UNIVERSITY OF CYPRUS
DEPARTMENT OF BIOLOGICAL SCIENCES

The Department of Biological Sciences cordially invites you to the thesis defense of the PhD candidate

Andrea Christofides
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entitled

"The potential role of miR-548c-5p in regulating *FOXC2* transcription in differentiating human podocytes"

Abstract

Podocytes are highly differentiated epithelial cells outlining glomerular vessels. FOXC2 is a transcription factor essential for inducing podocyte differentiation, development and maturation, and is considered to be the earliest podocyte marker. It has been found that microRNAs, a class of short non-coding single-stranded RNA molecules with a prominent role in the regulation of gene expression, are essential for podocyte function, as podocyte specific dicer knockouts fail to maintain glomerular function. miRNA prediction analysis revealed a full-length target site for the primate-specific miR-548c-5p at a genomic region >8kb upstream of *FOXC2*. Interestingly, both miR-548c-5p and this specific target site are absent from non-primates. It was hypothesised that the transcription rates of *FOXC2* during podocyte differentiation might be tuned by miR-548c-5p through this target site. Experiments were performed with cultured human podocytes, transfected with luciferase reporter constructs bearing this target site region within an enhancer element of the native plasmid. The results confirmed a seed-region driven targeting potential by the miRNA, with mimics downregulating and inhibitors enhancing luciferase activity. Introducing mutations into the miRNA target seed region abolished the expected response. Moreover, also in cultured podocytes, FOXC2 mRNA and protein levels responded to miR-548c-5p abundance in a very coordinated manner before and after induction of differentiation, with high statistical significance. Ago-ChIP experiments using a Pan-Ago antibody revealed occupancy of the miRNA target site by miRNA/RISC in undifferentiated cells and its release when differentiation is initiated. The target site is thus allowed to interact with the gene's promoter region leading to amplification of *FOXC2* expression, as shown by chromosome conformation capture and qRT-PCR. Results are suggestive of an enhancing role for the region bearing the miRNA target site. Moreover, the expression

pattern of *FOXC2* during podocyte differentiation seems to be affected by miR-548c-5p, as removal of miR-548c-5p results in increased FOXC2 protein levels and cells resembling those undergoing differentiation. More specifically, in Western blot and immunofluorescence experiments, allowing hPCs to recover from the effect of several sequential miRNA mimic transfections, resulted in a rescue in protein levels and resumption of differentiation by examining the nuclear or cytoplasmic localization of FOXC2. Taking everything into account, a mechanism is proposed by which the RISC/miR-548c-5p complex influences the expression of FOXC2 and consequently affects podocyte differentiation. Collectively, results indicate a well-orchestrated regulatory model of *FOXC2* expression by a remote upstream target site for miR-548c-5p.

Tuesday, December 3, 2019 at 10:00
Building ΧΩΔ02, Room B107 (Panepistimioupoli Campus)

The presentation is open to the public.