Hemolytic Component in Sickle Leg Ulcer

Subjects: Hematology

Contributor: Edvan do Carmo Santos , Gabriela Imbassahy Valentim Melo , Paulo Vinícius Bispo Santana , Idaiara Graziele Silva Quadros , Sètondji Cocou Modeste Alexandre Yahouédéhou , Caroline Conceição da Guarda , Rayra Pereira Santiago , Luciana Magalhães Fiuza , Suéllen Pinheiro Carvalho , Elisângela Vitória Adorno , Carla Martins Kaneto , Teresa Cristina Cardoso Fonseca , Marilda Souza Goncalves , Milena Magalhães Aleluia

Sickle leg ulcers (SLU) are malleoli lesions with exuberant hemolytic pathophysiology. The microRNAs are potential genetic biomarkers for several pathologies. Thereby, the expression of circulating miR-199a-5p, miR-144, and miR-126 in association with hemolytic biomarkers in SLU was assessed.

sickle cell disease | sickle leg ulcer | microRNA | hemolysis | biomarkers

1. Introduction

Sickle cell disease (SCD) is a common genetic disorder in Brazil, with 3500 new cases annually, and characterized by intravascular hemolysis, sterile inflammation, reduced nitric oxide (NO) bioavailability, and endothelial dysfunction [1]. The chronic hemolysis leads to vaso-occlusive episodes and clinical manifestations, such as painful crises, acute chest syndrome, stroke, and sickle leg ulcers (SLU) [2].

The SLU are cutaneous lesions with raised edges that frequently affect the malleolus of SCD patients [3]. SLU wound bed may present unviable tissues, such as necrotic tissue due to accumulation of dead cells or sloughy tissue without vascularization and constituted by cells fragments. In addition, the SLU wound bed may present viable granulation tissue with the proliferation of blood capillaries and epithelized tissue consisting of regenerated and dry epidermis [3][4].

The SLU occurrence has been associated with morbidity, work disability, social restriction, frequent recurrence, and recalcitration [2][5]. Thus, the evaluation of these factors is insufficient to understand the SLU clinical profile. Molecular biology techniques revealed the existence of microRNAs (miRNAs) which may modulate pathophysiology mechanisms [6]. miRNAs are small non-coding RNA molecules which act as positive or negative regulators in several pathologies, including SCD [6][7]. In this sense, miR-199a-5p has been associated with NO metabolism in vitro [8] and miR-144 may regulate fetal hemoglobin (HbF) production through gene silencing [6]. miR-126 has been associated with protective mechanisms against vascular damage in vitro and in vivo [9]. However, the role of these miRNAs in the intravascular hemolysis related to SLU occurrence is unknown.

2. Circulating miRNAs Expression in SCD Patients and between HbSS and HbSC Genotype with and without SLU

Regarding SLU, there was higher miR-199a-5p (**Figure 1**a) and miR-144 (**Figure 1**b) expression, as well as lower miR-126 (**Figure 1**c) expression in SLU+ patients ($p < 0.05$).

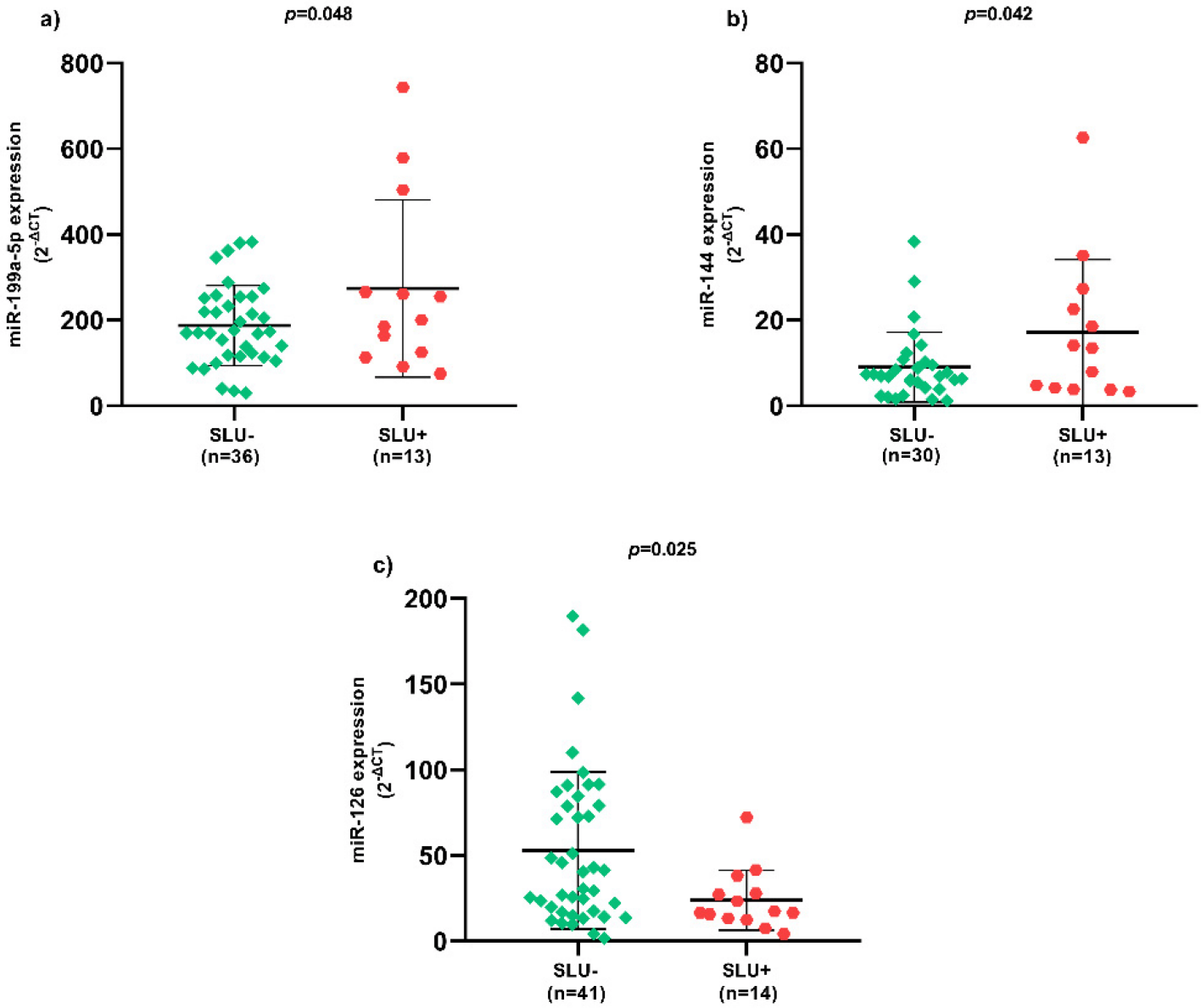


Figure 1. Expression of circulating miRNAs between SLU- and SLU+ patients. (**a**) Expression of circulating miR-199a-5p between SLU- and SLU+ patients; (**b**) Expression of circulating miR-144 between SLU- and SLU+ patients; (**c**) Expression of circulating miR-126 between SLU- and SLU+ patients. SLU-, patients without sickle leg ulcer; SLU+, patients with active sickle leg ulcer or previous history; miR, microRNA; *p*-value obtained using independent *t*-test.

In SLU- patients with HbSC genotype, there was higher expression of circulating miR-199a-5p, miR-144, and miR-126. In addition, circulating miR-126 expression was higher in SLU+ patients with HbSC genotype.

3. Correlation Coefficients between Hemolytic Biomarkers and Circulating miRNAs Expression in SLU+ Patients

miR-199a-5p expression was positively correlated with NOm, LDH, AST, GGT, iron, and ferritin levels, and negatively correlated with red blood cell (RBC) count and hemoglobin levels ($p < 0.05$) (**Figure 2**a). Moreover, there were positive correlations between miR-144 expression and NOm, LDH, indirect bilirubin, AST, GGT, iron, and ferritin levels, as well as negative correlations with RBC count and hemoglobin levels ($p < 0.05$) (**Figure 2**b). Regarding miR-126 expression, there were negative correlations with indirect bilirubin, iron and ferritin levels ($p < 0.05$) (**Figure 2**c).

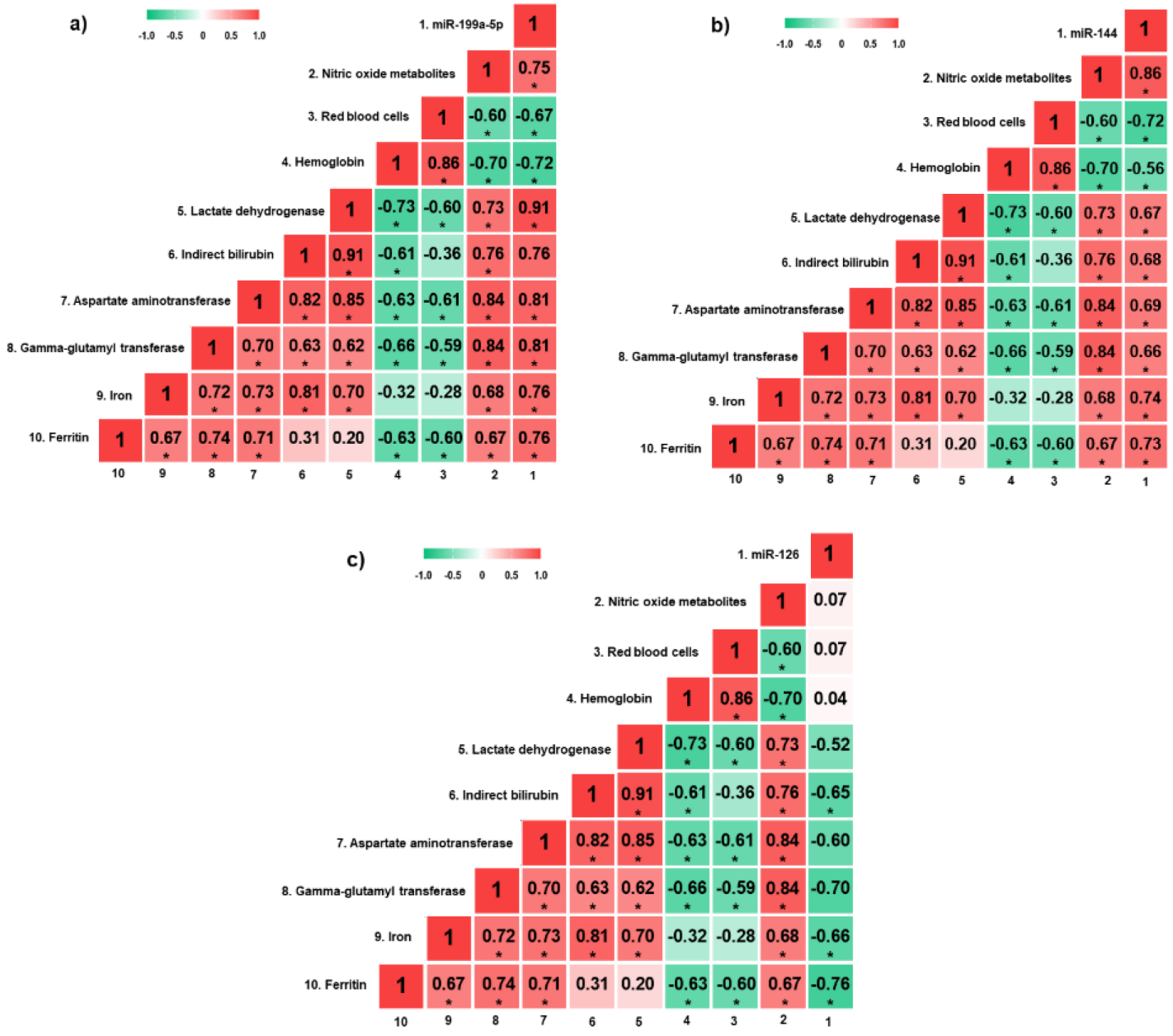


Figure 2. Heatmap of correlation coefficients between hemolytic biomarkers and circulating miRNAs in SLU+ patients. (**a**) Correlation analyses between hemolytic biomarkers and circulating miR-199a-5p; (**b**) correlation analyses between hemolytic biomarkers and circulating miR-144; (**c**) correlation analyses between hemolytic

biomarkers and circulating miR-126. miRNAs, microRNAs; SLU+, patients with active sickle leg ulcer or previous history. * $p < 0.05$. *p*-value obtained by Pearson correlation.

4. Circulating miRNAs Expression in Active SLU

Patients whose SLU wound bed was constituted by unviable tissues, such as necrotic and sloughy tissues, presented higher miR-144 expression (**Figure 3**a) and lower miR-126 expression (**Figure 3**b) ($p < 0.05$). Patients with more than one SLU simultaneously presented higher miR-144 (**Figure 3**c) and miR-199a-5p (**Figure 3**d) expression ($p < 0.05$). In addition, SLU recurrence between 4 to 7 episodes presented higher miR-199a-5p expression (**Figure 3**e) and lower miR-126 expression (**Figure 3**f) ($p < 0.05$). SLU with sick edges were associated with higher miR-199a-5p (**Figure 3**g) and miR-144 (**Figure 3**h) expression ($p < 0.05$).

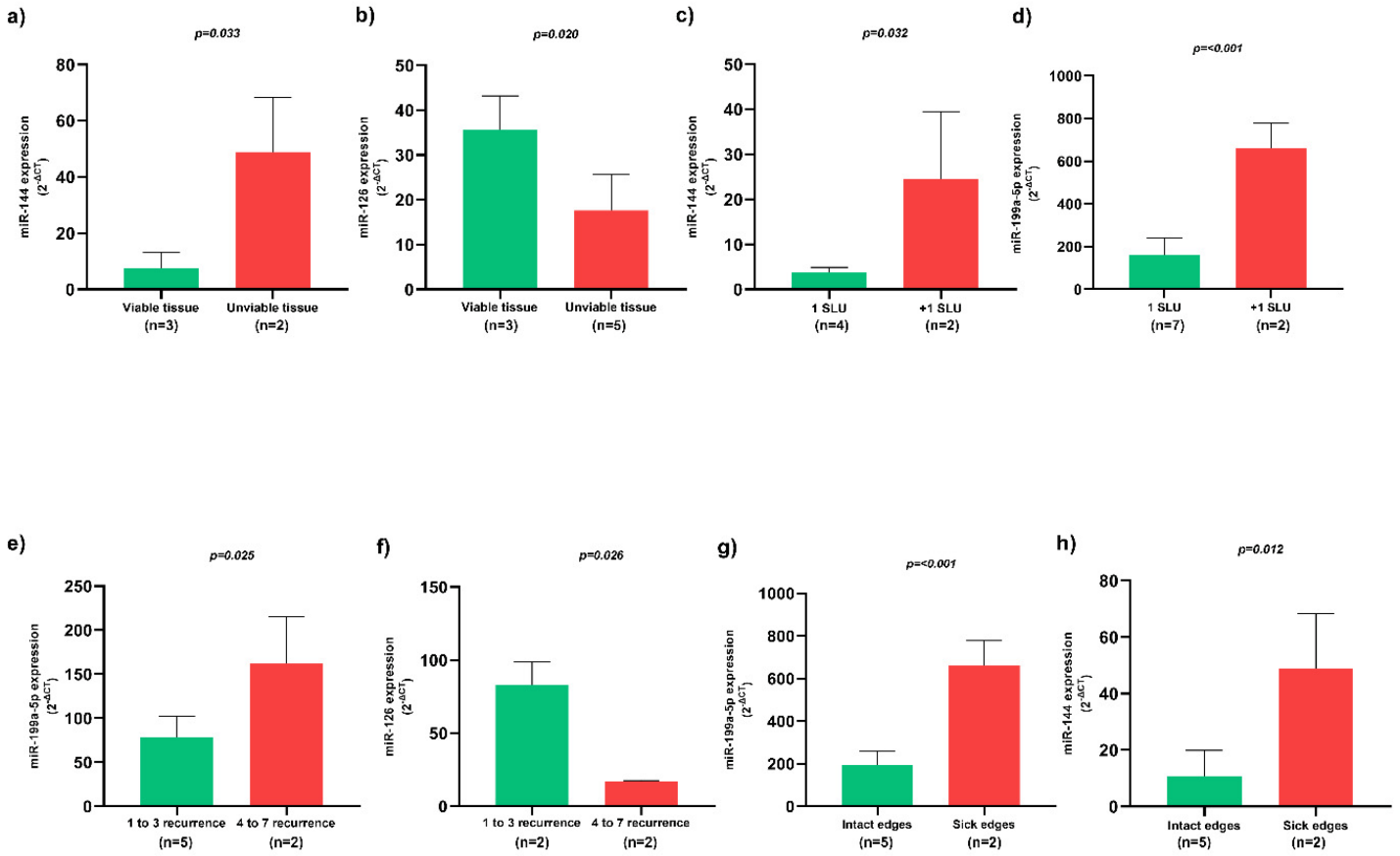


Figure 3. Association of circulating miRNAs and clinical characteristics of active SLU. (**a**) Expression of circulating miR-144 in SLU with viable and unviable tissues; (**b**) Expression of circulating miR-126 in SLU with viable and unviable tissues; (**c**) Expression of circulating miR-144 in patients with one and more than one SLU; (**d**) Expression of circulating miR-199a-5p in patients with one and more than one SLU; (**e**) Expression of circulating miR-199a-5p in patients with multiple SLU recurrence; (**f**) Expression of circulating miR-126 in patients with multiple SLU recurrence; (**g**) Expression of circulating miR-199a-5p in SLU with intact and sick edges; (**h**) Expression of circulating miR-144 in SLU with intact and sick edges. SLU, sickle leg ulcers; miR, miRNA. 1 SLU, one sickle leg ulcer; +1 SLU, more than one sickle leg ulcer; *p*-value obtained using independent *t*-test.

5. Association of HU with Circulating miRNAs Expression in Patients with and without SLU

Two MLR models were performed with HU as dependent variable. In SLU- patients, miR-199a-5p and miR-126 expression were independently associated with HU (R^2 = 0.486; $p < 0.05$), while in SLU+ patients only miR-126 expression presented independent association (R^2 = 0.779; $p < 0.05$) (**Table 1**).

Table 1. Multivariate linear regression models of hydroxyurea in association with confounding variables.

	Independent Variables	Dependent Variable	*p*-Value	β	R^2	*p*-Value of the Model
SLU- patients N = 43	Circulating miR-199a-5p	**Hydroxyurea**	**0.007**	0.695	**0.486**	**0.022**
	Circulating miR-144		0.096	0.468		
	Circulating miR-126		**0.009**	−0.834		
SLU+ patients N = 15	Circulating miR-199a-5p		0.485	0.191	**0.779**	**0.043**
	Circulating miR-144		0.997	−0.001		
	Circulating miR-126		**0.011**	−0.885		

SLU-, patients without sickle leg ulcers; SLU+, patients with active sickle leg ulcers or previous history; miR, microRNA; R^2: coefficient of determination; β: coefficient of regression; Bold p-values indicate significance at p < 0.05.

6. Target Gene Prediction with Biological Processes of miR-199a-5p to SCD Patients

The results demonstrate interaction networks of miR-199a-5p and target genes using miRWalk analysis (**Figure 4**a). Correlation between level significance by the score for these genes is presented (**Figure 4**b), as well as biological processes (**Figure 4**c).

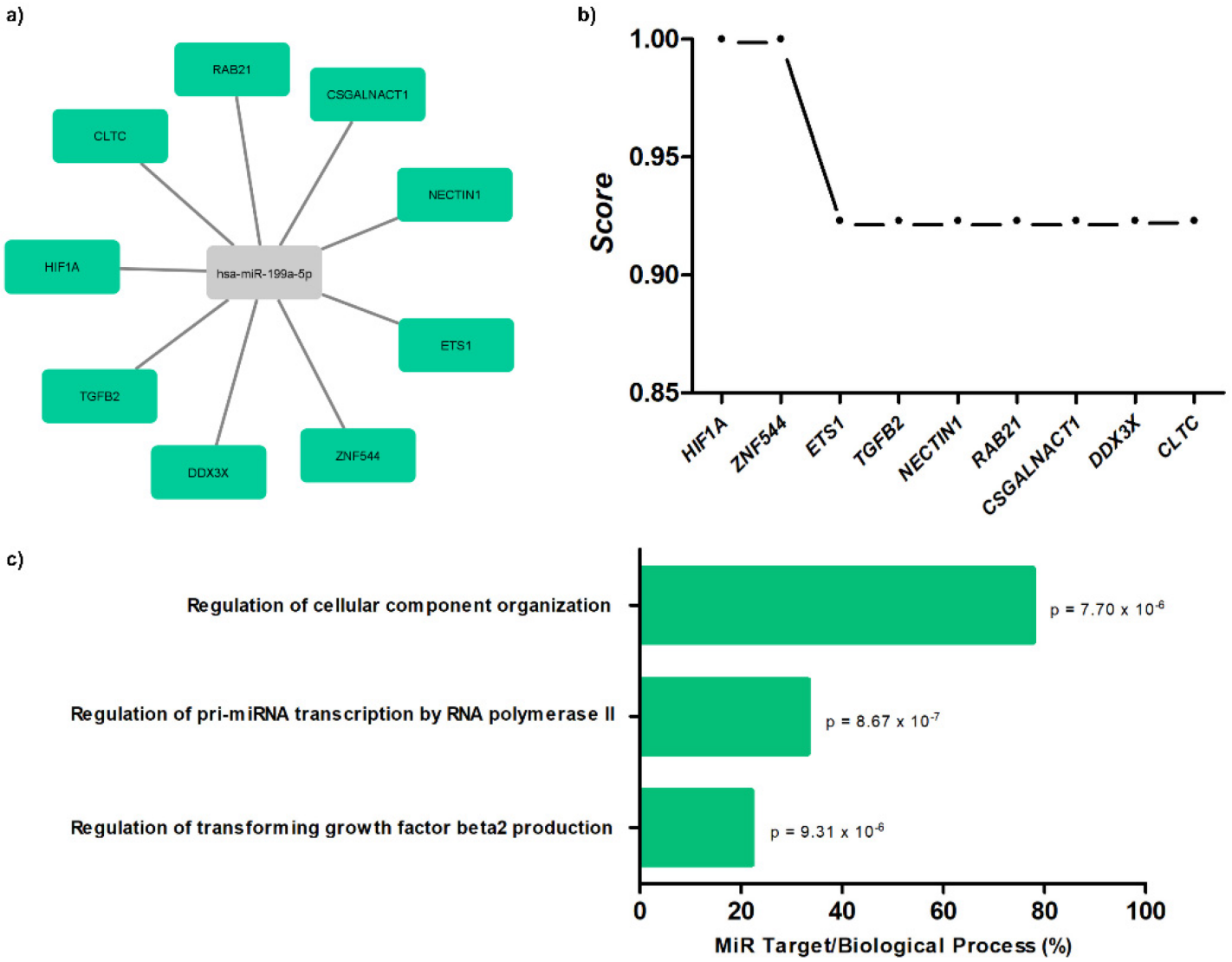


Figure 4. Target gene prediction with biological processes of miR-199a-5p. (**a**) Interaction networks of miR-199a-5p and target genes using miRWalk analysis; (**b**) correlation between level significance by score for genes; (**c**) biological processes for miR-199a-5p with *p*-values.

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