Experimental Cell Research 339 (2015) 360–366

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Research Article

Aberrant miR-21 and miR-200b expression and its pro-fibrotic potential in hypertrophic scars

Renpeng Zhou [a], Qi Zhang [b], Yun Zhang [c], Shibo Fu [a], Chen Wang [a,*]

[a] Department of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhi Zao Ju Road, Shanghai 200011, China
[b] Department of Burn and Plastic Surgery, People's Hospital of Dancheng County, 42 Ren Min Road, Zhoukou City, Henan Province 477150, China
[c] Institute of Arthritis Research, Shanghai Academy of Chinese Medical Sciences and Guanghua Integrative Medicine Hospital, China

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form
10 October 2015
Accepted 19 October 2015
Available online 20 October 2015

Keywords:
Hypertrophic scar
miR-21
miR-200b
TGF -β1
Fibrosis

ABSTRACT

The post-traumatic hypertrophic scar (HS) is a fibrotic disease with excessive extracellular matrix (ECM) production by fibroblasts in response to tissue injury. Although dysregulation of miRNAs is known to be involved in a variety of pathophysiologic processes, the role of miRNA in hypertrophic scar formation is unclear. Abnormal expression of miRNA in fibrosis has been investigated in several studies. The transforming growth factor β1 (TGF-β1) promotes fibroblasts proliferation, the synthesis of collagen and other extracellular matrix, and ultimately leads to the formation of the HS by inducing excessive deposition of ECM. We identified two miRNAs whose expression was correlated with fibrotic diseases: miR-21 and miR-200b. This study further confirmed that after stimulation with TGF-β1, the expression of miR-21 was increased, whereas the mRNA level of SMAD7 was decreased in fibroblasts. TGF-β1 reduced the expression of miR-200b, while it augmented that of Zinc finger E-box-binding homeobox 1(Zeb1). Our experiments demonstrated that the expression of miR-21 and miR-200b are related to a disorder, and the TGF-β/miR-21/Smad7 and TGF-β/miR200b/Zeb1 pathways might participate in the pathogenesis of HS. Thus, a novel, beyond the traditional methods, approach for HS treatment via miRNA therapeutics could have been provided.

1. Introduction

Hypertrophic scar (HS) is a dermal fibroproliferative disorder which results from a deep thermal or traumatic injury of the skin. It is characterized by excessive extracellular matrix (ECM) deposition (Fig. 1 A and B) [1].

MicroRNAs (miRNAs) are short 21–23-nucleotide-long noncoding RNAs, which bind to the 3' untranslated regions (UTRs) of target genes and regulate gene expression by translational repression or mRNA degradation [2]. Aberrant expression of miRNAs promoted several pathologic processes, including the fibrosis and ECM metabolism in organs, such as the heart, kidneys, liver, and lungs [3]. However, the function of miRNAs in HS remain still poorly understood. miR-21 expression was upregulated selectively in fibroblasts in failing hearts and promoted interstitial fibrosis and cardiac hypertrophy [4], and the altered miR-21 expression could play an important role in multiple critical signaling events involved in pulmonary fibrosis [5]. Another microRNA, miR-200b was also reported attenuate the fibrosis in Crohn's disease and pulmonary disorders [6,7]. A recent study revealed that Zeb1, the target of miR-200b, was not only overexpressed in the alveolar epithelial cells of pulmonary fibrosis patients, but was also related to a higher fibrosis score [8]. Thus, the altered miRNAs expression is favorable for fibrotic tissue formation and has attracted increasingly attention as a new target for gene therapy. However, the miRNAs regulation and their molecular mechanism of action in hypertrophic scar formation remain unknown.

TGF-β1 was shown to exert a critical influence in the production and deposition of collagen [9], and was also identified as the molecular target for antifibrotic therapy. Although the interactions between TGFβ1 and miR-21 in carcinoma-associated fibroblasts (CAF) formation have been studied [10], their expression and interaction in hypertrophic scar fibroblasts has not yet been investigated. Therefore, determining the roles of specific miRNAs participating in the pathogenesis of fibrosis (especially in the TGF-β signaling pathway) contributes to the elucidation of the pathogenesis of HS more clearly and to the development of novel methods for the diagnosis and treatment of HS.

Therefore, in this examination, we first analyzed the aberrant miRNAs expression in hypertrophic scar tissues, and then we investigated the interaction between the altered miRNAs and TGF-β1 in fibroblasts.

* Corresponding author. Fax: +86 21 23271699.
E-mail address: wangchen2369@163.com (C. Wang).

http://dx.doi.org/10.1016/j.yexcr.2015.10.018
0014-4827/

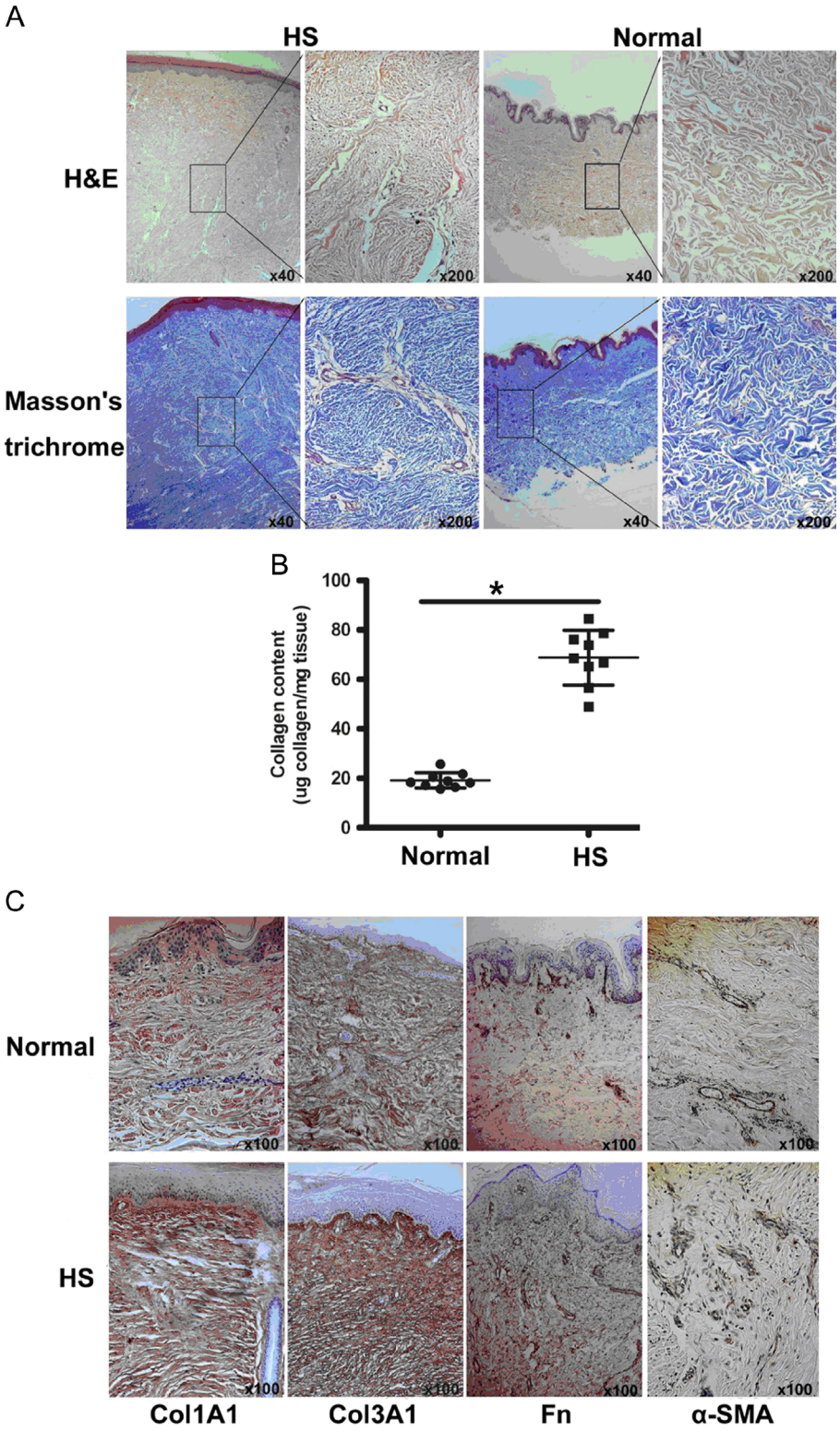

Fig. 1. H&E staining and Masson's trichrome staining for collagen. HS was characterized histopathologically by excessive and disorganized deposition of collagen. Scale bar = 100 μm. (A). Collagen content in the specimens was determined; all results are given as ug collagen/mg tissue (dry weight) based on a conversion factor of 7.46 ($n = 5$); (B). The immunohistochemical analyses of the skin and HS tissues showed upregulation of Col1A, Col3A, Fn, and α-SMA in HS tissues; (C). * indicated $P < 0.05$. HS: hypertrophic scar. Normal: the normal skin tissue.

2. Materials and methods

2.1. Patients, resected specimens, and cell culture

All nine subjects signed the informed consent approved by the Ethic Committee of Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine. The patients met the classification criteria for scar assessment of "Patient reported facial scar assessment: directions for the professional (POSAS)" [11] and were further characterized as hypertrophic scars. Hypertrophic scar specimens were obtained from nine patients (Table 1) recovering from thermal injury who developed