Review Article

MicroRNA in keloid pathogenesis

Hajime Shimizu[1], Ramin Ghazizadeh[2], Mohammad Ghazizadeh[3]*

[1]*Department of Molecular Pathology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, Kawasaki, Japan*
[2]*Podiatric Medicine, Weiss Memorial Hospital, Chicago, Illinois, USA*
[3]*World Academy of Anti-aging and Regenerative Medicine, Imperial Tower, Tokyo, Japan*

Abstract. Keloid is a fibro-proliferative lesion that develops as a result of abnormal wound healing in susceptible individuals. Many factors such as skin tension, wound infection, racial difference and genetic predisposition have been implicated in the etiology of keloid. MicroRNAs are highly conserved noncoding small RNA sequences and are key posttranscriptional gene regulators that contribute to the maintenance of differentiated cell phenotype. In this review, we outline the results of the studies on the expression of various miroRNAs in keloid as well as their role in pathogenesis of this lesion.

Keywords: MicroRNA, keloid fibroblast, normal dermal fibroblast, mechanism, pathogenesis, type I procollagen

Keloid is a fibro-proliferative lesion characterized by excessive collagen deposition. It develops as a result of abnormal wound healing in susceptible individuals. Keloid is defined as a scar within the skin that grows beyond the confines of original wound. Many factors such as skin tension, wound infection, racial difference and genetic predisposition have been implicated in the etiology of keloid lesions [1]. They are characterized by overproduction of extracellular matrix (ECM) and invasiveness beyond the confines of original wound [2]. Excess deposition of ECM as collagen by fibroblasts is responsible for keloid but the etiology and mechanism are unknown still now.

A number of treatment modalities have been employed to overcome keloid. In general, surgical excision alone has proved unsatisfactory because of a recurrence rate of 45-100% [3]. This has led to the development of various adjuvant therapies including radiotherapy [4]. Recently, early postoperative electron beam (EB) irradiation has proved to be a well-tolerated and effective method in reducing the recurrence rate of keloid [5].

Furthermore, the molecular mechanisms behind the effect of EB irradiation in reducing keloid recurrences revealed that the involvement of the interleukin 6 (IL-6) synthesis and ECM gene expression in keloid was inhibited in response to EB irradiation [5]. However, continuous efforts are needed to explore more effective and less hazardous modes of therapy and prophylaxis for this lesions. Understanding the mechanisms behind the development of the keloid scar is highly desirable in order to establish such approaches. We determined a functional role of IL-6 signaling in keloid scars. Keloid fibroblasts (KF) and counterpart normal fibroblasts (NF) were subjected to induction or inhibition of IL-6 or its specific receptor IL-6 receptor alpha and detection of their effects on ECM gene expression [6]. It was concluded that IL-6 plays a pivotal role in the developmental mechanism(s) of keloid.

MiRNAs, highly conserved noncoding small RNAs of 19 to 26 nucleotides, are key posttranscriptional gene regulator that contribute to the maintenance of differentiated cell phenotype [7]. Mammalian miRNAs usually bind to the 3' UTR of target mRNAs, promoting mRNA degradation and/or inhibiting translation of the protein-code genes [8]. In mammals, miRNAs are predicted to control the activity of approximately 30% of all protein-coding genes and have been shown to participate in the regulation of almost every cellular process. It is also highly likely that mechanisms that control translation initiation will have a significant impact on miRNAs-regulated gene expression [9]. Recently, some miRNAs have been reported to participate in fibrosis and ECM metabolism. A number of miRNAs, such as the miR-21 and miR-29 families are emerging as common regulators of fibrosis and that directly target the translation of ECM components [10-14]. MiR-21 ratios are selectively increased in fibroblasts of the failing heart by the extent of interstitial fibrosis and cardiac hypertrophy. These findings

---

reveal that miR-21 as a disease target and antagomiR-21 as a therapeutic target in heart failure in mice model [14]. Administration of miR-21 antisense probes diminished the severity of experimental lung fibrosis in mice with bleomycin-induced fibrosis [15]. In addition, comparison of different miR-21 inhibitor as 8-mer or 22-mer oligonucleotide, treatment with 22-mer anti-miR-21 is ineffective in preventing cardiac disease in a mouse model [16]. Thus, miRNAs have important roles which suggest a new approach using miRNA therapeutics in fibrotic diseases. The mechanisms behind keloid pathogenesis remains unclear.

In a study, we attempted to clarify the differential analysis of miRNA expression in primary cell culture from KFs and NFs using miRNA microarray and then explored the function of miR-10a which showed the lowest expression level in KFs compared with NFs (unpublished data). We confirmed that miR-10a was statistically significantly under-expressed in KFs compared with NFs. Suppression and activation study of miR-10a by microRNA inhibitor and retinoic acid in KFs and NFs from the culture supernatant revealed that IL-6 and procollagen type I (PICP) secretions were significantly inhibited by miR-10a inhibitor and activated by retinoic acid (RA) treatment. KF and NF derived cells were transfected with microRNA oligonucleotide mimic and inhibitor for miR-10a. The secretion of PICP was reduced in miR-10a transfected KFs and NFs. HOXA1 was expressed at significantly higher levels in KFs compared to NFs. In RA treatment experiments, HOXA1 was increasing in KF at lower levels, but in miR-10a inhibitor treatment, HOXA1 expression was significantly inhibited. Downregulation of miR-10a was consistent with direct HOXA1 dependent regulation. MiR-10a expression was inversed to that of HOXA1 expression. We found that HOXA1 is a direct target of miR-10a. On the RA treatment, miR-10a was significant overexpressed in KFs and NFs. Treated fibroblasts in each well were harvested for miR-10a qPCR assay and the culture supernatant in each well was analyzed for IL-6 and PICP secretion by ELISA method. These ELISA assays indicated that IL-6 concentrations were markedly increased in KFs compared with NFs in RA treatment. The molecular mechanisms of RA-mediated transcription regulation is not well-defined and little is known about the influence of RA signaling on miRNA regulation. Our own data demonstrate MiR-10a regulates the secretion of collagen synthesis in KFs and NFs. The secretion of type I collagen was reduced in miR-10a transfected KFs and NFs. Especially, remarkably reduction was shown in miR-10a transfected KFs. Downregulation of miR-10a may be one of the mechanisms by which collagens are highly deposited in keloid tissues. These results suggested that miR-10a regulates the secretion of collagen synthesis in KFs and NFs. We concluded that miR-10a may be a promising new effective strategy for targeting keloid lesions.

Keloid scaring develops as a result of abnormal wound healing that grows beyond the wound margins and exhibits inflammation, fibroblastic cell proliferation, and accumulation of excessive collagen deposits. Our previous study have shown that IL-6 secretion is significantly increased in KF compared to NF. IL-6 induces collagen synthesis in fibroblasts. Several downstream targets including JAK1, ATAT3, RAF1, and ELK1 are upregulated at mRNA and protein levels in KFs compared to NFs. In the IL-6 signaling pathway are upregulated at transcriptional and translational levels [6]. It is possible that deregulation of the IL-6 signaling pathway might represent a common molecular mechanism that contribute to the pathogenesis in keloid. From a therapeutic point of view, the appreciation of dysfunction of IL-6 signaling as the underlying molecular mechanism of keloid immediately suggests that stabilization of IL-6 activity would have a potential therapeutic impact on keloid [17]. We have also shown postoperative adjuvant electron beam (EB) irradiation as successful tool to reduce keloid recurrences. The molecular mechanism behind the effect of EB irradiation might hinder keloid formation by regularizing disturbances in the homeostatic equilibrium between inducer and inhibitor activities in the matrix system most likely through the IL-6 pathway [5].

Recent evidence has shown that miRNA-21 had the highest fold change (6.87-fold) and miRNA-203 had the lowest expression level in keloid samples [18]. Another report demonstrated that downregulation of miRNA-196a may be one of the mechanisms by which collagens are highly deposited in keloid tissues [19]. MiRNA-19a/b played important role in the regulation of IL-6 and MMP3 release in rheumatoid fibroblast-like synoviocytes [20].

Similar to mRNA expression, miRNA expression has been found to be dysregulated in disease tissues in comparison with normal tissues. These dysregulated miRNA represent a novel pool of therapeutic targets and biomarkers, including those in tissue fibrosis. For example, the miR-29 family of miRNA is down-regulated in a mouse model of cardiac fibrosis following myocardial infarction [21]. In comparison with the normal skin tissues, miRNAs were aberrantly expressed in limited cutaneous scleroderma and diffuse cutaneous scleroderma skin tissues. MiRNAs whose expressions were correlated with systemic sclerosis fibrosis; miR-21, Mir-31, miR-146, miR-503, miR-145, and miR-29b were predicted to be involved [22].

The miRNAs, miR-10a and miR-10b, are close homologs, differing by a single central nucleotide only. The miR-10a interacts with the 5' untranslated region of mRNAs encoding ribosomal proteins to enhance their translation [23]. Down regulation of miR-10a may increase upstream stimulatory factor 2 and contribute to the increase in cell proliferation of CML. Implicating a miRNA in the abnormal behavior of CML [24]. MiR-10a is sharply down-regulated during megakaryocytic differentiation [25]. Significant low endothelial expression of miR-10a detected in regions of athero-susceptibility was investigated by a combination of genomic profiling, miRNA manipulations and molecular analyses in freshly isolated arterial endothelium and in cultured cells [26]. On the contrary, miR-10b is highly expressed in metastatic breast tumors initiate robust invasion and metastasis. The miR-10b induced by the transcription factor Twist

proceeds to inhibit translation of the messenger RNA encoding homeobox D10, resulting in increased expression of a well-characterized pro-metastatic gene, RHOC. Significantly, the level of miR-10b expression in primary breast carcinomas correlates with clinical progression [27]. A close relative of miR-10b, miR-10a has been recently reported to target HOX1, a gene that plays an oncogenec role in human mammary carcinoma cells [28], indicating that miR-10a might have an opposite rather than similar function in breast cancer. Homeobox A1 (HOXA1) is an experimentally validated downstream target of miR-10a, as shown by direct HOXA1 3′ UTR-dependent suppression during megakaryocytopoiesis.

During human megakaryotic differentiation, the main finding was down-regulation of miR-10a. Hypothetically, the down-regulation of microRNAs unblocks target genes involved in differentiation. MiR-10a expression is inverse to that of HOXA1, and we showed that HOXA1 is a direct target of miR-10a [25]. HOXA1 was expressed at significantly higher levels in KFs compared to NFs. In RA treatment, HOXA1 was increasing in KF at lower levels, but miR-10a inhibitor treatment, HOXA1 expression was significantly inhibited. Down-regulation of miR-10a consistent with direct HOXA1 dependent regulation.

On the RA treatment, little is known about the influence of RA signaling on microRNA regulation. Up-regulation of miR-10b was previously reported in RA-induced neuronal differentiation of human embryonal carcinoma NT2/D1 cells [29], and an increase in miR-10a could be detected during RA-induced differentiation of mouse embryonic stem cell into smooth muscle cells [30]. MiR-10a is a key mediator of metastatic behavior in pancreatic cancer. Inhibition of miR-10a expression (with retinoic acid receptor antagonists) or function (with specific inhibitor) is a promising attracting point for anti-metastatic therapies [31]. New technologies are emerging that utilize artificial miRNA target sites to exploit or inhibit endogenous miRNA regulation. This approach has been used to improve cell-specific targeting for gene and stem cell therapy studies and for animal transgenics and also to reduce the toxicity of oncolytic viruses and to attenuate viral vaccines [32].

Whereas microRNA-21 regulates the ERK-MAP kinase signaling pathway in cardiac fibroblasts, which has impacts on global cardiac structure and function, miR-21 levels are increased selectively in fibroblasts of the failing heart, augmenting ERK-MAP kinase activity through inhibition of sprout homologue 1. This mechanism regulates fibroblast survival and growth factor secretion, apparently controlling the extent of interstitial fibrosis and cardiac hypertrophy. Silencing of miR-21 by specific antagomir reduces cardiac ERK-MAP kinase activity, inhibits interstitial fibrosis and attenuates cardiac dysfunction [14].

If the future proves that miRNAs are major players in inflammatory disorders, a new generation of RNA drugs could include small antisense RNAs that antagonize miRNA function [33]. Such antagomirs can be synthetically made [34] or encoded by DNA [35] and may specifically target an overexpressed miRNA. As an example of such strategy, stabilized RNA antagomirs has been recently followed up by the use of miR-10b-directed antagomirs in cancer treatment including anti-metastatic therapy [36]. Downregulation of miR-10a may be one of the mechanisms by which collagens are highly deposited in keloid tissues. In this regard, both miR-10a inhibitors and RA receptor antagonists are promising effective strategy for targeting keloid therapies.

Conclusion

In conclusion, microRNAs regulate gene expression and modulate critical processes involved in keloid development. In keloid, a number of microRNAs act as pro-fibrotic molecules and their upregulation induce fibroblast proliferation and collagen synthesis. Others have anti-fibrotic function and their downregulation promote fibroblast proliferation and collagen synthesis. Thus a balance between pro-fibrotic and anti-fibrotic microRNAs has to be maintained in order to prevent keloid formation. Various inflammatory processes involving IL-6 signaling, TGF-beta signaling, ECM deposition, fibroblast proliferation and differentiation, and EMT play important role in keloid pathogenesis. Therapeutic strategies should consider methods to upregulate anti-fibrotic miRNAs or downregulate pro-fibrotic miRNAs or both in the management of keloid.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Ma S, Chang WP, Fang RH. Measurement of radiation-induced DNA double-strand breaks in human diploid fibroblasts from keloid and normal skin by single-cell gel electrophoresis. Plast Reconstr Surg 5:821-826, 1996.

2. Syed F, Ahmadi E, Iqbal SA, Singh S, McGrouther DA, Bayat A. Fibroblasts from the growing margin of keloid scars produce higher levels of collagen I and III compared with intralesional and extralesional sites: clinical implications for lesional site-directed therapy. Br J Dermatol 164:83-96, 2011.

3. Berman B, Bleley HC. Adjunct therapies to surgical management of keloids. Durmatol Surg 2282:126-130, 1996.

4. Ragoowansi R, Cormer PG, Moss AL, Gless JP. Treatment of keloids by surgical excision and immediate postoperative single-fraction radiotherapy. Plast Reconstr Surg 111:1853-1859, 2003.

5. Tosa M, Ghazizadeh M, Shimizu H, Hirai T, Hyakusoku H, Kawanami O. Global gene expression analysis of keloid fibroblasts in response to electron beam irradiation reveals the involvement of interleukin-6 pathway. J Invest Dermatol 124:704-713, 2005.

6. Ghazizadeh M, Tosa M, Shimizu H, Kawanami O. Functional implications of the IL-6 signaling pathway in keloid pathogenesis. J Invest Dermatol 127:98-105, 2006.

7. Ambrous V. The functions of animal microRNAs. Nature 16,431: 350-355, 2004.

8. Bartel DP. MicroNAs: Target recognition and

regulatory functions. Cell 136:215-233, 2009.

9. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 9: 102-114, 2008.

10. Van Rooiji E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Maeshall WS, Hill JA, Oison EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. PNAS 105: 13027-13032, 2008.

11. Jiang X, Tsitsiou E, Herrick SE, Lindsay MA. MicroRNAs and the regulation of fibrosis. FEBS J 277: 2015-2021, 2010.

12. Maurer B, Stanczyk J, Jüngel A, Akhmetshina A, Trenkmann M, Brock M, Kowal-Bielecka O, Gay RE, Michel BA, Distler JH, Gay S, Distler O. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. Arthritis Rheum 62(6):1733-1743, 2010.

13. Rodenburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, Janssen J, Koppe C, Knolle P, Castoldi M, Tacke F, Trautwein C, Luedde T. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology 53:209-218, 2011.

14. Thum T, Gross C, Fiedler J, Fischer T, Kissler S. et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signaling in fibroblasts. Nature 18,456:980-984, 2008.

15. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J Exp Med 207:1589-1597, 2010.

16. Thum T, Chau N, Bhat B, Gupta SK, Linsley PS, Bauersachs J, Engelhardt S. Comparison of different miR-21 inhibitor chemistries in a cardiac disease model. J Clin Invest 121:461-462, 2011.

17. Ghazizadeh M. (2007) Essential role of IL-6 signaling pathway in keloid pathogenesis. J Nippon Med Sch 74:11-22, 2007.

18. Liu Y, Yang D, Xiao Z, Zhang M. miRNA expression profiles in keloid tissue and corresponding normal skin tissue. Aesthetic Plast Surg 36:193-201, 2012.

19. Kashiyama K, Mitsutake N, Matsuse M, Ogi T, Saenco VA, Ujifuku K, Utani A, Hirano A, Yamashita S. (2012) miR-196a downregulation increases the expression of type I and III collagens in keloid fibroblasts. J Invest Dermatol 132:1597-1604, 2012.

20. Phillippe L, Alsaleh G, Suffert G, Meyer A, Georgel P, Sibilla J, Wachsmann D, Pfeffer S. TLR2 expression is regulated by microRNA miR-19 in rheumatoid fibroblast-like synoviocytes. J Immunol 188:454-461, 2012.

21. Chau BN, Brenner DA.What goes up must come down: the emerging role of microRNA in fibrosis. Hepatology 53:4-6, 2011.

22. Zhu H, Li Y, Qu S, Luo H, Zhou Y, Wang W, Zhao H, You Y, Xiao X, Zuo X. MicroRNA expression abnormalities in limited cutaneous scleroderma and diffuse cutaneous scleroderma. J Clin Immunol 32(3): 514-22, 2012.

23. Ørom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 30:460-471, 2008.

24. Agirre X, Jiménez-Velasco A, San José-Enériz E, et al. Down-regulation of hsa-miR-10a in chronic myeloid leukemia CD34+ cells increases USF2-mediated cell growth. Mol Cancer Res 6:1830-1840, 2008.

25. Garzon R, Pichiorri F, Palumbo T, Luliano R, Cimmino A, Aqeilan R, Volinia S, Bhatt D, Alder H, Marcucci G, Calin GA, Liu CG, Bloomfield CD, Andreeff M, Croce CM. MicroRNA fingerprints during human megakaryocytopoiesis. Proc Natl Acad Sci 103: 5078-5083, 2006.

26. Fang Y, Shi C, Manduchi E, Civelek M, Davies PF. MicroRNA-10a regulation of proinflammatory phenotype in athero-susceptible endothelium in vivo and in vitro. Proc Natl Acad Sci 107:13450-13455, 2010.

27. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449:682-688, 2007.

28. Zhang X, Zhu T, Chen Y, Mertani HC, Lee KO, Lobie PE. Human growth hormone-regulated HOXA1 is a human mammary epithelial oncogene. J Biol Chem 278:7580-7590, 2003.

29. Lee NS, Kim JS, Cho WJ, Lee MR, Steiner R, Gompers A, Ling D, Zhang J, Strom P, Behlke M, Moon SH, Salvaterra PM, Jove R, Kim KS. MiR-302b maintains "stemness" of human embryonal carcinoma cells by post-transcriptional regulation of Cyclin D2 expression. Biochem Biophys Res Commun 377:434-440, 2008.

30. Huang H, Xie C, Sun X, Ritchie RP, Zhang J, Chen YE. MiR-10a contributes to retinoid acid-induced smooth muscle cell differentiation. J Biol Chem 285: 9383-9389, 2010.

31. Weiss FU, Marques IJ, Woltering JM, Vlecken DH, Aghdassi A, Parteckle LI, Heidecke CD, Lerch MM, Bagowski CP. Retinoic acid receptor antagonists inhibit miR-10a expression and block metastatic behavior of pancreatic cancer. Gastroenterogy 137:2136-2145, 2009.

32. Brown BD, Naldini L. Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. Nat Rev Genet 10: 578-585, 2009.

33. Bak RO, Mikkelsen JG. Regulation of cytokines by small RNAs during skin inflammation. J Biomed Sci 17:53, 2010.

34. Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with 'antagomirs'. Nature 438:685-689, 2005.

35. Scherr M, Venturini L, Battmer K, Schaller Schoenitz M, Schaefer D, Dallmann I, Ganser A, Eder M. Lentivirus-mediated antagomir expression for specific inhibition of miRNAfunction. Nucleic Acids Res 35:e149, 2007.

36. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson E, Teruya-Feldstein J, Bell GW, Weinberg RA. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol 28:341-347, 2010.