MicroRNA-130a: a tumor suppressive miRNA in cutaneous squamous cell carcinoma

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Background

- Cutaneous squamous cell carcinoma (cSCC) is one of the fastest increasing malignancies that represents particular problem among immunosuppressed patients.
- MicroRNAs are short non-protein-coding RNAs which regulate the activity of gene networks by regulating gene expression at the posttranscriptional level.
- MiR-130a is one of the miRNAs whose expression was significantly decreased in cSCC according to our initial screening.

Aim

To determine the role of miR-130a in the pathogenesis of cSCC and identify potential target genes in the disease.

Results

I. miR-130a is down-regulated in cSCC and regulated by HRas

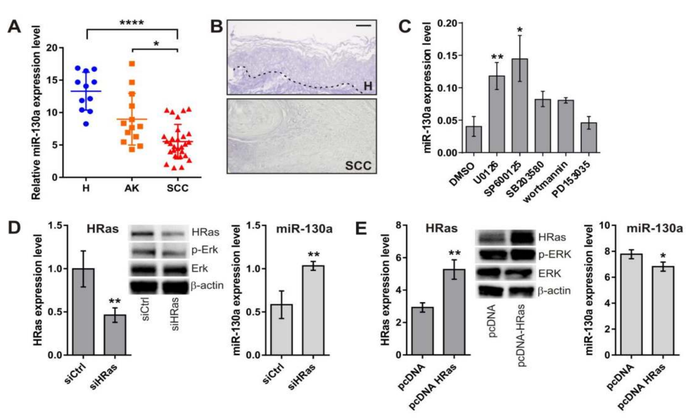
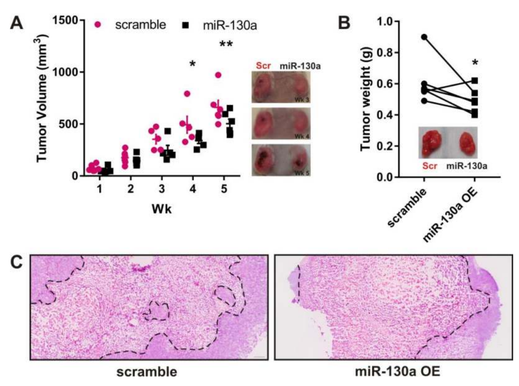


Figure 1. MiR-130a expression is decreased in cSCC and regulated by HRas. (A) qPCR analysis of miR-130a in heathy skin, actinic keratosis (AK) or cSCC. (B) *In situ* hybridization (ISH) analysis of miR-130a in heathy skin and cSCC. (C) Expression of miR-130a in A431 cells upon the treatment with different chemical inhibitors. ERK phosphorylation, HRas and miR-130a expression in human cSCC cell line upon (D) silencing or (E) transient overexpression of Hras were assessed by western blot and qPCR

II. MiR-130 suppresses primary tumor growth *in vivo*

Figure 2. Overexpression of miR-130 suppresses primary tumor growth. (A) Xenograft experiment was performed in NSG mice with miR-130a overexpressing UT-SCC-7 and control cells. (B) At the end of the experiment, tumors were excised and weighed. (C) Hematoxylin-Eosin staining of the harvested tumor.



III. MiR-130a inhibits the colony forming capacity and sphere formation in cSCC

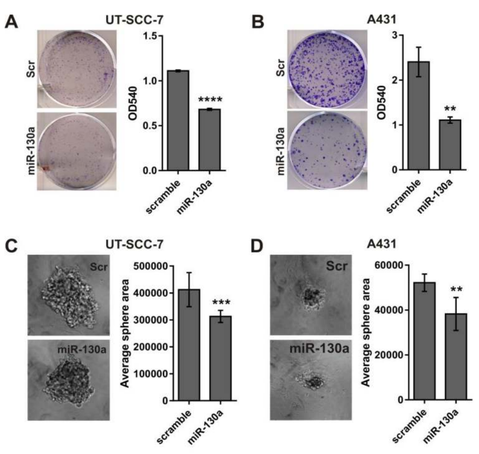


Figure 3. MiR-130a suppresses colony formation and self-renewal ability of cSCC. (A and B) The clonogenic capacity of two cutaneous SCC cell lines was assayed upon ectopic overexpression of miR-130a or control. (C and D) Tumor sphere formation assay was performed in UT-SCC-7 and A431 upon transfection with miR-130a or scramble ODNs.

IV. Overexpression of miR-130a decreases the migratory and invasive capacity of SCC cells

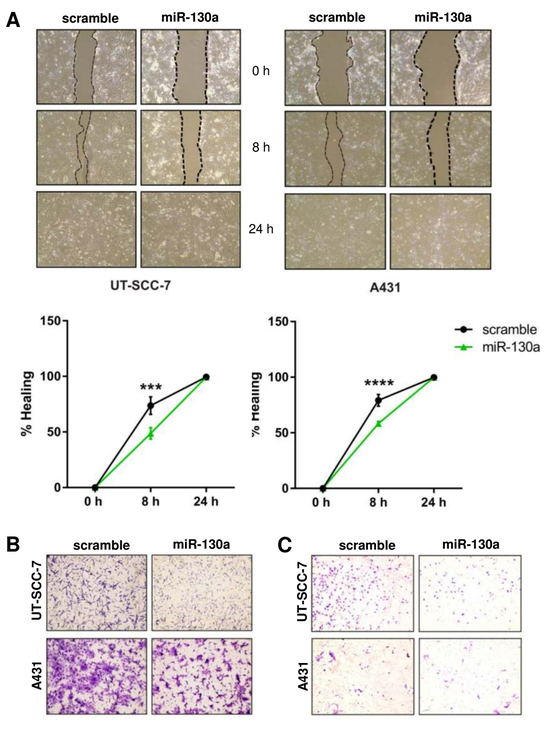


Figure 4. Overexpression of miR-130 suppresss cell motility in cSCC. (A) Scratch wound healing experiments were performed in cSCC cells upon overexpression of miR-130a. (B) Transwell migration and (C) Transwell invasion experiment of miR-130 or scramble overexpressing UT-SCC-7 and A431 SCC cell lines.

V. Activin A receptor type I (ACVR1)/SMAD1 signal is regulated by miR-130a

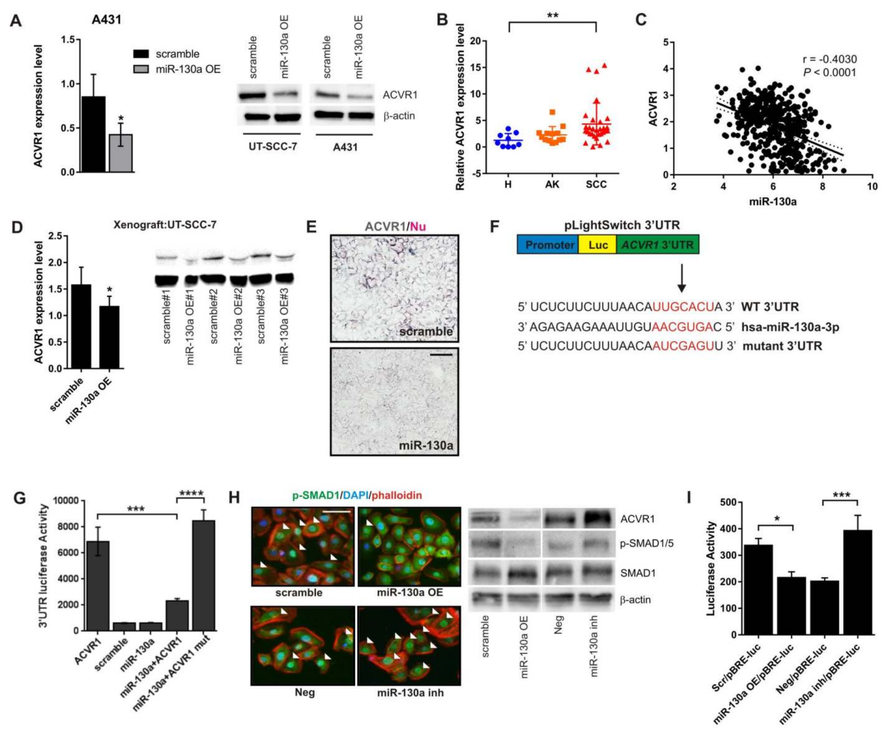


Figure 5. MiR-130a targets ACVR1 and dampens SMAD1-mediated BMP signal (A) ACVR1 expression level was in SCC cell lines transfected with miR-130a mimic. (B) ACVR1 expression in cSCC patient cohort. (C) Correlation of miR-130a and ACVR1 expression in TCGA HNSCC cohort. (D) ACVR1 expression in tumor harvested from mice in xenograft experiment. (E) IHC staining of ACVR1 in xenograft samples. (F) Predicted binding site of miR-130a in the 3'UTR of ACVR1. (G) 3'UTR luciferase assay in NHEK cells. (H) p-SMAD1 nuclear translocation in NHEK cells upon the transfection with miR-130a mimic or miR-130a inhibitor. (I) p-SMAD1/5/8 mediated BMP signalling was determined by luciferase reporter assay .

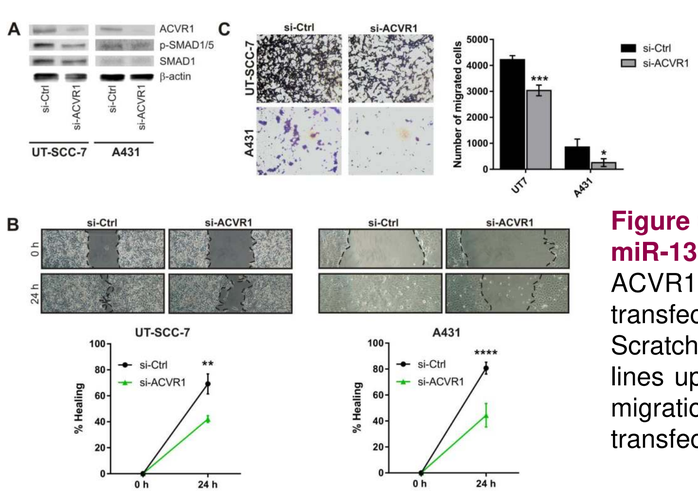


Figure 6. Knockdown of ACVR1 phenocopies miR-130a overexpression. (A) Decrease of ACVR1 in cSCC cell lines upon si-ACVR1 transfection was determined by western blot. (B) Scratch wound healing experiments in cSCC cell lines upon si-ACVR1 transfection. (C) Transwell migration experiment of cSCC cell lines transfected with si-ACVR1.

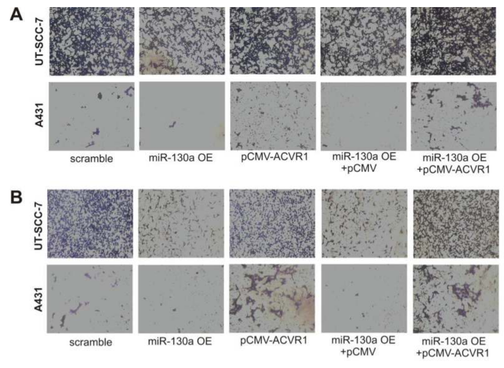


Figure 7. ACVR1 restoration rescues cell motility suppression mediated by miR-130a in cSCC cells. Double transfection of UT-SCC-7 and A431 cells with miR-130a together with pCMV or pCMV-ACVR1 was performed, followed by subjection to (A) Transwell migration or (B) Transwell invasion assay.

Conclusions

- MiR-130a expression is decreased in human cSCC
- Overexpression of miR-130a suppresses tumor growth *in vivo*
- MiR-130a regulates processes related to the self-renewal ability, cell motility, migration and invasion in cSCC
- Regulation of ACVR1/SMAD1 pathway was identified as an potential mechanism explaining the tumor suppressive effect of miR-130a in cSCC

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