TITLE:

Hsa-microRNA-1249-3p modulates human epithelial cell clonogenicity *via* Homeobox A13 gene regulation

Authors:
Chiara Mazziotta, Giada Badiale, Christian Felice Cervellera, Giulia Tonnini, Mauro Tognon, Fernanda Martini, John Charles Rotondo
(Full name and surname)

Affiliation:
Department of Medical Sciences, University of Ferrara

e-mail: mzzchr@unife.it
Phone: +390532455536

Keywords:

microRNA; cell-cell adhesion; HOXA13; hsa-miR-1249-3p; clonogenicity; human epithelial cells

Text (300 words max):

Hsa-miR-1249-3p is dysregulated in several neoplasms, including hepatocellular and breast carcinomas. However, its function in human epithelial cells is unknown. Herein, we functionally investigated the effect of hsa-miR-1249-3p on the proliferation, migration, clonogenicity and apoptosis of human epithelial cells and explored the underlying mechanism.

Droplet digital PCR was used to evaluate the hsa-miR-1249-3p expression in human keratinocyte cell lines HaCaT and NCTC and in control human uterine cervical carcinoma cell lines SiHa, CaSki and HeLa. Then, the hsa-miR-1249-3p mimic, inhibitor and negative/positive controls were transfected onto HaCaT cells. Upon transfections, cell proliferation, clonogenicity, migration and apoptosis were assessed by WST, clonogenic, wound healing and western blot assays, respectively.

Results indicate that hsa-miR-1249-3p is overexpressed in HaCaT and NCTC cell lines, respectively, compared to pooled human cervical carcinoma cell lines. Upon transfections, hsa-miR-1249-3p resulted as undetectable in miR-inhibitor HaCaT condition, while being strongly overexpressed miR-mimic HaCaT, compared to untreated cells. Hsa-miR-1249-3p inhibition modestly favored cell proliferation and migration potential in HaCaT cells, without perturbing apoptosis. Contrariwise, a strong clonogenic effect was detected in hsa-miR-1249-3p-inhibited HaCaT cells. Furthermore, *in silico* analyses conducted with TargetScan tool identified the oncogene *Homeobox A13* (*HOXA13*) as a hsa-miR-1249-3p downstream target. Mechanistically, hsa-miR-1249-3p inhibition prompted the up-regulation of HOXA13 transcript in HaCaT cells *in vitro*.

Our data indicate that hsa-miR-1249-3p can target *HOXA13* to regulate the clonogenic potential of HaCaT cells. These data will allow the set-up of further studies aimed in investigating the role of has-miR-1249-3p/HOXA13 axis in epithelial cell clonogenicity, such as evaluating the relationship between this miRNA/target gene axis and its downstream genes implicated in cell-cell adhesion pathways, i.e., *β-catenin*, *c-Met* and *c-Jun*.