Assessment of Micro RNAs 31 In Pathogenesis of Hypertrophic Scar

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Abstract

Introduction: A hypertrophic scar (HS) is a pathological result of wound healing. It is caused by the excessive accumulation of extracellular matrix components. MicroRNAs regulate protein-coding gene expression, participating in different biological processes. MiR-31 has a wide range of molecular targets and is specifically expressed in various tissues and organs.

Aim of the study: to evaluate the role of miR-31 in hypertrophic scar parthenogenesis.

Subjects and Methods: The study included 20 hyper-trophic scar patients and 20 healthy subjects as a control. The level of the microRNAs in serum was estimated using serum isolation Then CDNA will be formed using the RT Kit. PCR will be conducted by real-time PCR for the amplification of the genes.

Results: There was a highly statistically significant difference between the two groups of miR-31. The level of mir-31 was much higher in the patient group than in the normal control.

Conclusion: Our findings suggest that miR-31 is pro-fibrotic and that its up-regulation stimulates fibrosis. This may provide a new direction for the study of the parthenogenesis of hypertrophic scars.

Key words: Hypertrophic scar; MicroRNA; MiR-31.

1. Introduction

A hypertrophic scar (HS) is a pathological result of wound healing. It is characterized by erythematous, raised, and inflexible skin tissues [1]. Although HS is raised above the skin surface, it does not extend beyond the original wound. HS is caused by the excessive accumulation of extracellular matrix components due to the

recruitment of inflammatory cells and increased numbers of fibroblasts [2].

MicroRNAs are a class of endogenous, small, non-coding, single-stranded RNA molecules with a length of about 22 nucleotides. Growing evidence demonstrates that microRNAs have a crucial role in pathological wound healing and may be related to the development and progression of skin fibrosis [3].

MicroRNA-31 could regulate cell proliferation, apoptosis, and the cell cycle

progression of fibroblasts [4]. The miR-31 gene is located on chromosome band 9p21.3, ∼500 kb from the locus of the well-known tumor suppressors cyclin dependent kinase inhibitor (CDKN) 2A and CDKN2B, which encode the cell cycle inhibitor proteins p15 and p16. MiR-31 shows that it displays altered levels of expression in different tumors [5]. The functional role of miR-31 is extremely complex, and it possesses both tumor suppressive and oncogenic roles in different tumor types [6].

2. Subjects and methods

2.1.Subjects

This study was a case-control cross-sectional study conducted in the dermatology department of Fayoum University hospitals between March 2020 and October 2020. We explained the nature and purpose of the study, and participation in this study is voluntary. Also, oral consent was obtained from illiterate participants.

Inclusion criteria

Patients with hypertrophic scars of both sexes

Sample size

This study was a case-control cross-sectional study conducted in the Department of Dermatology, STDs, and Androgyny at Fayoum University Hospital. 20 patients were included in the study, and 20 were in the control group.

2.2. Study design

We collected all available data about the onset, course, duration, cause, and site of the scar, associated itching, pain, different thicknesses or colors within the scar, and the shape of the scar through the PSAS (Patient Scar Assessment Scale). Past history

included systemic and cutaneous diseases, previous medications, and operations.

2.3. Statistical Analysis

Data analysis was performed using the statistical package for social science (SPSS 17.0) on Windows 8.1.

Descriptive analysis was performed in the form of numbers and percentages for qualitative data. Arithmetic means were calculated as central tendency measurement, while standard deviation was a measure of dispersion for quantitative parametric data [7].

3. Results

The patient ages with a mean value of 24.80± 2.20 and the control ages with a mean value of 31.05±2.31 (**Table 1**). The patients included 18 females (90.0%) and 2 males (10.0%), and the control included 17 females (85.0%) and 3 males (15.0%) (**Table 1**). There was no statistically significant difference (*P > 0.05*) between the case and control groups as regards age and sex (**Table 1**). There was a highly statistically significant difference between patients (6.67±0.58) and the control (1.01±0.1) group regarding mirR-31 levels in serum, with a p-value of 0.0001.

There was no statistically significant difference between sex, site, skin type, OSAS baseline of mild and moderate hypertrophic scar, PASA baseline, and miR-31 levels in serum, with p-values equal to 0.378, 0.992, 0.027, 0.855, 0.020, and 0.406, respectively. There was no statistically significant difference between scar duration <85 months,

between 85 and 160 months, and miR-31 level in serum, with p-values of 0.859, 0.255, and 0.853, respectively. There was a statistically significant difference between skin type and the OSAS baseline of moderate and severe hypertrophic scars and miR-31 levels in serum, with p-values of 0,027, 0,020, and 0.003, respectively (**Table 2**).

There was no statistically significant difference between sex, skin type, site, OSAS baseline of mild hypertrophic scar, PASA baseline, and miR-29b levels in serum, with *P-values* equal to 0.146, 0.163, 0.902, 0.417, and 0.863, respectively.

The ROC for miR-31 in serum was found to give the maximum area under the curve, and it was seen that the level of miR-31 in serum was 0.98. The cut-off value of miR-31 in serum was 5.18, with a sensitivity

of 75% in serum and a specificity of 100% in

serum (**Table 3**).

Table 1: Age and gender distribution among patients and control.

Parameters		HS Patients (N=20)	Control (N=20)	*P-value*
Age		24.80 ±2.20	31.05 ±2.31	0.368
Sex	Male	18 (90%)	17 (85%)	0.1
	Female	2 (10%)	3 (15%)	

HS: hypertrophic scar.

Table 2: Relationship of miR-31 levels in serum for Patients with characteristics data.

Variables			*P-value*
Sex	Male	5.34±1.12	0.378
	Female	6.82±0.63	
Skin Type	3	6.89±0.76	0.027*
	4	6.0±0.404	
Scar Duration (Month)	<85	6.37±1.20	0.859
	85-160	6.10±0.89	0.255
	>160	7.69±0.92	0.853
Site	Arm/Elbow	6.66±0.87	0.992
	Other Sites	6.68±0.82	
OSAS Baseline	Mild	5.58±1.89	0.855
	Moderate	6.30±0.59	0.02*
	Severe	10.75±0.53	0.003*
PSAS Baseline	Mild	6.32±1.14	0.406
	Moderate	6.71±0.64	

*Significant.

Table 3: Sensitivity and Specificity percentages for miR-31 biomarker in serum in patients

Serum Biomarker	AUC	Cut-off value	Sensitivity	Specificity
miR-31 (Serum)	0.98	5.18	75%	100%

AUC: Area under the curve.

4. Discussion

HTS is a fibrotic disorder and shows features of increased cutaneous thickness, hypercellularity, excessive deposition of disorganized collagen, and increased vascularity. HTS is erythematous, raised, confined to its boundaries, and can regress over time. HTS can arise from deep lacerations, surgery, or individuals suffering from burns and has a strong, yet unknown, genetic predisposition [8].

miRNAs are small non-coding RNAs with 18–24 nucleotides in length. MiRNAs can bind to target mRNAs and negatively regulate gene expression. Particular miRNAs emerge as principal regulators that control major cell functions in various physiological and pathophysiological settings [9].

MicroRNA-31 is a highly conserved specific miRNA, which has a wide range of molecular targets and is specifically expressed in various tissues and organs. Mir-31 regulates different cell development processes by targeting genes involved in cell

proliferation, apoptosis, cell differentiation and cell movement [10].

This study was a case control cross-section study, conducted in Dermatology Departments of Fayoum and Cairo University Hospitals. The patients group included 20 patients; 18 females and 2 males. The second group included 20 healthy control, 17 females and 3 males. Serum and tissue microRNA levels were measured for both groups using ELISA technique.

There was highly statistically significant difference between mir-31 levels in patients and control in serum and tissue with p-value 0.0001. The level of mir-31 was much higher in patients' group than normal control that could be the role of miR -31 in pathogenesis of hypertrophic scar.

Wang et al., (2015) showed hsa-miR31-5p was the most highly expressed miRNA in HS. HS and NS samples from 12 patients were obtained to verify the

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expression of hsa-miR31-5p by qRT-PCR, and hsa-miR31-5p was highly expressed in HS tissues compared to NS tissues [11].

Conclusion

The recently identified miRNAs regulate gene expression and modulate key processes involved in skin fibrosis: TGF beta signaling, ECM deposition, fibroblast proliferation and differentiation, and EMT. MiR-31 is profibrotic and their up regulation stimulates fibrosis. This may provide a new direction for the study of the pathogenesis of hypertrophic scar. In treating skin fibrosis, therapies may be directed toward up regulating anti-fibrotic miRNAs or down regulating profibrotic miRNAs.

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