MicroRNA-128-3p-mediated depletion of Drosha promotes lung cancer cell migration

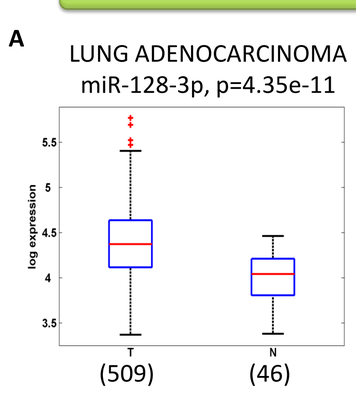
31

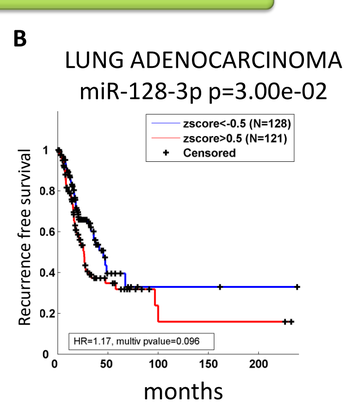
Tania Frixa[1], Andrea Sacconi[1], Mario Cioce[1], Giuseppe Roscilli[2], Fabiana Fosca Ferrara[2], Luigi Aurisicchio[2], Claudio Pulito[3], Stefano Telera[4], Mariantonia Carosi[5], Paola Muti[6], Sabrina Strano[3,6], Sara Donzelli[1,*] and Giovanni Blandino[1,6,*]

[1]Oncogenomic and Epigenetic Unit, Italian National Cancer Institute 'Regina Elena', 00144 Rome, Italy, [2]Takis s.r.l., via Castel Romano 100, 00128 Rome, Italy, [3]Molecular Chemoprevention Group, Italian National Cancer Institute 'Regina Elena', 00144 Rome, Italy, [4]Department of Neurosurgery, Italian National Cancer Institute 'Regina Elena', 00144 Rome, Italy, [5]Department of Pathology, Italian National Cancer Institute 'Regina Elena', 00144 Rome, Italy and [6]Department of Oncology, Juravinski Cancer Center, McMaster University Hamilton, Hamilton, ON L8V 5C2 Ontario, Canada

Alteration in microRNAs (miRNAs) expression is a frequent finding in human cancers. In particular, widespread miRNAs down-regulation is a hallmark of malignant transformation. In the present report, we showed that the miR-128-3p, which is up-regulated in lung cancer tissues, has Drosha and Dicer, two key enzymes of miRNAs processing, as the main modulation targets leading to the widespread down-regulation of miRNA expression. We observed that the miRNAs down-regulation induced by miR-128-3p contributed to the tumorigenic properties of lung cancer cells. In particular, miR-128-3p-mediated miRNAs down-regulation contributed to aberrant SNAIL and ZEB1 expression thereby promoting the epithelial-to-mesenchymal transition (EMT) program. Drosha also resulted to be implicated in the control of migratory phenotype as its expression counteracted miR-128-3p functional effects. Our study provides mechanistic insights into the function of miR-128-3p as a key regulator of the malignant phenotype of lung cancer cells. This also enforces the remarkable impact of Drosha and Dicer alteration in cancer, and in particular it highlights a role for Drosha in non-small cell lung cancer cells migration.

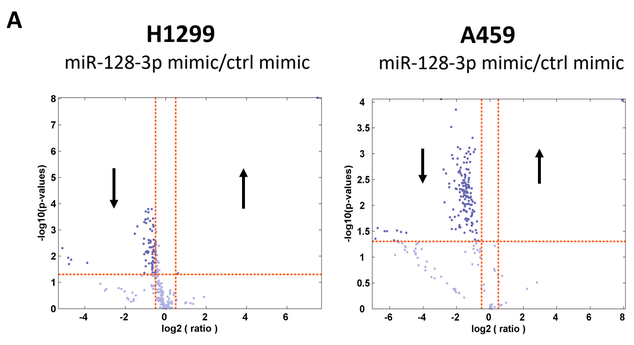
1. MiR-128-3p expression is up-regulated in NSCLC tissues

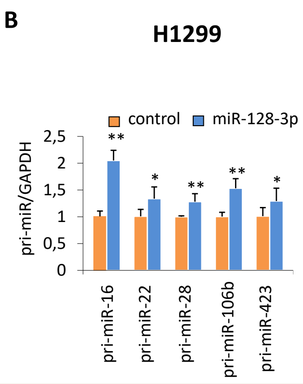




MiR-128-3p resulted to be significantly up-regulated in 509 tumoral tissues when compared to 46 non-tumoral lung tissues in a large lung adenocarcinoma patient cohort from The Cancer Genome Atlas (TCGA) database **(A)**. The analysis of recurrence-free survival of TCGA lung cancer patients showed a positive trend between high levels of miR-128-3p and probability of recurrence **(B)**. Altogether these data mirror an oncogenic role of miR-128-3p in lung cancer.

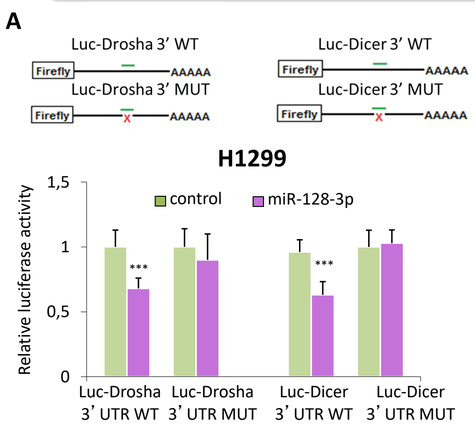
2. MiR-128-3p expression triggers a global down-regulation of miRNAs

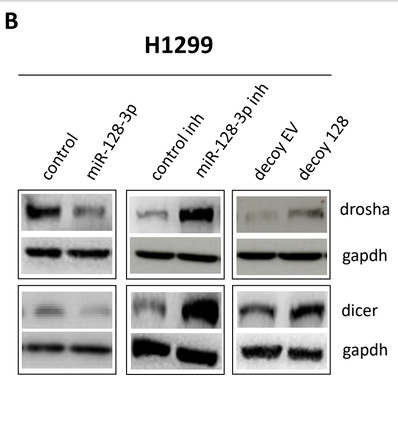




We found that ectopic expression of miR-128-3p caused a pronounced global down-regulation of miRNAs in both H1299 and A459 cells **(A).** Additionally, we observed an accumulation of primary miRNAs (pri-miRs) in miR-128-3p over-expressing cells, suggesting a specific effect of miR-128-3p on miRNAs maturation steps rather than on miRNAs transcription **(B)**.

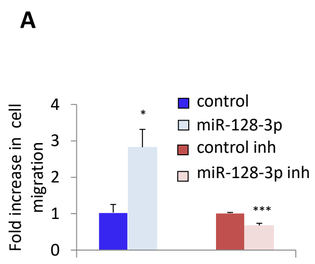
3. The miRNA processing enzymes Drosha and Dicer are novel targets of miR-128-3p

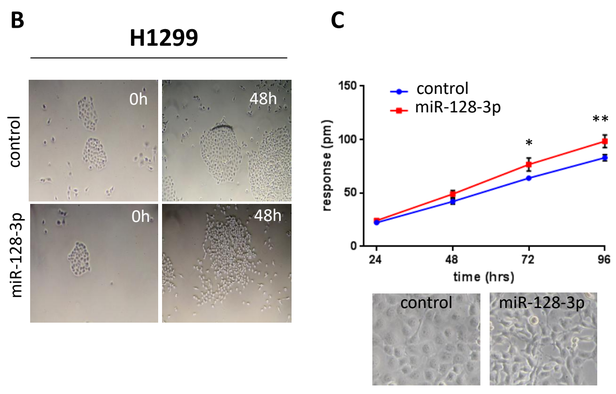




In silico analysis revealed that, among the putative targets of miR-128-3p, Drosha and Dicer were scoring high. By performing luciferase assays, we observed that miR-128-3p significantly reduced the relative luciferase activity of the wild-type reporters but not that of mutant reporters with the full-length 3'UTR of Drosha or Dicer. Moreover, miR-128-3p ectopic expression reduced the protein levels of Drosha and Dicer, and, conversely, its depletion determined increased Drosha and Dicer protein levels (**B**) These findings indicated a direct inhibitory effect of miR-128-3p on Drosha and Dicer expression.

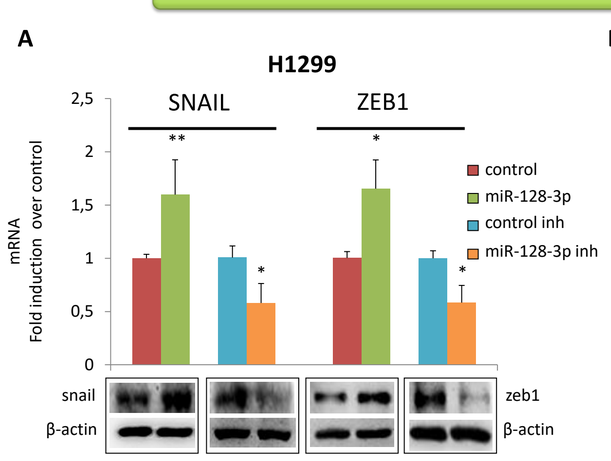
4. Ectopic expression of miR-128-3p promotes migratory phenotype in lung cancer cells

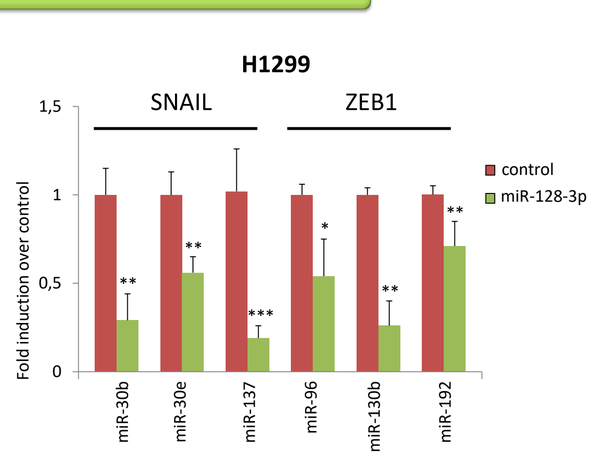


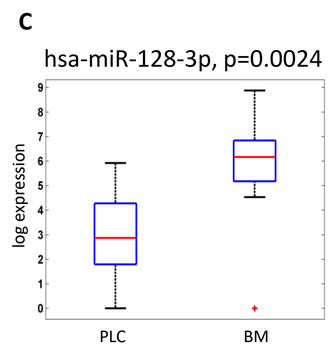


By performing both trans-well and wound-healing migration assays in H1299 and A459 cells, we found that miR-128-3p-expressing cells were hyper-migratory (**A**). Conversely, miR-128-3p depletion strongly discouraged the migration of H1299 cells (**A**). Moreover, in NSCLC cells expressing ectopic miR-128-3p, we observed a striking change in cell morphology, consisting of a shift from a cobblestone shape, typical of epithelial phenotype, into a spindle-fibroblast-like morphology, with extensive cellular scattering (**B**). Such changes were quantitatively addressed by means of label-free assays and were not due to any alteration in cells viability (**C**).

5. MiR-128-3p-mediated miRNAs down-regulation promotes EMT program

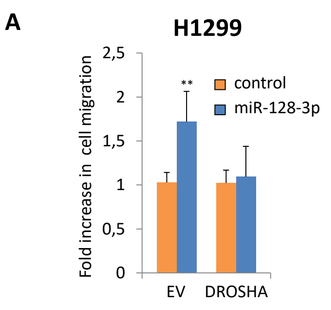


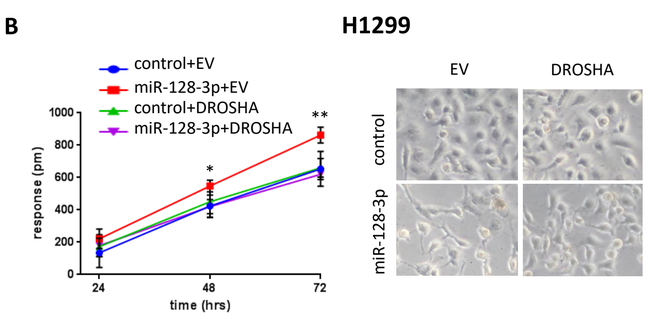


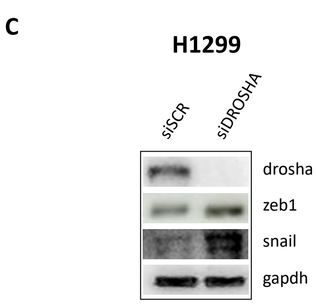


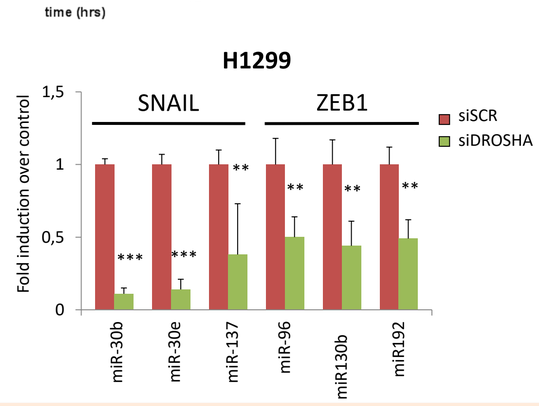
We found that the expression levels of two key transcription factors, SNAIL and ZEB1 were significantly increased in H1299 cells upon miR-128-3p over-expression (**A**). On the contrary, miR-128-3p depletion in H1299 cells determined a significant reduction of both SNAIL and ZEB1 mRNA and protein levels (**A**).
Moreover, upon miR-128-3p ectopic expression, there was a significant decrease in the expression levels of all of the anti-EMT miRNAs previously reported to target SNAIL (miR-30b, miR-30e, miR-137) and ZEB1 (miR-96, miR-130b, miR-192) (**B**).
In support of a key role for miR-128-3p in determining metastatic potential of lung cancer cells, we also had an *in vivo* evidence: in a casuistry of 13 primitive lung adenocarcinoma tissues and their matched brain metastasis, that we previously profiled for miRNAs expression, we observed a significant up-regulation of miR-128-3p in brain metastases compared to primary lesions (**C**).

6. Drosha depletion in lung cancer cells mimics miR-128-3p effects on cell migration and EMT



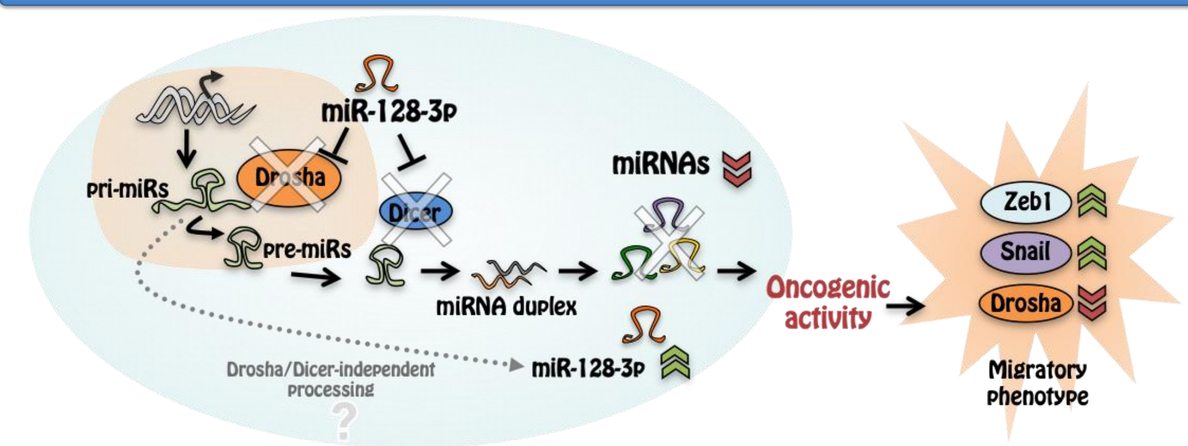






Ectopic expression of Drosha in miR-128-3p-expressing H1299 cells rescued the migratory phenotype (**A**). Moreover, morphological changes were rescued in miR-128-3p-expressing H1299 cells upon ectopic expression of Drosha (**B**) All these data suggested an implication of Drosha in miR-128-3p-mediated EMT. Drosha depletion determined also an increase in SNAIL and ZEB1 protein levels concomitantly with a reduction in the expression of miRNAs targeting SNAIL or ZEB1 (**C-D**).

Conclusions



In lung cancer cells, the oncogenic miR-128-3p directly binds to Drosha and Dicer 3'UTR determining the inhibition of their expression. The consequence is a significant alteration in miRNAs biogenesis, characterized by a global down-regulation in miRNAs expression, a feature known to promote tumorigenesis. Among the down-regulated miRNAs by miR-128-3p, there are a group of miRNAs that target SNAIL and ZEB1,two of the major players of EMT. This event leads to an up-regulation of SNAIL and ZEB1 and to a consequent induction of EMT that promotes metastatic potential of lung cancer cells. MiR-128-3p levels might be kept high through alternative processing mechanisms that are Drosha/Dicer-independent.