MicroRNA-130a (miR-130a) regulates self-renewal capacity and tumor growth in cutaneous squamous cell carcinoma

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Background

- Cutaneous squamous cell carcinoma (cSCC) is the third most common cancer in Sweden (1).
- cSCC is the fastest increasing cancer type in Sweden - its incidence has doubled in the past decade.
- MicroRNAs are short non-protein-coding RNAs which regulate the activity of gene networks by regulating gene expression at the posttranscriptional level.
- Our group has been studying the role of microRNAs in cSCC. Our initial screen identified miR-130a as a miRNA whose expression was significantly decreased in cSCC (2).

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 downregulated in human cSCCs

Figure 1. Analysis of miR-130a expression in human cSCCs. (A) The expression of miR-130a was analyzed by quantitative real-time PCR in healthy skin (HS) and cSCC samples. (B) qPCR analysis of miR-130a expression in healthy skin and in well-differentiated (WD) and moderately differentiated (MD) cSCCs. (C) *In situ* hybridization analysis of miR-130a expression in healthy skin and cSCC. Epidermal staining was observed for miR-130a in healthy skin, while it was absent in cSCC. Mann Whitney-test; two-tailed; * $P < 0.05$.

II. miR-130a regulates the long-term growth capacity of SCC cells

Figure 2. miR-130a suppresses the colony formation ability of SCC cells *in vitro*. Colony formation assays were performed with two human cSCC cell lines – UT-SCC-7 and A431. The transient overexpression of miR-130a significantly decreased the colony formation ability of both cell lines. Optical density of crystal violet-stained colonies was plotted and analyzed. Student's t-test, two-tailed; *** $P < 0.001$, **** $P < 0.0001$.

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

Figure 3. Transient overexpression of miR-130a suppresses the migratory and invasive capacity of SCC cells *in vitro*. (A) UT-SCC-7 and A431 transfected with miR-130a mimic or scrambled ODNs were subjected to scratch assay. Images were taken at 0, 8 and 24 hours after the monolayer was scratched. Depicted are representative images and indications of the migration distances. The percentage of healing, which represents random cell migration was calculated and presented as a graph. (B and C) Transwell (Corning Life Sciences, New York, NY) migration and Matrigel (Corning Life Sciences) invasion assay of miR-130a mimic- or scrambled ODN-transfected UT-SCC-7 and A431 cells. Data shown are means of cell counts obtained from three separate wells, and error bars represent standard deviations. Student's t-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

IV. Inhibition of miR-130a increases the migratory and invasive capacity of SCC cells

Figure 4. Inhibition of miR-130a increases the migratory and invasive capacity of SCC cells *in vitro*. (A) UT-SCC-7 and A431 transfected with miR-130a inhibitor or scrambled ODNs were subjected to scratch assay. Images were taken at 0, 8 and 24 hours after the monolayer was scratched. Depicted are representative images and indications of the migration distances. Percentage of healing, which represents random cell migration was calculated and presented as a graph. (B and C) Transwell migration and Matrigel invasion assays of miR-130a inhibitor or scrambled ODNs transfected UT-SCC-7 and A431 cells. Data shown are means of cell counts obtained from three separate wells, and error bars represent standard deviations. Student's t-test; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

V. Overexpression of miR-130a suppresses tumor growth in xenograft model of cSCC

Figure 5. Overexpression of miR-130a suppresses the growth of cSCC in xenograft model. (A) UT-SCC-7 cell lines with stable overexpression of miR-130a or scrambled ODNs were established by lentiviral transduction. The overexpression of miR-130a was confirmed by quantitative real-time PCR. (B) The functionality of stably overexpressed miR-130a was confirmed in Transwell migration assay. Similar to the results obtained by transient transfection, the migratory capacity of miR-130a overexpressing cells was significantly decreased. (C) Ten million scramble- or miR-130a-overexpressing UT-SCC-7 cells were injected into the left and right flank of each mouse, respectively (n = 6). (D) Tumor volume was calculated as (width² × length)/2 at the indicated times. (E) Harvested xenografts (left panel) and comparison tumor weights (right panel) measured at the end of the experiment. Student's t-test; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

VI. Identification of miR-130a targets in cSCC

Figure 6. Identification of miR-130a targets in cSCC. (A) Differentially expressed genes between healthy skin and cSCC were identified by RNAseq (NextSeq500). Unsupervised hierarchical clustering based on the significantly differentially expressed (determined by LIMMA) separated healthy skin from SCCs. (B) Intersection analysis of genes whose expression was increased in cSCC and predicted target genes (TargetScan 7.1) of miR-130a. (C) Pathway enrichment analysis of predicted targets of miR-130a, which were upregulated in SCC (n = 151; EnrichR).

Conclusions

- miR-130a expression is downregulated in human cSCC
- miR-130a regulates processes related to the self-renewal ability of SCC cells, cell motility, migration and invasion
- Overexpression of miR-130a suppresses tumor growth *in vivo*
- Several predicted target genes of miR-130a are upregulated in SCC