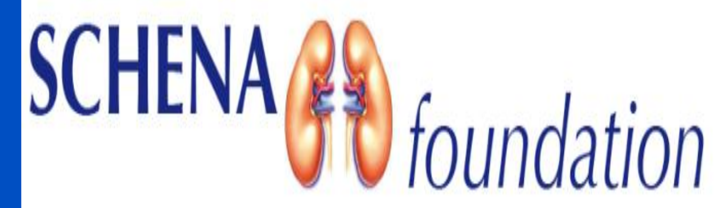
microRNA PROFILING USING NEXT-GENERATION SEQUENCING IN RENAL CANCER STEM CELLS: A NEW REGULATORY MECHANISM

Grazia Serino[1], Fabio Sallustio[2], Vanessa Galleggiante[3], Monica Rutigliano[3], Claudia Curci[2], Giuseppe Lucarelli[3], Pasquale Ditonno[3], Sharon N. Cox[4], Michele Battaglia[3], Francesco Paolo Schena[2,4]

[1]IRCCS "de Bellis", Castellana Grotte, BA, Italy
[2]Nephrology, Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari, Italy.
[3]Urology, Andrology and Kidney Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari
[4]Schena Foundation, Research Center for Renal Diseases, Bari, Italy.



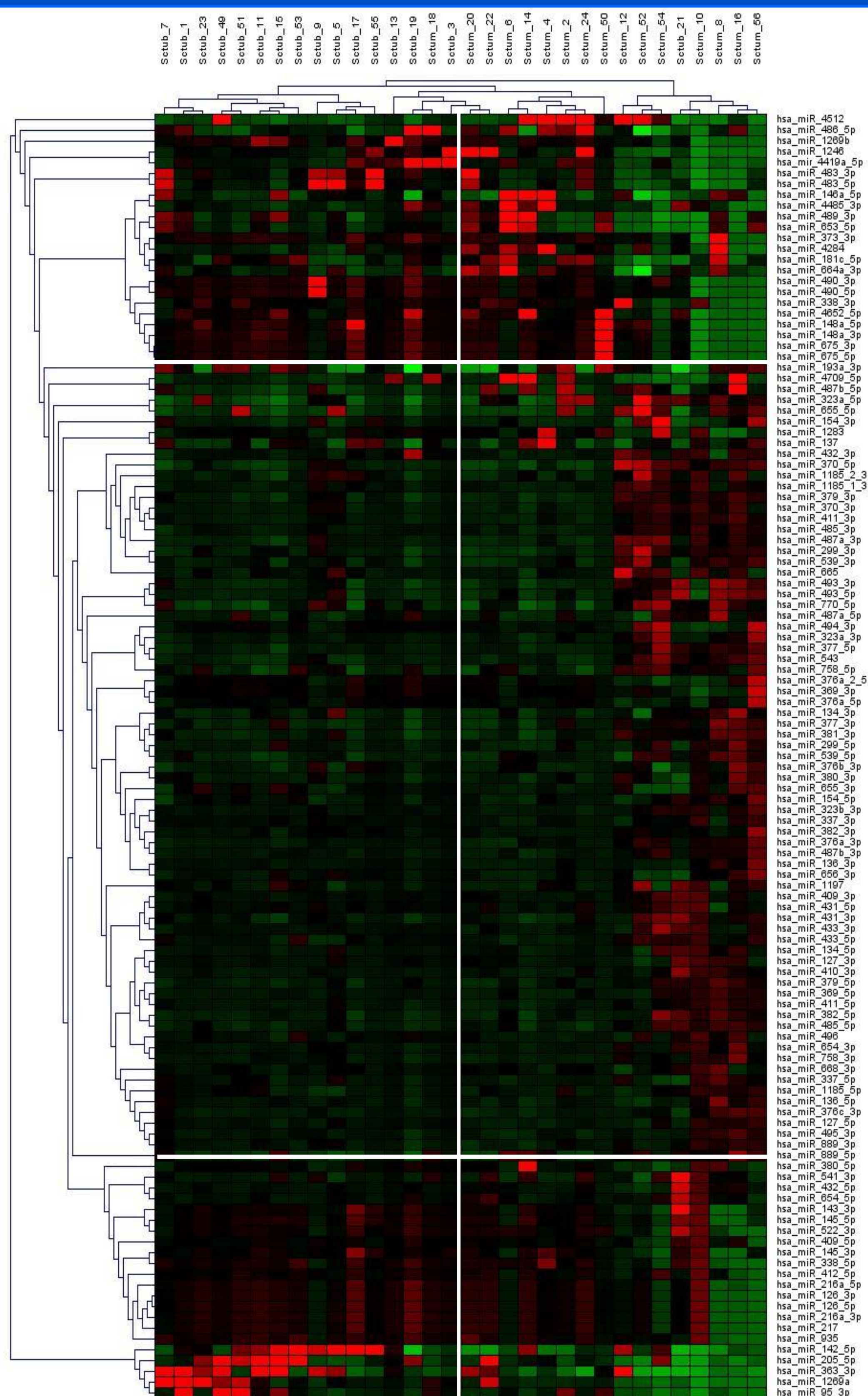
Introduction and Objectives

Clear cell renal cell carcinoma (ccRCC) is the most common form of kidney cancer in adults. Recent evidences show that in several human cancers a small subset of tumor cells called cancer stem cells (CSCs) is present. These cells regulate self-renewal and differentiation of cancer cells, they are also responsible of tumor formation, growth, metastasis, drug resistance and recurrence. CSCs have been found and characterized also in ccRCC tissue.
To date, the role of microRNAs (miRNAs) in ccRCC has been extensively studied, but their function in renal CSCs has not yet been reported.
The goal of this study was to identify and characterize deregulated miRNAs in renal CSCs compared to healthy tubular Adult Renal Stem/Progenitor Cells (tARPCs).

Methods

We isolated CD133+/CD24+ cells from healthy and tumor renal tissue of 28 patients who underwent nephrectomy for ccRCC. Cells were characterized for their mesenchymal phenotype and stemness proteomic profile. The global miRNA profile was identified using small RNA-Seq (llumina) from 16 tARPCs and 16 renal CSCs. miRNA differential expression analysis was done using Bioconductor edgeR software. To study the molecular mechanisms in which the miRNAs were involved, we performed a bioinformatic analysis to predict target genes and specific deregulated pathways. Sequencing results were confirmed by qRT-PCR.

Results I



miRNA differential expression analysis

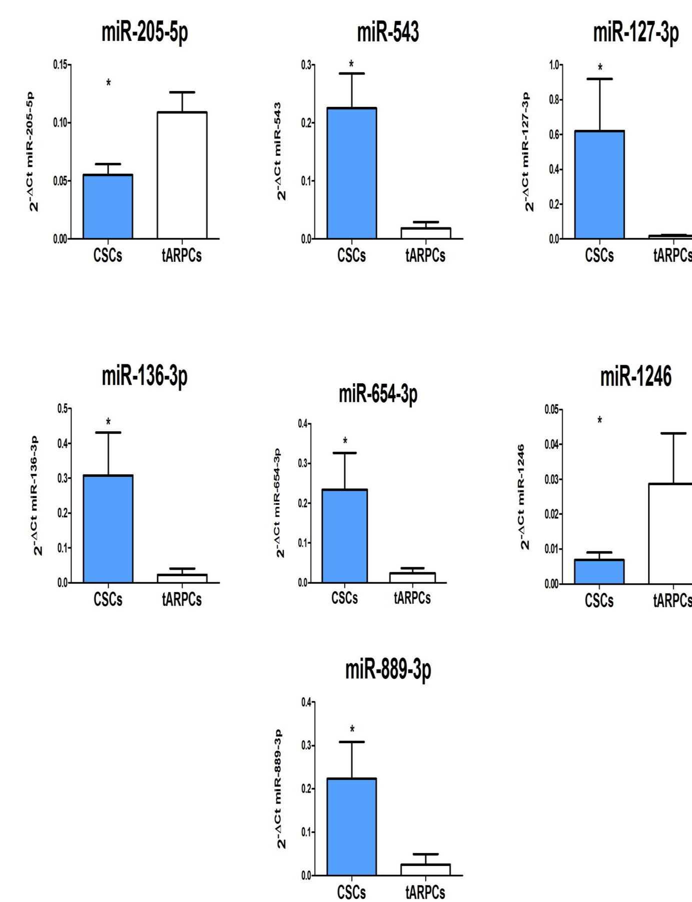
We performed a global miRNA expression profile on 16 tARPCs and 16 renal CSCs and identified 120 differentially expressed miRNAs in renal CSCs compared to the healthy counterpart, with an adjusted p-value <0.05 and a Log Fold Change >1.5. Of these miRNAs 12 were downregulated and 108 were upregulated.
Many of these deregulated miRNAs are involved in several pathways typical of cancer and renal cell carcinoma, as TGF-β signaling, PI3K-AKT signaling, WNT signaling.

Results II

Bioinformatic analysis of miRNA target genes

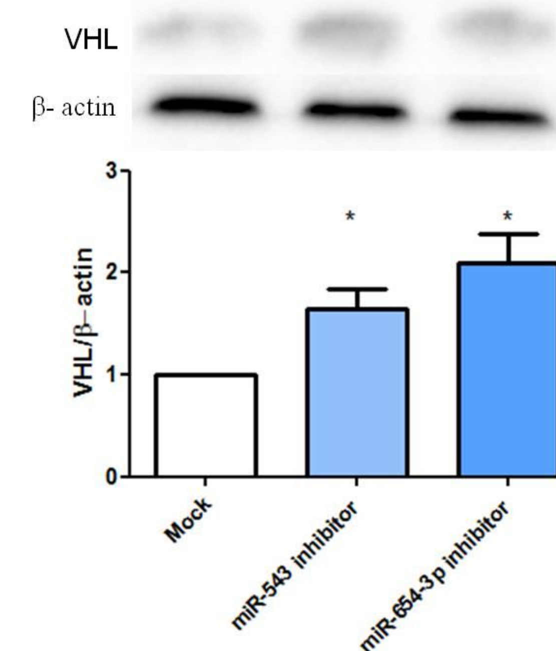
We found that 48 upregulated miRNAs codified all together in a cluster on chr14:101341370-101533138, near the region 14q LOH that is associated with tumor progression of ccRCC.
Moreover, as shown in sequence alignment of 3' UTR of VHL mRNA, some of these miRNAs putatively regulated this gene that is involved in the ccRCC pathogenesis.

Validation of sequencing data with Real-Time PCR



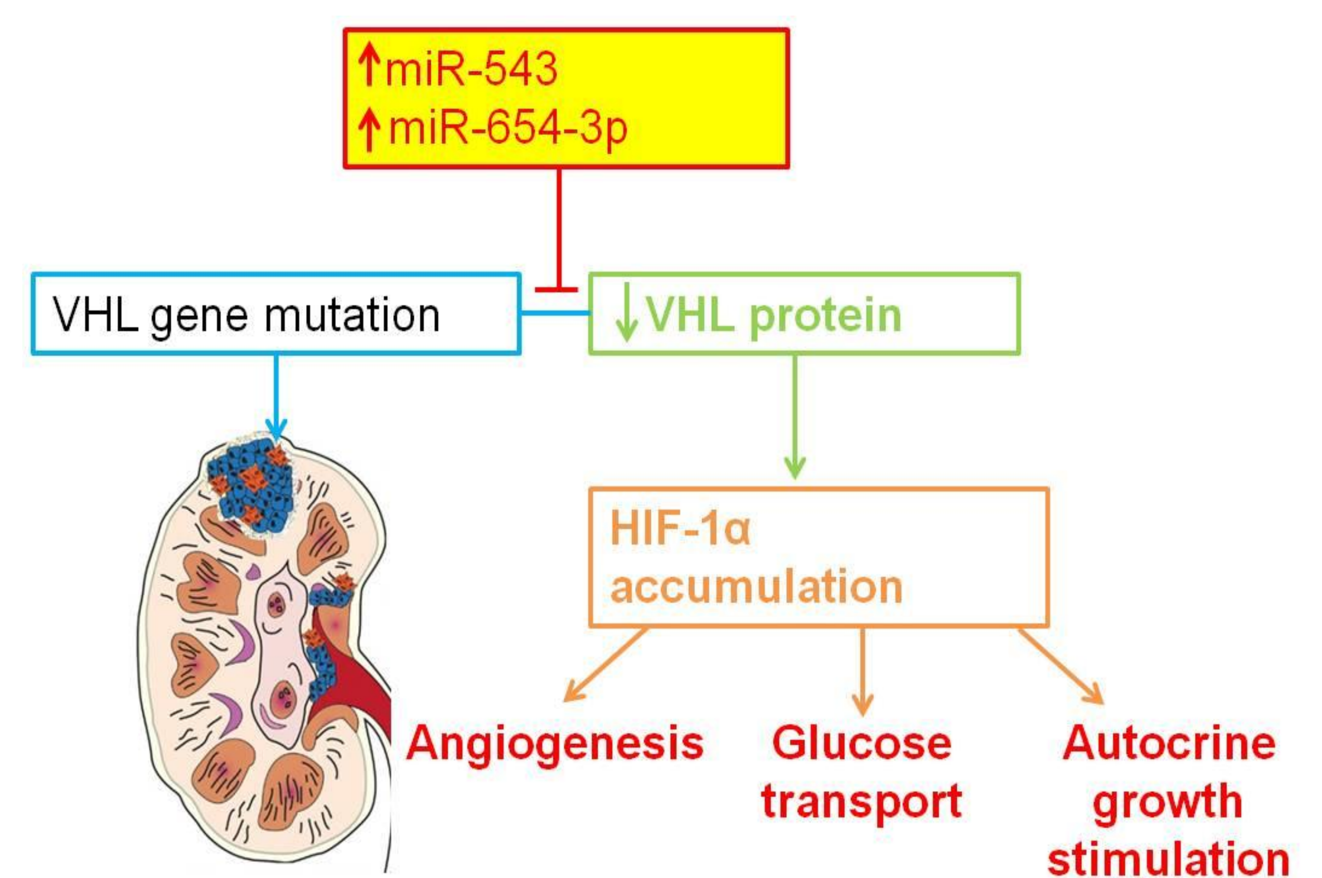
We confirmed data obtained from sequencing with Real-time PCR on miR-205-5p, miR-543, miR-127-3p, miR-136-3p, miR-654-3p, miR-1246 and miR-889-3p in renal stem cells (tARPCs and CSCs) isolated from an independent cohort of 12 ccRCC patients.

Functional validation of miR-543 and 654-3p target



We demonstrated that the expression of VHL depends on high levels of miR-543 and miR-654-3p and that blocking the 2 miRNAs , VHL protein expression increase.
Transfection of CSCs with 100 nM of miR-543 inhibitor or miR-654-3p inhibitor resulted in a 1.6 and 2 fold increase of VHL protein expression, respectively, as shown by Western blot. β-actin was used as endogenous control. * $p<0.05$

Conclusions



Our results demonstrate an additional mechanism that regulates the production of VHL protein in ccRCC. We show that the upregulation of miR-543 and miR-654-3p lead to VHL protein reduction. Therefore, VHL protein cannot target and degrade HIF-1α that overaccumulates and causes increased transcription of downstream genes.
These new molecules could provide a potential target for new therapeutic approaches in ccRCC.

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