with SSc. Indeed, we observed that pitting scars, a result of the excess fibrous tissue formation and one of the minor criteria commonly seen in SSc [32,51] were accompanied by significantly lower serum levels of miRNA-133 contrasted to patients without scars ($p = 0.009$). This was previously observed with miRNA-196 as well [34].

In the current work, patients with DM, arrhythmias, and vasculitis had significantly lower serum levels of miRNA-133 compared to patients without these findings. The lower miRNA-133 in patients with DM may be explained by insulin's inhibitory effect on miRNA-133 via sterol regulatory element binding protein 1c and myocyte enhancer factor 2C [52]. In concordance with our results, Abdellatif [53] demonstrated that the downregulation of miRNA-133 is a prerequisite for the development of apoptosis, fibrosis, and prolongation of the QT interval and hence arrhythmias.

Interestingly, SSc patients treated with MMF had their miRNA-133 expression less downregulated than patients on other treatments signifying the beneficial role of MMF in the treatment of SSc. It would be intriguing to investigate the beneficial effect of MMF Vs. other SSc medications in the management of the co-morbidities, we report to be associated with lower miRNA-133 levels.

The innate immunity-related lncRNA-H19 is involved in the biological activities of the skin via its role in the stimulation of the Wnt/β-catenin signaling pathway [54]. H19 aberrantly modulates the proliferation and differentiation of various fibroblasts, particularly dermal fibroblasts, RA synovial fibroblasts, and fibroblast-like cells, such as pulmonary artery smooth muscle cells [55]. Reminiscent of SLE, RA, and osteoarthritis (OA) [56,57], implies that H19 may have a fundamental role in SSc pathogenesis as well through induction of ECM differentiation and myofibroblast production. Thus, it was not surprising to detect its upregulation in our SSc patients compared to the control group ($p = 0.0001$). Interestingly, as DNA methylation can affect the expression of lncRNAs, the H19 upregulation we observed may be related to a hypomethylation of the promoter of the H19 gene or the methylation of CpG islands in promoter regions which is a mutual way for the deactivation of some genes as HLA-DRB1 which present significantly in patients with SSc [58,59].

Significantly higher serum levels of H19 were detected in patients under 40 ($p = 0.002$). In the same manner, significantly higher serum expression levels of H19 were observed among patients who developed SSc before 14 years of age ($p = 0.018$). This was also seen in mice models where younger mice had a higher average H19 expression than older mice [60]. Further investigations of these findings seem relevant, particularly with the fact that younger patients with SSc ≤30 years frequently present with more disseminated disease [61,62]. Skin manifestations of SSc, including digital ulcers, calcinosis, and telangiectasia as well as respiratory manifestations were associated with significantly higher serum levels of H19 compared to patients without these findings. The H19's immune regulatory and aging protective functions may explain these associations [63]. Nonetheless, contradictory observations regarding H19 levels and both ILD and arthritis had been formerly narrated by Wan et al. [64] and Fu et al. [65]. Such differences may be related to differences in the selection/recruitment of patients, race, other epigenetic factors, or sample size.

The present study detected a positive correlation between H19 and PKM2 ($r = 0.429$, $p = 0.032$) and this was consistent with previous studies in liver and ovarian cancers, and it was hypothesized that H19 may induce and activate tumor-specific PKM2 which is essential for gene expression during tumorigenesis [23,66]. On the contrary, Chen et al. [67] reported that H19 overexpression reduces PKM2 protein levels through ubiquitin-mediated degradation. Similarly, there are conflicting results regarding the relation between H19 and TGF-β. In our work and that of Zhang et al. [68], there is a negative correlation between those variables with the latter authors stating that TGF-β signaling inhibits expression of H19 in liver cancer. In contrast, Wang et al. [69] demonstrated a positive correlation between TGF-β and H19. These contradictions deserve further research in different clinical and immunological settings.

While aerobic glycolysis directly influences how lymphocytes, such as T, B, and NK cells, differentiate and function [70], this process encompasses a sequence of cellular enzymes; the most important key enzyme is pyruvate kinase (PK) [10]. In the majority of cell types, PK can be found as the M1 or M2 isoform or even both. Alternative splicing is implemented to PK muscle (PKM), in order to create the PKM1 or PKM2 isoforms, which include exon 9 or 10, respectively [12,13].

We observed that the expression level of PKM2 was significantly upregulated in serum samples of SSc patients compared to the control group ($p = 0.005$). In agreement with our study, cultured dermal fibroblasts of SSc patients in a glucose-free medium, demonstrate a higher glycolytic metabolism compared with normal fibroblasts [71]. PKM2 was also shown to facilitate fibrosis progression in murine models [72]. Such observations suggest its possible participation in SSc pathogenesis.

PKM2 promotes Th17 cell differentiation and autoimmune inflammation through STAT3 activation with the production of further cytokines and chemokines, such as IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) [73,74] with their previously addressed role of these cytokines in SSc pathogenesis [31]. This Th17/IL-17 promoting effect of PKM2 may also be why it tended to be more upregulated in patients with dry eyes, reminiscent of Sjogren's Syndrome (SS) where an increased Th17 quantity and IL-17 expression is evident [75].

Although PKM2 may function as a target for miRNA-133 and the latter suppresses glycolysis within lung cancer cells by targeting PKM2 [76], our results didn't show such correlation. PKM2 was significantly higher in female patients compared to males ($p = 0.014$) possibly due to estrogen's ability to activate PKM2 and further increase its expression [77]. On the other hand, it was unexpected that although PKM2 promotion of neutrophil activation may be responsible for bronchiectasis [78], our results showed that patients with bronchiectasis had significantly lower PKM2 serum expression levels compared to patients without bronchiectasis.

Until the role of PKM2 in cardiomyocyte cell cycle and cardiac regeneration is studied. We hypothesize that the positive correlation we detected between H19 and PKM2 may explain why patients with higher PKM2 showed an increased incidence of pericardial effusion and arrhythmia.

5. Conclusion

In brief, our study shows upregulation of key molecules; TGF-β, H19, and PKM2, involved in the fibrotic and immunological process responsible for the development of SSc and its associated abnormalities. We also demonstrate the downregulation of the anti-fibrogenic/inflammatory protein miRNA-133 which may possibly be involved in the various pathogenic mechanisms associated with SSc. The targeted pharmacological modulation of these molecules is an interesting field of research and innovation and may prove to be a beneficial option for patients with SSc.

Furthermore, in this patients' cohort, MMF seemed to be associated with miRNA-133 upregulation, and methotrexate with lower H19 expression in comparison to other examined treatments. We suggest further studies exploring these biomarkers and pharmacologic

mechanisms through which pulmonary and extra-pulmonary symptoms in SSc patients show better improvement when treated by a combination of MMF and low-dose methotrexate as described by Gonzalez-Nieto et al. [79].

Additional experimental studies are needed to illuminate the key roles of miRNA-133, H19, PKM2, and TGF-β in the pathogenesis of autoimmune diseases. The lack of tissue verification of these differentially expressed miRNA-133 and H19 and relevant pathways is the study's principal limitation. The minimal number of SSc patients for quantitative PCR verification is another restriction.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ahmed MB. Khedr: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Olfat G. Shaker:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Mohamed HM. EL-Komy:** Writing – review & editing, Supervision, Conceptualization. **Amul M. Badr:** Writing – review & editing, Supervision. **Randa Erfan:** Writing – review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2023.12.003.

References

[1] U.A. Walker, A. Tyndall, L. Czirják, C. Denton, D. Farge-Bancel, O. Kowal-Bielecka, U. Müller-Ladner, C. Bocelli-Tyndall, M. Matucci-Cerinic, Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group database, Ann. Rheum. Dis. 66 (2007) 754–763, https://doi.org/10.1136/ard.2006.062901.

[2] A. Daigeler, M. Meyer, H. Joneidi-Jafari, C. Möcklinghoff, H.-U. Steinau, M. Lehnhardt, [Manifestations of scleroderma at the hand–options for hand surgery in an interdisciplinary concept], Handchir. Mikrochir. Plast. Chir. : Organ der Deutschsprachigen Arbeitsgemeinschaft fur Handchirurgie : Organ der Deutschsprachigen Arbeitsgemeinschaft fur Mikrochirurgie der Peripheren Nerven und Gefasse : Organ der V 39 (2007) 128–134, https://doi.org/10.1055/s-2006-924536.

[3] M. Hinchcliff, J. Varga, Systemic sclerosis/scleroderma: a treatable multisystem disease, Am. Fam. Physician 78 (2008) 961–968.

[4] B. Zhou, X.X. Zuo, Y.S. Li, S.M. Gao, X.D. Dai, H.L. Zhu, H. Luo, Integration of microRNA and mRNA expression profiles in the skin of systemic sclerosis patients, Sci. Rep. 7 (2017) 1–9, https://doi.org/10.1038/srep42899.

[5] Y. Allanore, R. Simms, O. Distler, M. Trojanowska, J. Pope, C.P. Denton, J. Varga, Systemic sclerosis, Nat. Rev. Dis. Prim. 1 (2015), 15002, https://doi.org/10.1038/nrdp.2015.2.

[6] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: therapeutic translation for fibrotic disease, Nat. Med. 18 (2012) 1028–1040, https://doi.org/10.1038/nm.2807.

[7] Z. Szekanecz, A.E. Koch, Endothelial cells in inflammation and angiogenesis, Curr. Drug Targets - Inflamm. Allergy 4 (2005) 319–323, https://doi.org/10.2174/1568010054022187.

[8] V. Viswanath, M.M. Phiske, V. V Gopalani, Systemic sclerosis: current concepts in pathogenesis and therapeutic aspects of dermatological manifestations, Indian J. Dermatol. 58 (2013) 255, https://doi.org/10.4103/0019-5154.113930.

[9] F. Klingberg, B. Hinz, E.S. White, The myofibroblast matrix: implications for tissue repair and fibrosis, J. Pathol. 229 (2013) 298–309, https://doi.org/10.1002/path.4104.

[10] R.A. Gatenby, R.J. Gillies, Why do cancers have high aerobic glycolysis? Nat. Rev. Cancer 4 (2004) 891–899, https://doi.org/10.1038/nrc1478.

[11] L. Jiang, L. Xiao, H. Sugiura, X. Huang, A. Ali, M. Kuro-o, R.J. Deberardinis, D. A. Boothman, Metabolic reprogramming during TGFβ1-induced epithelial-to-mesenchymal transition, Oncogene 34 (2015) 3908–3916, https://doi.org/10.1038/onc.2014.321.

[12] K.A. Olson, J.C. Schell, J. Rutter, Pyruvate and metabolic flexibility: illuminating a path toward selective cancer therapies, Trends Biochem. Sci. 41 (2016) 219–230, https://doi.org/10.1016/j.tibs.2016.01.002.

[13] C. V Clower, D. Chatterjee, Z. Wang, L.C. Cantley, M.G. Vander Heiden, A. R. Krainer, The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoform expression and cell metabolism, Proc. Natl. Acad. Sci. U.S. A. 107 (2010) 1894–1899, https://doi.org/10.1073/pnas.0914845107.

[14] D. Wang, H. Zhang, M. Li, M.G. Frid, A.R. Flockton, B.A. McKeon, M.E. Yeager, M. A. Fini, N.W. Morrell, S.S. Pullamsetti, S. Velegala, W. Seeger, T.A. McKinsey, C. C. Sucharov, K.R. Stenmark, MicroRNA-124 controls the proliferative, migratory, and inflammatory phenotype of pulmonary vascular fibroblasts, Circ. Res. 114 (2014) 67–78, https://doi.org/10.1161/circresaha.114.301633.

[15] C. Chen, Y. Zhou, J. Wang, Y. Yan, L. Peng, W. Qiu, Dysregulated microRNA involvement in multiple sclerosis by induction of T helper 17 cell differentiation, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.01256.

[16] K. Taniguchi, Y. Ito, N. Sugito, M. Kumazaki, H. Shinohara, N. Yamada, Y. Nakagawa, T. Sugiyama, M. Futamura, Y. Otsuki, K. Yoshida, K. Uchiyama, Y. Akao, Organ-specific PTB1-associated microRNAs determine expression of pyruvate kinase isoforms, Sci. Rep. 5 (2015) 1–8, https://doi.org/10.1038/srep08647.

[17] H. Shan, Y. Zhang, Y. Lu, Y. Zhang, Z. Pan, B. Cai, N. Wang, X. Li, T. Feng, Y. Hong, B. Yang, Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines, Cardiovasc. Res. 83 (2009) 465–472, https://doi.org/10.1093/cvr/cvp130.

[18] E. Dangelmaier, S.B. Lazar, A. Lal, Long noncoding RNAs: P53's secret weapon in the fight against cancer? PLoS Biol. 17 (2019) 1–11, https://doi.org/10.1371/journal.pbio.3000143.

[19] Y. Zhang, M. Zhang, W. Xu, J. Chen, X. Zhou, The long non-coding RNA H19 promotes cardiomyocyte apoptosis in dilated cardiomyopathy, Oncotarget 8 (2017) 28588–28594, https://doi.org/10.18632/oncotarget.15544.

[20] Y. Liu, G. Li, J. fang Zhang, The role of long non-coding RNA H19 in musculoskeletal system: a new player in an old game, Exp. Cell Res. 360 (2017) 61–65, https://doi.org/10.1016/j.yexcr.2017.09.007.

[21] A. Bhan, S.S. Mandal, Long Noncoding RNAs : Emerging Stars in Gene Regulation , Epigenetics and Human Disease, 2014, pp. 1932–1956, https://doi.org/10.1002/cmdc.201300534.

[22] X. Zhang, D.Y. Li, M.P. Reilly, Long intergenic noncoding RNAs in cardiovascular diseases: challenges and strategies for physiological studies and translation, Atherosclerosis 281 (2019) 180–188, https://doi.org/10.1016/j.atherosclerosis.2018.09.040.

[23] H. Li, J. Li, S. Jia, M. Wu, J. An, Q. Zheng, W. Zhang, D. Lu, miR675 upregulates long noncoding RNA H19 through activating EGR1 in human liver cancer, Oncotarget 6 (2015) 31958–31984, https://doi.org/10.18632/oncotarget.5579.

[24] F. van den Hoogen, D. Khanna, J. Fransen, S.R. Johnson, M. Baron, A. Tyndall, M. Matucci-Cerinic, R.P. Naden, T.A.J. Medsger, P.E. Carreira, G. Riemekasten, P. J. Clements, C.P. Denton, O. Distler, Y. Allanore, D.E. Furst, A. Gabrielli, M. D. Mayes, J.M. van Laar, J.R. Seibold, L. Czirjak, V.D. Steen, M. Inanc, O. Kowal-Bielecka, U. Müller-Ladner, G. Valentini, D.J. Veale, M.C. Vonk, U.A. Walker, L. Chung, D.H. Collier, M. Ellen Csuka, B.J. Fessler, S. Guiducci, A. Herrick, V. M. Hsu, S. Jimenez, B. Kahaleh, P.A. Merkel, S. Sierakowski, R.M. Silver, R. W. Simms, J. Varga, J.E. Pope, Classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative, Ann. Rheum. Dis. 72 (2013) (2013) 1747–1755, https://doi.org/10.1136/annrheumdis-2013-204424.

[25] Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee, Arthritis Rheum. 23 (1980) 581–590, https://doi.org/10.1002/art.1780230510.

[26] C.W. Wasson, G. Abignano, H. Hermes, M. Malaab, R.L. Ross, S.A. Jimenez, H. Y. Chang, C.A. Feghali-Bostwick, F. Del Galdo, Long non-coding RNA HOTAIR drives EZH2-dependent myofibroblast activation in systemic sclerosis through miRNA 34a-dependent activation of NOTCH, Ann. Rheum. Dis. 79 (2020) 507–517, https://doi.org/10.1136/annrheumdis-2019-216542.

[27] T.D. Ly, A. Kleine, R. Plümers, B. Fischer, V. Schmidt, D. Hendig, J.H.W. Distler, J. Kuhn, C. Knabbe, I. Faust, Cytokine-mediated induction of human xylosyltransferase-I in systemic sclerosis skin fibroblasts, Biochem. Biophys. Res. Commun. 549 (2021) 34–39, https://doi.org/10.1016/j.bbrc.2021.02.092.

[28] T.W. Henry, F.A. Mendoza, S.A. Jimenez, Role of microRNA in the pathogenesis of systemic sclerosis tissue fibrosis and vasculopathy, Autoimmun. Rev. 18 (2019), 102396, https://doi.org/10.1016/j.autrev.2019.102396.

[29] X. Zhao, J.Y.Y. Kwan, K. Yip, P.P. Liu, F.F. Liu, Targeting metabolic dysregulation for fibrosis therapy, Nat. Rev. Drug Discov. 19 (2020) 57–75, https://doi.org/10.1038/s41573-019-0040-5.

[30] I. Szabo, L. Muntean, T. Crisan, V. Rednic, C. Sirbe, S. Rednic, Novel concepts in systemic sclerosis pathogenesis: role for mirnas, Biomedicines 9 (2021) 1–18, https://doi.org/10.3390/biomedicines9101471.

[31] H. Zhu, H. Luo, X. Zuo, MicroRNAs: their involvement in fibrosis pathogenesis and use as diagnostic biomarkers in scleroderma, Exp. Mol. Med. 45 (2013), https://doi.org/10.1038/emm.2013.71 e41–e41.

[32] J. Varga, C.P. Denton, F.M. Wigley, Y. Allanore, M. Kuwana, Scleroderma: from Pathogenesis to Comprehensive Management, second ed., 2016, https://doi.org/10.1007/978-3-319-31407-5.

[33] E. Pachera, S. Assassi, G.A. Salazar, M. Stellato, F. Renoux, A. Wunderlin, P. Blyszczuk, R. Lafyatis, F. Kurreeman, J. de Vries-Bouwstra, T. Messemaker, C. A. Feghali-Bostwick, G. Rogler, W.T. Van Haaften, G. Dijkstra, F. Oakley, M. Calcagni, J. Schniering, B. Maurer, J.H.H.W. Distler, G. Kania, M. Frank-Bertoncelj, O. Distler, Long noncoding RNA H19X is a key mediator of TGF-β-driven fibrosis, J. Clin. Invest. 130 (2020) 4888–4905, https://doi.org/10.1172/JCI135439.

[34] N. Honda, M. Jinnin, I. Kajihara, T. Makino, K. Makino, S. Masuguchi, S. Fukushima, Y. Okamoto, M. Hasegawa, M. Fujimoto, H. Ihn, TGF-β–Mediated downregulation of MicroRNA-196a contributes to the constitutive upregulated type I collagen expression in scleroderma dermal fibroblasts, J. Immunol. 188 (2012) 3323–3331, https://doi.org/10.4049/jimmunol.1100876.

[35] H. Li, R. Yang, X. Fan, T. Gu, Z. Zhao, D. Chang, W. Wang, C. Wang, MicroRNA array analysis of microRNAs related to systemic scleroderma, Rheumatol. Int. 32 (2012) 307–313, https://doi.org/10.1007/s00296-010-1615-y.

[36] M. Etoh, M. Jinnin, K. Makino, K. Yamane, W. Nakayama, J. Aoi, N. Honda, I. Kajihara, T. Makino, S. Fukushima, H. Ihn, MicroRNA-7 down-regulation mediates excessive collagen expression in localized scleroderma, Arch. Dermatol. Res. 305 (2013) 9–15, https://doi.org/10.1007/s00403-012-1287-4.

[37] K. Makino, M. Jinnin, A. Hirano, K. Yamane, M. Eto, T. Kusano, N. Honda, I. Kajihara, T. Makino, K. Sakai, S. Masuguchi, S. Fukushima, H. Ihn, The downregulation of microRNA let-7a contributes to the excessive expression of type I collagen in systemic and localized scleroderma, J. Immunol. 190 (2013) 3905–3915, https://doi.org/10.4049/jimmunol.1200822.

[38] P. Moinzadeh, K. Kuhr, E. Siegert, U. Mueller-Ladner, G. Riemekasten, C. Günther, I. Kötter, J. Henes, N. Blank, G. Zeidler, C. Pfeiffer, A. Juche, I. Jandova, J. Ehrchen, M. Schmalzing, L. Susok, T. Schmeiser, C. Sunderkoetter, J.H.W. W. Distler, M. Worm, A. Kreuter, T. Krieg, N. Hunzelmann, Older age onset of systemic sclerosis - accelerated disease progression in all disease subsets, Rheumatology 59 (2020) 3380–3389, https://doi.org/10.1093/rheumatology/keaa127.

[39] D. Frydecka, B. Misiak, E. Pawlak-Adamska, L. Karabon, A. Tomkiewicz, P. Sedlaczek, A. Kiejna, J.A. Beszłej, Sex differences in TGFB-β signaling with respect to age of onset and cognitive functioning in schizophrenia, Neuropsychiatric Dis. Treat. 11 (2015) 575–584, https://doi.org/10.2147/NDT.S74672.

[40] N. Ziller, R. Kotolloshi, M. Esmaeili, M. Liebisch, R. Mrowka, A. Baniahmad, T. Liehr, G. Wolf, I. Loeffler, Sex differences in diabetes- and TGF-β1-induced renal damage, Cells 9 (2020) 1–26, https://doi.org/10.3390/cells9102236.

[41] J.R. Urban, B. King, Divalproex sodium: a potential therapy for scleroderma digital ulcers, JAAD Case Rep 1 (2015) 44–45, https://doi.org/10.1016/j.jdcr.2014.11.004.

[42] S. Segawa, D. Goto, Y. Yoshiga, M. Sugihara, T. Hayashi, Y. Chino, I. Matsumoto, S. Ito, T. Sumida, Inhibition of transforming growth factor-β signalling attenuates interleukin (IL)-18 plus IL-2-induced interstitial lung disease in mice, Clin. Exp. Immunol. 160 (2010) 394–402, https://doi.org/10.1111/j.1365-2249.2010.04094.x.

[43] A. Saito, M. Horie, T. Nagase, TGF-β signaling in lung health and disease, Int. J. Mol. Sci. 19 (2018) 1–18, https://doi.org/10.3390/ijms19082460.

[44] G. Epstein Shochet, E. Brook, B. Bardenstein-Wald, D. Shitrit, TGF-β pathway activation by idiopathic pulmonary fibrosis (IPF) fibroblast derived soluble factors is mediated by IL-6 trans-signaling, Respir. Res. 21 (2020) 1–10, https://doi.org/10.1186/s12931-020-1319-0.

[45] D. Hori, B. Dunkerly-Eyring, Y. Nomura, D. Biswas, J. Steppan, J. Henao-Mejia, H. Adachi, L. Santhanam, D.E. Berkowitz, C. Steenbergen, R.A. Flavell, S. Das, MiR-181b regulates vascular stiffness age dependently in part by regulating TGF-β signaling, PLoS One 12 (2017) 1–20, https://doi.org/10.1371/journal.pone.0174108.

[46] X. Ma, Z. Cui, Z. Du, H. Lin, Transforming growth factor-β signaling, a potential mechanism associated with diabetes mellitus and pancreatic cancer? J. Cell. Physiol. 235 (2020) 5882–5892, https://doi.org/10.1002/jcp.29605.

[47] F. Heydarpour, S. Sajadimajd, E. Mirzarazi, P. Haratipour, T. Joshi, M.H. Farzaei, H. Khan, J. Echeverría, Involvement of TGF-β and autophagy pathways in pathogenesis of diabetes: a comprehensive review on biological and pharmacological insights, Front. Pharmacol. 11 (2020) 1–21, https://doi.org/10.3389/fphar.2020.498758.

[48] L. Zhao, Y. Zou, F. Liu, Transforming growth factor-beta1 in diabetic kidney disease, Front. Cell Dev. Biol. 8 (2020) 1–10, https://doi.org/10.3389/fcell.2020.00187.

[49] A. von Borstel, J.S. Sanders, A. Rutgers, C.A. Stegeman, P. Heeringa, W. H. Abdulahad, Cellular immune regulation in the pathogenesis of ANCA-associated vasculitides, Autoimmun. Rev. 17 (2018) 413–421, https://doi.org/10.1016/j.autrev.2017.12.002.

[50] N. Li, H. Zhou, Q. Tang, miR-133: a suppressor of cardiac remodeling? Front. Pharmacol. 9 (2018) https://doi.org/10.3389/fphar.2018.00903.

[51] C.P. Denton, D. Khanna, Systemic sclerosis, Lancet 390 (2017) 1685–1699, https://doi.org/10.1016/S0140-6736(17)30933-9.

[52] A. Granjon, M.P. Gustin, J. Rieusset, E. Lefai, E. Meugnier, I. Güller, C. Cerutti, C. Paultre, E. Disse, R. Rabasa-Lhoret, M. Laville, H. Vidal, S. Rome, The microRNA signature in response to insulin reveals its implication in the transcriptional action of insulin in human skeletal muscle and the role of a sterol regulatory element-binding protein-1c/myocyte enhancer factor 2C pathway, Diabetes 58 (2009) 2555–2564, https://doi.org/10.2337/db09-0165.

[53] M. Abdellatif, The role of microRNA-133 in cardiac hypertrophy uncovered, Circ. Res. 106 (2010) 16–18, https://doi.org/10.1161/CIRCRESAHA.109.212183.

[54] C.M. Lin, Y. Liu, K. Huang, X.C. Chen, B.Z. Cai, H.H. Li, Y.P. Yuan, H. Zhang, Y. Li, Long noncoding RNA expression in dermal papilla cells contributes to hairy gene regulation, Biochem. Biophys. Res. Commun. 453 (2014) 508–514, https://doi.org/10.1016/j.bbrc.2014.09.119.

[55] M. Rusek, D. Krasowska, Non-coding RNA in systemic sclerosis: a valuable tool for translational and personalized medicine, Genes 12 (2021) 1296, https://doi.org/10.3390/genes12091296.

[56] X. Chen, X. Luo, Y. Wei, H. Sun, L. Dai, Y. Tangzhou, H. Jin, Z. Yin, LncRNA H19 induces immune dysregulation of BMMSCs, at least partly, by inhibiting IL-2 production, Mol. Med. 27 (2021), https://doi.org/10.1186/s10020-021-00326-y.

[57] Y.C. Chen, P.Y. Kuo, Y.C. Chou, H.E. Chong, Y.T. Hsieh, M.L. Yang, C.L. Wu, A. L. Shiau, C.R. Wang, Up-regulated expression of pro-apoptotic long noncoding RNA lincrna-P21 with enhanced cell apoptosis in lupus nephritis, Int. J. Mol. Sci. 22 (2021) 1–18, https://doi.org/10.3390/ijms22010301.

[58] Y. Luo, Y. Wang, Q. Wang, R. Xiao, Q. Lu, Systemic sclerosis: genetics and epigenetics, J. Autoimmun. 41 (2013) 161–167, https://doi.org/10.1016/j.jaut.2013.01.012.

[59] E.Y. Lee, J.M. Song, H.J. Kim, H.R. Park, Hypomethylation of lncRNA H19 as a potential prognostic biomarker for oral squamous cell carcinoma, Arch. Oral Biol. 129 (2021), 105214, https://doi.org/10.1016/j.archoralbio.2021.105214.

[60] M. Vander Roest, C. Krapp, J.L. Thorvaldsen, M.S. Bartolomei, W.D. Merryman, H19 is not hypomethylated or upregulated with age or sex in the aortic valves of mice, Phys. Rep. 7 (2019) 1–9, https://doi.org/10.14814/phy2.14244.

[61] P.D. Sampaio-Barros, A.B. Bortoluzzo, R.G. Marangoni, L.F. Rocha, A.P.T. Del Rio, A.M. Samara, N.H. Yoshinari, J.F. Marques-Neto, Survival, causes of death, and prognostic factors in systemic sclerosis: analysis of 947 Brazilian patients, J. Rheumatol. 39 (2012) 1971–1978, https://doi.org/10.3899/jrheum.111582.

[62] P.E. Carreira, L. Carmona, B.E. Joven, E. Loza, J.L. Andréu, G. Riemekasten, S. Vettori, A. Balbir-Gurman, P. Airò, U. Walker, N. Damjanov, M. Matucci-Cerinic, L.P. Ananieva, S. Rednic, L. Czirják, O. Distler, D. Farge, R. Hesselstrand, A. Corrado, P. Caramaschi, M. Tikly, Y. Allanore, Differences associated with age at onset in early systemic sclerosis patients: a report from the EULAR Scleroderma Trials and Research Group (EUSTAR) database, Scand. J. Rheumatol. 48 (2019) 42–51, https://doi.org/10.1080/03009742.2018.1459830.

[63] B. Wang, C.W. Suen, H. Ma, Y. Wang, L. Kong, D. Qin, Y.W.W. Lee, G. Li, The roles of H19 in regulating inflammation and aging, Front. Immunol. 11 (2020) 1–11, https://doi.org/10.3389/fimmu.2020.579687.

[64] X. Wan, X. Tian, J. Du, Y. Lu, Y. Xiao, Long non-coding RNA H19 deficiency ameliorates bleomycin-induced pulmonary inflammation and fibrosis, Respir. Res. 21 (2020) 1–12, https://doi.org/10.1186/s12931-020-01534-6.

[65] X. Fu, G. Song, R. Ni, H. Liu, Z. Xu, D. Zhang, F. He, G. Huang, lncRNA-H19 silencing suppresses synoviocytes proliferation and attenuates collagen-induced arthritis progression by modulating miR-124a, Rheumatology 60 (2021) 430–440, https://doi.org/10.1093/rheumatology/keaa395.

[66] X. Zheng, Y. Zhou, W. Chen, L. Chen, J. Lu, F. He, X. Li, L. Zhao, Ginsenoside 20(S)-Rg3 prevents PKM2-Targeting miR-324-5p from H19 sponging to antagonize the warburg effect in ovarian cancer cells, Cell. Physiol. Biochem. 51 (2018) 1340–1353, https://doi.org/10.1159/000495552.

[67] C. Chen, W.R. Liu, B. Zhang, L.M. Zhang, C.G. Li, C. Liu, H. Zhang, Y.S. Huo, Y. C. Ma, P.F. Tian, Q. Qi, J.J. Li, Z. Tang, Z.F. Zhang, G. Giaccone, D.S. Yue, C. L. Wang, LncRNA H19 downregulation confers erlotinib resistance through upregulation of PKM2 and phosphorylation of AKT in EGFR-mutant lung cancers, Cancer Lett. 486 (2020) 58–70, https://doi.org/10.1016/j.canlet.2020.05.009.

[68] J. Zhang, C. Han, N. Ungerleider, W. Chen, K. Song, Y. Wang, H. Kwon, W. Ma, T. Wu, A transforming growth factor-β and H19 signaling Axis in tumor-initiating hepatocytes that regulates hepatic carcinogenesis, Hepatology 69 (2019) 1549–1563, https://doi.org/10.1002/hep.30153.

[69] X. Wang, Z. Cheng, L. Dai, T. Jiang, L. Jia, X. Jing, L. An, H. Wang, M. Liu, Knockdown of long noncoding RNA H19 represses the progress of pulmonary fibrosis through the transforming growth factor β/smad3 pathway by regulating MicroRNA 140, Mol. Cell Biol. 39 (2019) 1–12, https://doi.org/10.1128/mcb.00143-19.

[70] M. Chen, H. Liu, Z. Li, A.L. Ming, H. Chen, Mechanism of PKM2 affecting cancer immunity and metabolism in Tumor Microenvironment, J. Cancer 12 (2021) 3566–3574, https://doi.org/10.7150/JCA.54430.

[71] E. Andreucci, F. Margheri, S. Peppicelli, F. Bianchini, J. Ruzzolini, A. Laurenzana, G. Fibbi, C. Bruni, S. Bellando-Randone, S. Guiducci, E. Romano, M. Manetti, M. Matucci-Cerinic, L. Calorini, Glycolysis-derived acidic microenvironment as a driver of endothelial dysfunction in systemic sclerosis, Rheumatology 60 (2021) 4508–4519, https://doi.org/10.1093/rheumatology/keab022.

[72] G. Satyanarayana, R.C. Turaga, M. Sharma, S. Wang, F. Mishra, G. Peng, X. Deng, J. Yang, Z.R. Liu, Pyruvate kinase M2 regulates fibrosis development and progression by controlling glycine auxotrophy in myofibroblasts, Theranostics 11 (2021) 9331–9341, https://doi.org/10.7150/thno.60385.

[73] L.E.A. Damasceno, D.S. Prado, F.P. Veras, M.M. Fonseca, J.E. Toller-Kawahisa, M. H. Rosa, G.A. Públio, T.V. Martins, F.S. Ramalho, A. Waismaz, F.Q. Cunha, T. M. Cunha, J.C. Alves-Filho, PKM2 promotes Th17 cell differentiation and autoimmune inflammation by fine-tuning STAT3 activation, J. Exp. Med. 217 (2020), https://doi.org/10.1084/JEM.20190613.

[74] E.H. El-Morsy, A.A. Eid, H. Ghoneim, K.A. Al-Tameemi, Serum level of interleukin-17A in patients with alopecia areata and its relationship to age, Int. J. Dermatol. 55 (2016) 869–874, https://doi.org/10.1111/ijd.12994.

[75] A. Wang, J. Liu, S. Yu, X. Liu, X. Zhuang, Y. Liu, X. Chen, Exosomes Derived from Stem Cells from Apical Papilla Ameliorate Sjogren ' S Syndrome by Suppressing Th17 Cell Differentiation, 2021, pp. 1–23, https://doi.org/10.21203/rs.3.rs-864685/v1.

[76] G. Liu, Y. Li, X. Gao, Overexpression of microRNA-133b sensitizes non-small cell lung cancer cells to irradiation through the inhibition of glycolysis, Oncol. Lett. 11 (2016) 2903–2908, https://doi.org/10.3892/ol.2016.4316.

[77] Y. Lu, X. Liu, E. Zhang, E.J. Kopras, E.P. Smith, A. Astreinidis, C. Li, Y.K. Leung, S. M. Ho, J.J. Yu, Estrogen activates pyruvate kinase M2 and increases the growth of TSC2-deficient cells, PLoS One 15 (2020) 1–20, https://doi.org/10.1371/journal.pone.0228894.

[78] N. Dhanesha, R.B. Patel, P. Doddapattar, M. Ghatge, G.D. Flora, M. Jain, D. Thedens, H. Olalde, M. Kumskova, E.C. Leira, A.K. Chauhan, PKM2 promotes neutrophil activation and cerebral thromboinflammation: therapeutic implications for ischemic stroke, Blood 139 (2022) 1234–1245, https://doi.org/10.1182/blood.2021012322.

[79] J.A. Gonzalez-Nieto, I.J. Martin-Suarez, F.L. Gil-Muñoz, Efficacy of mycophenolate associated with methotrexate as a maintenance treatment for systemic sclerosis-associated interstitial lung disease, Arch. Bronconeumol. 47 (2011) 575, https://doi.org/10.1016/j.arbr.2011.06.013.