miR-26a targets identification in prostate cancer cell lines using miRNA pull-out assay

G. Berti[1], M. Evangelista[1], G. Rainaldi[1], F. Russo[2], R. D'Aurizio[2], M. Pellegrini[2], M. Rizzo[1]

1 Institute of Clinical Physiology CNR, Pisa, Italy 2 Laboratory of Integrative Systems Medicine Institute of Informatics and Telematics and Institute of Clinical Physiology CNR, Pisa, Italy

Introduction. The re-expression of tumor suppressor (TS) miRNAs in cancer cells in which they are downregulated (miRNA replacement therapy) has been evaluated as a promising approach to inhibit tumor proliferation both in in vitro and in vivo models. As a single miRNA simultaneously targets hundreds of genes, it derives that the TS-miRNA re-expression gives rise to a broad inhibition of pro-tumorigenic genes and pathways. In this work we investigated the molecular mechanism at the basis of the antiproliferative potential of the TS-miR-26a by identifying all its targets in prostate cancer (PCa) cell lines using the miRNA pull-out assay.

Material and methods.miR-26a was transfected using the appropriate transfectant for each tumor cell lines. Cell proliferation was detected with crystal violet staining. miRNA pull-out assay was performed using biotinylated synthetic version of miR- 26a and the miRNA/target complexes isolated with streptvidine sepharose high performance (GE Health care). RNA-seq was performed with TruSeq stranded total RNA sample preparation kit and sequenced with HiSeq 2000 (Illumina).

Results and discussion. We rst demonstrated that miR-26a was downregulated in several tumor cell lines (including PCa cell lines). By overexpressing miR-26a in some of these tumor cell lines we established its tumor suppressor activity in that it was able to inhibit cell proliferation. In particular, it was able to affect cell proliferation in both PC-3 and DU-145 PCa cells. To identify the miR-26a targets in PCa, the miRNA pull-out assay was performed in DU-145 cells. Using this approach we were able to isolate the miRNA/target complexes that we sequenced using high-throughput technology. We obtained 1423 transcripts and we found that 85% of them presented canonical miRNA binding sites predicted by more than one predictive algorithms, suggesting that the miRNA/targets isolation was successful. These results were reinforced by the fact that some of the identi ed transcripts were miR-26a targets already validated in other biological contests. Finally, the isolated targets were signi cantly enriched of transcript belonging to biological processes relevant for cancer proliferation.

Conclusion. The results indicate that the TS-miRNA pull-out assay protocol may be useful for the identi cation of TS-miRNA targets involved in key anti-tumorigenic processes and for that possible targets for anticancer therapy.