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A combinatorial study to identify microRNAs and their targets in Polycystic Kidney Diseases

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MiRNAs are short noncoding RNAs of about 22nt length that have recently been shown to play important roles in mammalian gene expression They induce posttranscriptional gene repression by blocking protein translation (by binding to the 3' UTR of their target genes) or by inducing mRNA degradation, and have the potential to play central roles in physiological and pathological conditions. Recently, it has been shown that miRNAs can also increase translation. The physiological conditions of a cell seem to affect the recruitment of regulatory proteins, which can alter the effect of a miRNA. MiRNAs are involved in diverse processes, including cellular differentiation, proliferation and apoptosis. Recent evidence suggests also their importance for cancerogenesis. By far the most important model systems in cancer research are mammalian organisms. Thus, we decided to compile comprehensive information on mammalian miRNAs, their origin and regulated target genes in an exhaustive, curated database called Argonaute (http://argonaute.uni-hd.de/). Argonaute collects latest information from both literature and other databases. It hosts information on origin of a miRNA, the tissue specificity of its expression and its known or proposed function, its potential target genes including GeneOntology annotation, as well as miRNA families and proteins known to be involved in miRNA processing. Additionally, target genes are linked to an information retrieval system that provides comprehensive information from sequence databases and a simultaneously search of MEDLINE with all synonyms of a given gene.

Additionally, we have developed a search tool on the principle of perfect seed pairing that allows to search with a set of potential target sequences to get information which miRNAs from Argonaute match with their seed regions, being the region between nucleotides 1 and 9 that share 7 to 8 consecutive, perfectly complementary bases.

We have explored the possible roles of miRNAs in genetic regulation in Polycystic Kidney Disease (PKD) using Argonaute. In a well known rat model system (Han:SPRD cy/+ rat model), we used a combinatorial approach involving prediction with two different tools, data mining, microarray analysis and further verification by qPCR, to profile the miRNAs involved in PKD. In parallel we also sketched the changes in mRNA transcript patterns during PKD using Affymetrix arrays. When we compared the mRNA transcript profile differentially regulated during PKD, with the Argonaute, there were few genes reported as miRNA target. We predicted miRNAs for differentially regulated genes using two tools, miRanda and TargetScan. The individual predictions showed that there were several miRNAs targeting these genes, but the overlap between these two prediction exercises was not very strong. We resorted to verify these with LNA-based miRNA micorarrays. But to our surprise, when we profiled the miRNAs using microarray, only thirty miRNAs were found differentially regulated. To further verify some (miR-7, miR-21, miR-31, miR-34b, miR-150, miR-185 and miR-214) of the miRNAs found differentially regulated by miRNA-chip analysis we used the qPCR technique. Only four of the seven miRNAs tested could be verified by qPCR.

While this work establishes a multi-layered approach for analysis of miRNA mode of genetic regulations of PKD, a lot more awaits further. We predict that several of the differentially regulated genes are miRNA targets and some miRNAs like miR-21, miR-31, miR-150 and miR-214 seem to be important players in such interaction. It is interesting to note that miR-31, miR-150 and miR-214 have not been previously reported in kidney. Only miR-21 has been reported in kidney but its function has yet not been established. In order to properly understand Cystic Diseases of Kidney development and design new therapeutic measures, knockout and over-expression studies would provide detail insight into the regulatory interaction of these miRNAs with their targets. Antagomirs could be used to silence specific miRNAs like miR-31 or miR-21 in kidneys to study the molecular function of these miRNAs *in vivo*.