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Genomic and functional analysis of microRNAs and PIWI-interacting RNAs in human cancers

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ABSTRACT

Small non-coding RNAs, including microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs), play an important role in gene expression regulation and are involved in many biological and pathological processes. Study of small RNA expression and function in cancer cells could contribute to greater understanding of how small RNAs are involved in cancer development. The main goal of this thesis work was to investigate the expression and functions of these small RNAs in adrenocortical carcinoma (ACC) and testicular germ cell tumors (TGCTs).

In **Paper I**, we identified a set of miRNAs that could distinguish ACC from its normal and benign counterparts. *miR-483-3p*, *miR-483-5p*, *miR-21* and *miR-210* expressions were higher, while *miR-195* and *miR-497* were lower in ACC. Suppression of *miR-483-3p* and over-expression of *miR-195* or *miR-497* reduces cell proliferation and increases apoptosis in ACC cells. The protein expression of PUMA, a target of *miR-483-3p,* is down-regulated in ACC and inversely correlated with the increased expression of *miR-483-3p.* Additionally, increased expressions of *miR-503*, *miR-1202*, and *miR-1275* are associated with short overall survival of ACC patients.

In **Paper II**, we evaluated the expression levels of core components of miRNA biogenesis in ACC. We observed significant increased expressions of TARBP2, DICER and DROSHA in ACC as compared to benign tumors or adrenal cortices. Higher *TARBP2* mRNA is a strong predictor for the discrimination of ACC from the non-carcinoma cases. Suppression of TARBP2 decreases cell proliferation and increases apoptosis in ACC cells. We also demonstrate that copy number gain of *TARBP2* gene and its regulation by *miR-195* and *miR-497* could contribute to TARBP2 overexpression in ACC.

In **Paper III**, we identified a set of deregulated miRNAs (including reduced expression of *miR-506~514* cluster and increased expressions of *miR-21* and *miR-223*) in TGCT. Overexpression of *miR-514a-3p*, a member of *miR-506~514* cluster, inhibits cell proliferation and induces apoptosis in TGCT cell lines. The apoptotic effect of *miR-514a-3p* is mediated through direct regulation of PEG3. Silencing of PEG3 or overexpression of *miR-514a-3p* leads to reduced nuclear accumulation of p50 and NF-κB reporter activity, suggesting that *miR-514a-3p* and PEG3 play a role in NF-κB pathway. Importantly, high expression of PEG3 and nuclear p50 were found in a large proportion of TGCT samples.

In **Paper IV**, we show that global piRNA expression is down-regulated in both human and mouse TGCTs compared to normal testes. Using high-throughput sequencing approaches, we demonstrate that most piRNA precursor transcripts were present and transcriptionally active in TGCTs. Our RNA sequencing data indicate that the piRNA biogenesis factors *MOV10L1*, *DDX4*, *MAEL*, *PIWIL3*, and *TDRD6* were significantly down-regulated in TGCTs, suggesting loss of piRNA expression in TGCT is likely due to impaired processing of piRNA precursors.

Overall, this thesis work describes the biological and clinical role of small RNAs and deregulation of their processing factors in both human ACC and TGCTs.