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ORIGINAL ARTICLE

MicroRNA-346 functions as an oncogene in cutaneous squamous cell carcinoma

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Abstract Cutaneous squamous cell carcinoma (cSCC) is an epidermal keratinocyte-derived skin tumor, which is the second most common skin cancer in the general population. Recently, studies showed that microRNAs (miRNAs) played an important role in the development of cancer. In our study, we showed that the expression of SRCIN1 was lower in cSCC tissues than in the matched normal tissues. Moreover, there was significant inversed correlation between miR-346 and SRCIN1 in cSCC tissues. The luciferase reporter assay data showed that miR-346 can target the SRCIN1 message via the 3′-untranslated region (UTR) of SRCIN1. Overexpression of miR-346 inhibited the messenger RNA (mRNA) and protein expression of SRCIN1 in the A431 cells. In addition, ectopic expression of miR-346 promoted the A431 cell proliferation and migration. Meanwhile, SRCIN1 overexpression inhibited the A431 cell proliferation and migration. Rescue experiment has showed that SRCIN1 overexpression reduced the miR-346-induced A431 cell proliferation and migration. Herein, this study may provide miR-346 as a new therapeutic target for cSCC.

Keywords Cutaneous squamous cell carcinoma · miR-346 · SRCIN1 · Oncogene

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Introduction

Cutaneous squamous cell carcinoma (cSCC) is an epidermal keratinocyte-derived skin tumor, which is the second most common skin cancer in the general population [1–3]. The incidence of cSCC was increased over the last decades [4–6]. The risk factors of cSCC include endogenous genetic factors and exogenous factors such as chemical carcinogens, sun exposure, ionizing radiation, and other environmental factors [2, 7–9]. cSCC has a substantial risk of metastasis and was responsible for approximately 20 % of skin cancer-related deaths [10–12]. Therefore, it is important to understand the molecular mechanism of cSCC for the early diagnosis and treatment of cSCC.

Small noncoding microRNAs (miRNAs) are a class of 22-nucleotide noncoding RNAs that silence protein expression by either translational inhibition or messenger RNA (mRNA) degradation [13–16]. More and more evidences showed that miRNAs play pivotal roles in a wide array of biological processes, including cell development, proliferation, differentiation, invasion, and apoptosis [17–20]. Emerging evidence strongly suggests that deregulated miRNA expression is a common and important feature of various cancers including renal cell carcinoma, gastric cancer, breast cancer, ovarian carcinoma, rhabdomyosarcoma, and uveal melanoma

[21–27]. miRNAs can function as tumor suppressors or oncogenes in these cancers [28–31]. So far, the functions of cSCC-related miRNAs were still not clear.

Previous studies demonstrated that miR-346 played a crucial role in anti-inflammatory pathways [32–34]. The expression of miR-346 was increased in rheumatoid inflammation patients, exerting anti-inflammatory effects [35]. Xiao et al. [36] showed that miR-346 was upregulated in podocytes and participated in the epithelial–mesenchymal transition. However, the role of miR-346 in cSCC was still unknown. In this study, we demonstrated that the expression of miR-346 was higher in cSCC tissues than in the matched normal tissues. Ectopic expression of miR-346 promoted the cSCC cell proliferation and migration through directly targeting SRCIN1.

Materials and methods

Ethics statement

Our study was approved by the Ethical Review Committee of Shanghai Ninth People's Hospital. All patients have written informed consent.

Clinical samples and cell culture transfections

cSCC tissues and adjacent normal tissues were collected from cSCC patients in our hospital. The CSCC line (A431) was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine

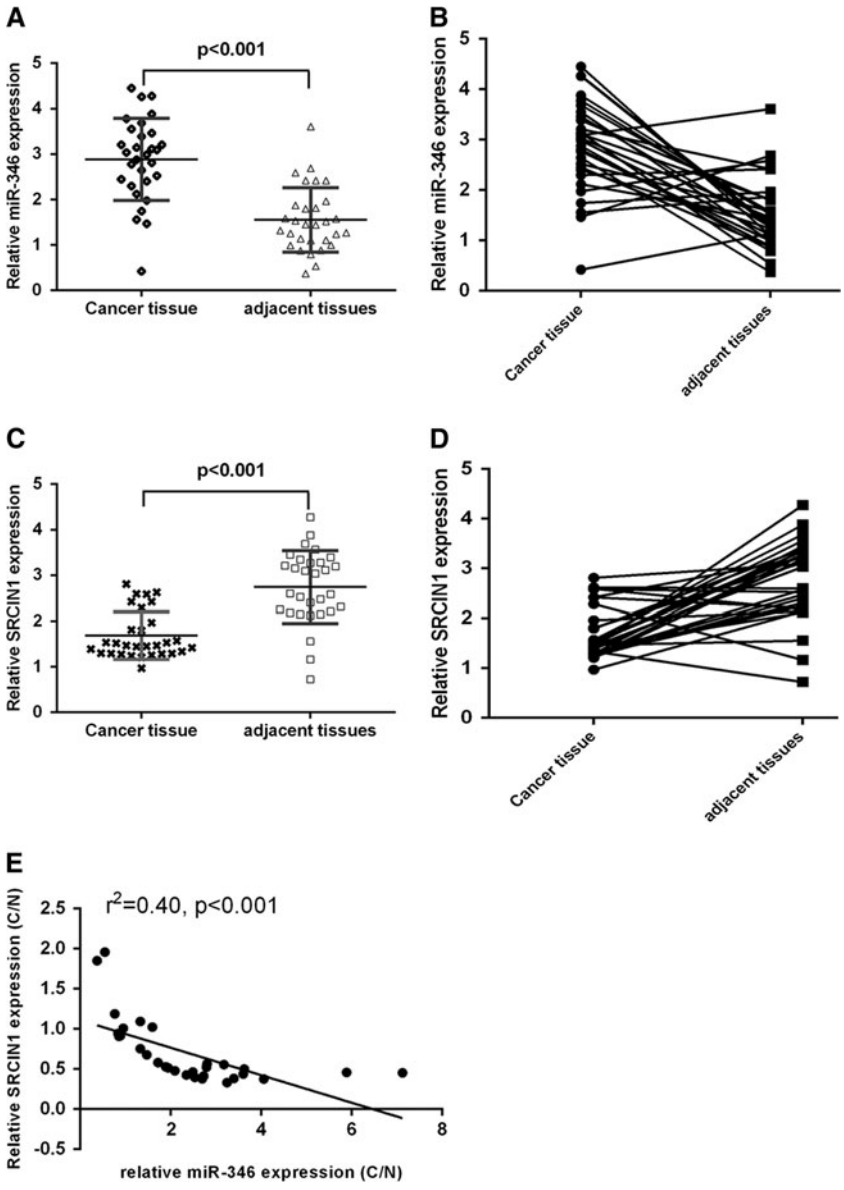

Fig. 1 The expression of miR-346 was upregulated and was inversed with the expression of SRCIN1 in cSCC. **a** The expression of miR-346 was higher in cSCC tissues than in the matched normal tissues. **b** miR-346 expression was increased in 77 % (23/30) of the patients with cSCC tissues compared to the matched normal tissues. **c** The expression of SRCIN1 was measured by qRT-PCR. **d** SRCIN1 expression was increased in 80 % (24/30) of the patients with cSCC tissues compared to the matched normal tissues. **e** There was significant correlation between miR-346 and SRCIN1 in cSCC tissues

serum. miR-364 mimic and scramble is synthesized from GenePharma (Shanghai, China). Cell transfections were transfected using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol.

Quantitative real-time PCR

Total RNA of tissues or cells was extracted using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed using the SYBR Green (TaKaRa, Dalian, China) in the ABI 7900HT thermocycler (Applied Biosystems). The primer sequences used were as follows: SRCIN1 (forward: AGCCCCGACAAAAGCAAAC; reverse: CCAAAGGAAGTCAATACAGGGATAG) and GAPDH (forward: TTGCCATCAATGACCCCTTCA; reverse: CGCCCCACTTGATTTTGGA). U6 and GAADH were used as an internal control.

Migration and proliferation assay

Wound healing assay was used to measure cell migration. A wound was done using a 1000-ll micropipette tip when cell confluence. Photographs were obtained immediately after 36 or 48 h. CCK-8 (Dojin Laboratories, Japan) was carried out to measure the cell proliferation at 0, 24, 48, and 72 h after transfection. Absorbance was performed at 450 nm.

Luciferase reporter assays

Using Lipofectamine 2000 (Invitrogen), the cell was transfected with pGL3-SRCIN1-3′-untranslated region (UTR)-MUT or pGL3-SRCIN1-3′-UTR-WT constructs along with miR-346 mimic or scramble and pRL-TK vector (Promega). The luciferase activity was done using the Dual-Luciferase Reporter kit (Promega, Madison, WI, USA) after 48 h. Renilla luciferase was done for endogenous normalization.

Western blot

BCA protein kit was used to extract protein from cell or tissues. Protein was separated by 12 % SDS–PAGE and then transferred to membranes (PVDF; Millipore, Danvers, MA, USA). Then, membranes were blocked with non-fat milk and were incubated with antibody SRCIN1 (Cell Signaling Technology) or GAPDH (Santa Cruz, CA, USA). The enhanced chemiluminescence system (ECL) (Millipore, USA) was used to measure the band.

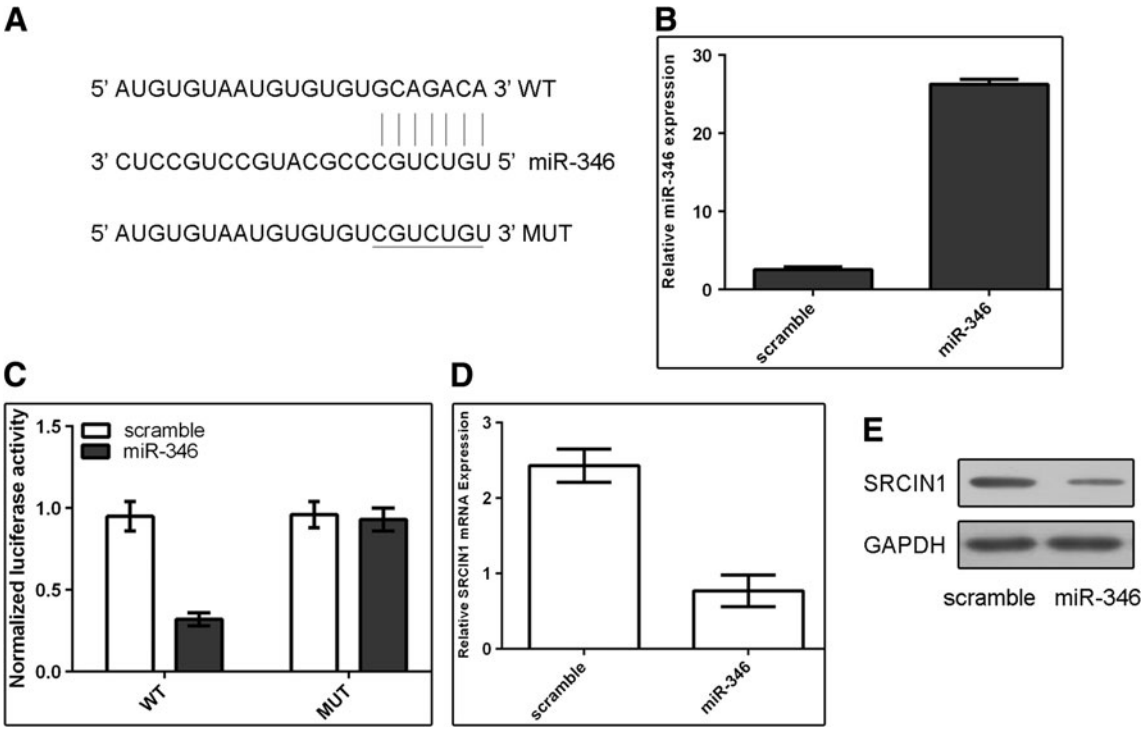

Fig. 2 miR-346 directly targets SRCIN1. **a** The 3′-UTR of SRCIN1 mRNA contains a putative site partially complementary to miR-346. **b** qRT-PCR analysis demonstrated the successful overexpression of miR-346 by transfected miR-346 mimic in UT-SCC-7 cells. **c** Overexpression of miR-346 inhibited the luciferase activity of the wt reporter plasmid, but not the mut reporter plasmid. **d** Upregulation of miR-346 inhibited the mRNA and protein expression of SRCIN1. **e** The protein of SRCIN1 after treated by miR-346 mimics was measured by western blot

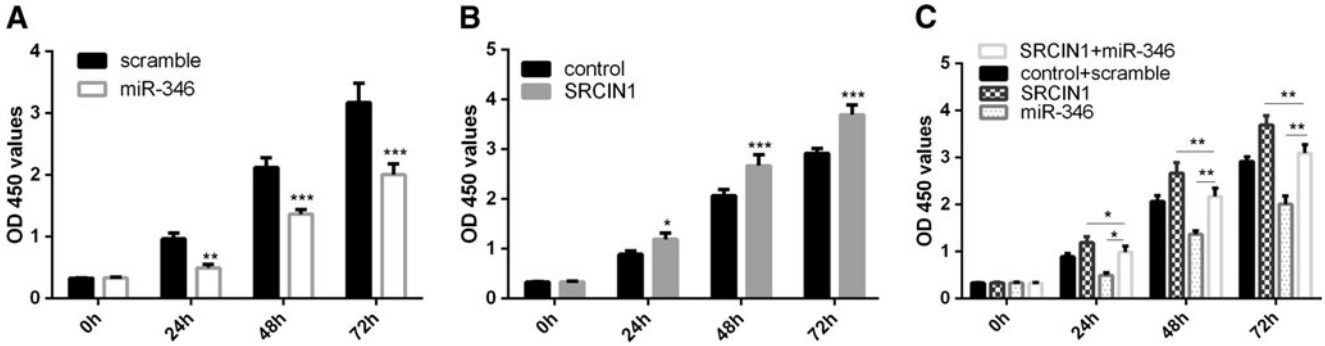

Fig. 3 miR-346 promoted cSCC cell proliferation through targeting SRCIN1. **a** Cell proliferation was measured by CCK-8 analysis. **b** Overexpression of SRCIN1 inhibited the UT-SCC-7 cell proliferation. **c** When miR-346 and pcDNA-SRCIN1 were cotransfected into UT-SCC-7 cells, SRCIN1 overexpression reduced the miR-346-induced UT-SCC-7 cell proliferation

Statistical analysis

Data are shown as the mean±SD. Mann–Whitney *U* test or Student's *t* test was used to determine the statistical significance. The relationship expression between SRCIN1 and miR-346 was measured by Spearman's correlation.

Result

The expression of miR-346 was upregulated and was inversed with the expression of SRCIN1 in cSCC

The expression of miR-346 was higher in cSCC tissues than in the matched normal tissues (Fig. 1a). miR-346

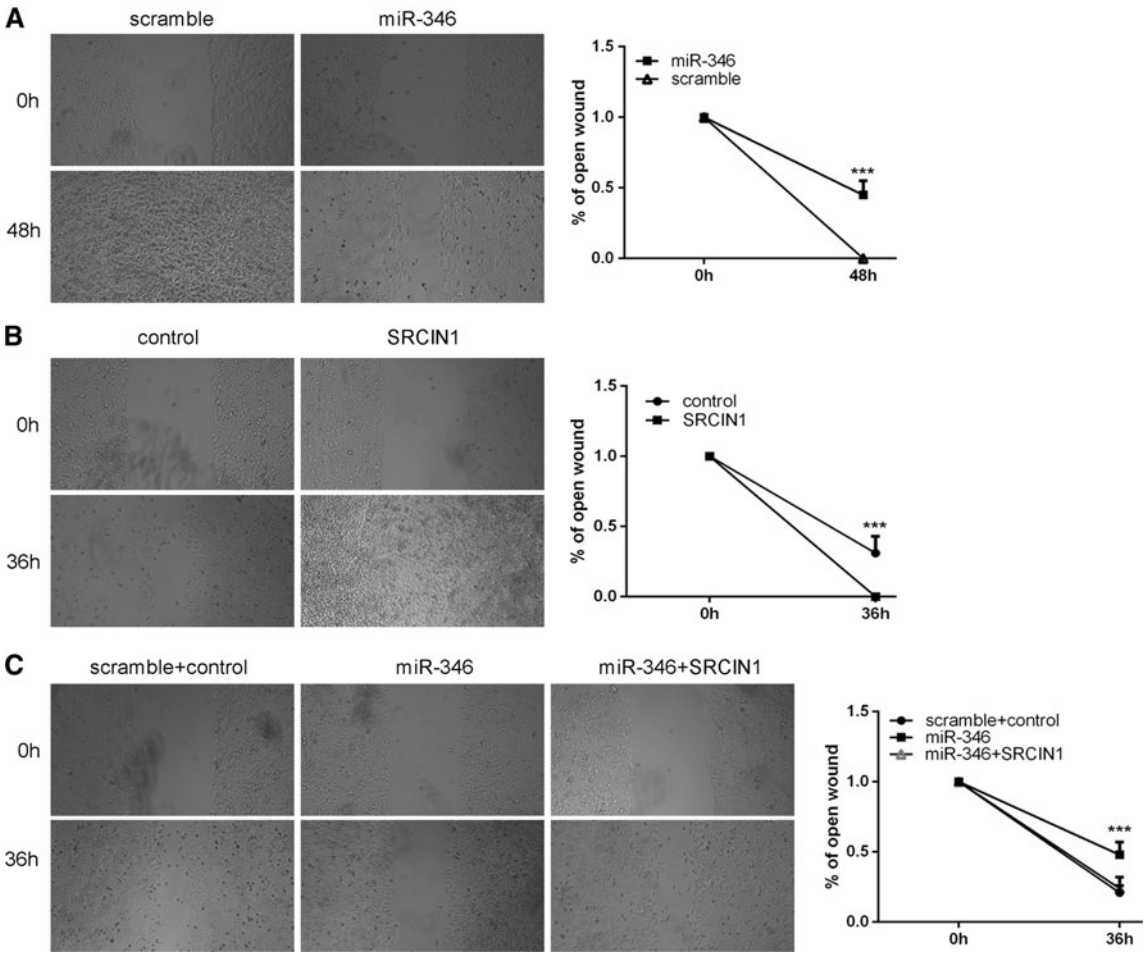

Fig. 4 miR-346 promoted cSCC cell migration through targeting SRCIN1. **a** Upregulation of miR-346 promoted the UT-SCC-7 cell migration. **b** SRCIN1 overexpression inhibited the UT-SCC-7 cell migration. **c** When miR-346 and pcDNA-SRCIN1 were cotransfected into UT-SCC-7 cells, SRCIN1 overexpression reduced the miR-346-induced UT-SCC-7 cell migration

expression was increased in 77 % (23/30) of the patients with cSCC tissues compared to the matched normal tissues (Fig. 1b). In contrast, the expression of SRCIN1 was lower in cSCC tissues than in the matched normal tissues (Fig. 1c). SRCIN1 expression was increased in 80 % (24/30) of the patients with cSCC tissues compared to the matched normal tissues (Fig. 1d). Interestingly, there was significant inversed correlation between miR-346 and SRCIN1 in cSCC tissues (Fig. 1e).

miR-346 directly targets SRCIN1

The 3′-untranslated region (UTR) of SRCIN1 mRNA contains a putative site partially complementary to miR-346 (Fig. 2a). Quantitative real-time PCR (qRT-PCR) analysis demonstrated the successful overexpression of miR-346 by transfected miR-346 mimic in A431 cells (Fig. 2b). Overexpression of miR-346 inhibited the luciferase activity of the wt reporter plasmid, but not the mut reporter plasmid (Fig. 2c). Upregulation of miR-346 inhibited the mRNA and protein expression of SRCIN1 (Fig. 2d, e).

miR-346 promoted cSCC cell proliferation through targeting SRCIN1

Upregulation of miR-346 promoted the A431 cell proliferation (Fig. 3a). SRCIN1 overexpression inhibited the A431 cell proliferation (Fig. 3b). When miR-346 and pcDNA-SRCIN1 were cotransfected into A431 cells, SRCIN1 overexpression reduced the miR-346-induced A431 cell proliferation (Fig. 3c).

miR-346 promoted cSCC cell migration through targeting SRCIN1

Upregulation of miR-346 promoted the A431 cell migration (Fig. 4a). SRCIN1 overexpression inhibited the A431 cell migration (Fig. 4b). When miR-346 and pcDNA-SRCIN1 were cotransfected into A431 cells, SRCIN1 overexpression reduced the miR-346-induced A431 cell migration (Fig. 4c).

Discussion

In this study, we identified miR-346 as a novel oncogene in cSCC. We found that the expression of miR-346 was higher in cSCC tissues than in the matched normal tissues. We determined that the expression of SRCIN1 was lower in cSCC tissues than in the matched normal tissues. Moreover, there was significant inversed correlation between miR-346 and SRCIN1 in cSCC tissues. The luciferase reporter assay data showed that miR-346 can target the SRCIN1 message via the 3′-UTR of SRCIN1. Overexpression of miR-346 inhibited the mRNA and protein expression of SRCIN1 in the A431 cells. In addition, ectopic expression of miR-346 promoted the A431 cell proliferation and migration. Meanwhile, SRCIN1 overexpression inhibited the A431 cell proliferation and migration. Rescue experiment has showed that SRCIN1 overexpression reduced the miR-346-induced A431 cell proliferation and migration.

Previous studies demonstrated that miR-346 plays an important role in anti-inflammatory pathways [32–34]. The expression of miR-346 was upregulated in rheumatoid inflammation patients, exerting anti-inflammatory effects [35]. Xiao et al. [36] demonstrated that miR-346 was upregulated in podocytes and participated in the epithelial–mesenchymal transition. However, the role of miR-346 in cSCC was still unknown. In our study, we found that the expression of miR-346 was higher in cSCC tissues than in the matched normal tissues. Moreover, ectopic expression of miR-346 promoted the A431 cell proliferation and migration.

SRCIN1, also known as p140CAP, contains two amino acids, two proline-rich regions, and two coiled-coil domains [37–39]. SRCIN1 acts as a regulator protein and has played an important role in cancer development [40]. Previous study showed that SRCIN1 overexpression inhibited the cell spreading, invasion, and migration in breast cancer [41]. Cao et al. [42] showed that miR-150 promoted migration, proliferation, and invasion through the inhibition of SRCIN1 expression in lung cancer. Moreover, Xu et al. [43] demonstrated that miR-374a increased the gastric cancer cell proliferation, migration, and invasion by inhibiting SRCIN1 expression. In line with these results, we also found that miR-346 promoted cSCC cell proliferation and migration by regulating SRCIN1 expression. Overexpression of SRCIN1 inhibited the cell proliferation and migration. We also identified SRCIN1 as a direct target of miR-346. These data suggest that SRCIN1 is a functional regulator for miR-346a in cSCC.

In conclusion, we demonstrated that the expression of miR-346 was higher in cSCC tissues than in the matched normal tissues. Ectopic expression of miR-346 promoted the cSCC cell proliferation and migration through directly targeting SRCIN1. This study may provide a new therapeutic target for cSCC.

Conflicts of interest None

References

1. Wang N, Xu ZW, Wang KH. Systematical analysis of cutaneous squamous cell carcinoma network of microRNAs, transcription factors, and target and host genes. Asian Pac J Cancer Prev: APJCP. 2014;15:10355–61.
2. Greenberg ES, Chong KK, Huynh KT, Tanaka R, Hoon DS. Epigenetic biomarkers in skin cancer. Cancer Lett. 2014;342:170–7.

3. Gastaldi C, Bertero T, Xu N, Bourget-Ponzio I, Lebrigand K, Fourre S, et al. miR-193b/365a cluster controls progression of epidermal squamous cell carcinoma. Carcinogenesis. 2014;35:1110–20.
4. Bruegger C, Kempf W, Spoerri I, Arnold AW, Itin PH, Burger B. MicroRNA expression differs in cutaneous squamous cell carcinomas and healthy skin of immunocompetent individuals. Exp Dermatol. 2013;22:426–8.
5. Zhou M, Zhou L, Zheng L, Guo L, Wang Y, Liu H, et al. miR-365 promotes cutaneous squamous cell carcinoma (CSCC) through targeting nuclear factor I/B (NFIB). PLoS ONE. 2014;9, e100620.
6. Ning MS, Andl T. Custodians of the transcriptome: how microRNAs guard stemness in squamous epithelia. Stem Cells. 2014;33(4):1047–54.
7. Sand M, Skrygan M, Georgas D, Sand D, Hahn SA, Gambichler T, et al. Microarray analysis of microRNA expression in cutaneous squamous cell carcinoma. J Dermatol Sci. 2012;68:119–26.
8. Ning MS, Kim AS, Prasad N, Levy SE, Zhang H, Andl T. Characterization of the Merkel cell carcinoma miRNome. J Skin Cancer. 2014;2014, 289548.
9. Yamane K, Jinnin M, Etoh T, Kobayashi Y, Shimozono N, Fukushima S, et al. Down-regulation of miR-124/-214 in cutaneous squamous cell carcinoma mediates abnormal cell proliferation via the induction of ERK. J Mol Med (Berl). 2013;91:69–81.
10. Kanitz A, Imig J, Dziunycz PJ, Primorac A, Galgano A, Hofbauer GF, et al. The expression levels of microRNA-361-5p and its target VEGFA are inversely correlated in human cutaneous squamous cell carcinoma. PLoS ONE. 2012;7, e49568.
11. Wang A, Landen NX, Meisgen F, Lohcharoenkal W, Stahle M, Sonkoly E, et al. MicroRNA-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell motility and colony formation ability of tumor cells. PLoS ONE. 2014;9, e103206.
12. Hameetman L, Commandeur S, Bavinck JN, Wisgerhof HC, de Gruijl FR, Willemze R, et al. Molecular profiling of cutaneous squamous cell carcinomas and actinic keratoses from organ transplant recipients. BMC Cancer. 2013;13:58.
13. Yu X, Li Z. MicroRNAs regulate vascular smooth muscle cell functions in atherosclerosis (review). Int J Mol Med. 2014;34:923–33.
14. Li Z, Lei H, Luo M, Wang Y, Dong L, Ma Y, et al. DNA methylation downregulated mir-10b acts as a tumor suppressor in gastric cancer. Gastric Cancer. 2015;18:43–54.
15. Li Z, Yu X, Shen J, Jiang Y. MicroRNA dysregulation in uveal melanoma: a new player enters the game. Oncotarget. 2015;6(7):4562–8.
16. Liu G, Liu R, Li Q, Tang X, Yu M, Li X, et al. Identification of microRNAs in wool follicles during anagen, catagen, and telogen phases in Tibetan sheep. PLoS ONE. 2013;8, e77801.
17. Li Z, Yu X, Shen J, Chan MT, Wu WK. MicroRNA in intervertebral disc degeneration. Cell Prolif. 2015;48(3):278–83.
18. Li Z, Yu X, Wang Y, Shen J, Wu WK, Liang J, et al. By downregulating TIAM1 expression, microRNA-329 suppresses gastric cancer invasion and growth. Oncotarget. 2014;6(19):17559–69.
19. Yu X, Li Z, Shen J, Wu WK, Liang J, Weng X, et al. MicroRNA-10b promotes nucleus pulposus cell proliferation through RhoC-Akt pathway by targeting HOXD10 in intervertebral disc degeneration. PLoS ONE. 2013;8, e83080.
20. Rao SA, Arimappamagan A, Pandey P, Santosh V, Hegde AS, Chandramouli BA, et al. miR-219-5p inhibits receptor tyrosine kinase pathway by targeting EGFR in glioblastoma. PLoS ONE. 2013;8, e63164.
21. Wu X, Weng L, Li X, Guo C, Pal SK, Jin JM, et al. Identification of a 4-microRNA signature for clear cell renal cell carcinoma metastasis and prognosis. PLoS ONE. 2012;7, e35661.
22. Li BS, Zhao YL, Guo G, Li W, Zhu ED, Luo X, et al. Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection. PLoS ONE. 2012;7, e41629.
23. Jung CJ, Iyengar S, Blahnik KR, Ajuha TP, Jiang JX, Farnham PJ, et al. Epigenetic modulation of miR-122 facilitates human embryonic stem cell self-renewal and hepatocellular carcinoma proliferation. PLoS ONE. 2011;6, e27740.
24. Vrba L, Munoz-Rodriguez JL, Stampfer MR, Futscher BW. Mirna gene promoters are frequent targets of aberrant DNA methylation in human breast cancer. PLoS ONE. 2013;8, e54398.
25. Denoyelle C, Lambert B, Meryet-Figuiere M, Vigneron N, Brotin E, Lecerf C, et al. miR-491-5p-induced apoptosis in ovarian carcinoma depends on the direct inhibition of both BCL-XL and EGFR leading to BIM activation. Cell Death Dis. 2014;5, e1445.
26. Rao PK, Missiaglia E, Shields L, Hyde G, Yuan B, Shepherd CJ, et al. Distinct roles for miR-1 and miR-133a in the proliferation and differentiation of rhabdomyosarcoma cells. FASEB J. 2010;24:3427–37.
27. Worley LA, Long MD, Onken MD, Harbour JW. Micro-RNAs associated with metastasis in uveal melanoma identified by multiplexed microarray profiling. Melanoma Res. 2008;18:184–90.
28. Yu X, Li Z, Liu J. MiRNAs in primary cutaneous lymphomas. Cell Prolif. 2015;48(3):271–7.
29. Li Z, Yu X, Shen J, Wu WK, Chan MT. MicroRNA expression and its clinical implications in Ewing's sarcoma. Cell Prolif. 2015;48:1–6.
30. Bemis LT, Chen R, Amato CM, Classen EH, Robinson SE, Coffey DG, et al. MicroRNA-137 targets microphthalmia-associated transcription factor in melanoma cell lines. Cancer Res. 2008;68:1362–8.
31. Langevin SM, Stone RA, Bunker CH, Grandis JR, Sobol RW, Taioli E. MicroRNA-137 promoter methylation in oral rinses from patients with squamous cell carcinoma of the head and neck is associated with gender and body mass index. Carcinogenesis. 2010;31:864–70.
32. Alsaleh G, Suffert G, Semaan N, Juncker T, Frenzel L, Gottenberg JE, et al. Bruton's tyrosine kinase is involved in miR-346-related regulation of IL-18 release by lipopolysaccharide-activated rheumatoid fibroblast-like synoviocytes. J Immunol. 2009;182:5088–97.
33. Chen Y, Du J, Zhang Z, Liu T, Shi Y, Ge X, et al. MicroRNA-346 mediates tumor necrosis factor alpha-induced downregulation of gut epithelial vitamin d receptor in inflammatory bowel diseases. Inflamm Bowel Dis. 2014;20:1910–8.
34. Chen J, Tian J, Tang X, Rui K, Ma J, Mao C, et al. MiR-346 regulates $CD4^{+}CXCR5^{+}$ T cells in the pathogenesis of Graves' disease. Endocrine. 2015;49(3):752–60.
35. Semaan N, Frenzel L, Alsaleh G, Suffert G, Gottenberg JE, Sibilia J, et al. miR-346 controls release of TNF-alpha protein and stability of its mRNA in rheumatoid arthritis via tristetraprolin stabilization. PLoS ONE. 2011;6, e19827.
36. Xiao J, Liu D, Jiao W, Guo J, Wang X, Zhang X, et al. Effects of microRNA-346 on epithelial-mesenchymal transition in mouse podocytes. Gene. 2015;560:195–9.
37. Cabodi S, del Pilar C-LM, Di Stefano P, Defilippi P. Integrin signalling adaptors: not only figurants in the cancer story. Nat Rev Cancer. 2010;10:858–70.
38. Repetto D, Aramu S, Boeri Erba E, Sharma N, Grasso S, Russo I, et al. Mapping of p140Cap phosphorylation sites: the EPLYA and EGLYA motifs have a key role in tyrosine phosphorylation and Csk binding, and are substrates of the Abl kinase. PLoS ONE. 2013;8, e54931.
39. Yamauchi M, Sudo K, Ito H, Iwamoto I, Morishita R, Murai T, et al. Localization of multidomain adaptor proteins, p140Cap and vinexin, in the pancreatic islet of a spontaneous diabetes mellitus model, Otsuka Long-Evans Tokushima fatty rats. Med Mol Morphol. 2013;46:41–8.
40. Sharma N, Repetto D, Aramu S, Grasso S, Russo I, Fiorentino A, et al. Identification of two regions in the p140Cap adaptor protein

that retain the ability to suppress tumor cell properties. Am J Cancer Res. 2013;3:290–301.

41. Di Stefano P, Damiano L, Cabodi S, Aramu S, Tordella L, Praduroux A, et al. p140Cap protein suppresses tumour cell properties, regulating Csk and Src kinase activity. EMBO J. 2007;26:2843–55.

42. Cao M, Hou D, Liang H, Gong F, Wang Y, Yan X, et al. miR-150 promotes the proliferation and migration of lung cancer cells by targeting SRC kinase signalling inhibitor 1. Eur J Cancer. 2014;50(5):1013–24.

43. Xu X, Wang W, Su N, Zhu X, Yao J, Gao W, et al. miR-374a promotes cell proliferation, migration and invasion by targeting SRCIN1 in gastric cancer. FEBS Lett. 2015;589:407–13.