“Functional characterization of *Disrupted in Renal Carcinoma 3* (*DIRC3*) long non-coding RNA in differentiated thyroid cancers”

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ABSTRACT

Differentiated thyroid cancers (DTCs) are endocrine malignancies with a strong but ill-defined hereditary predisposition. Genome-wide association studies highlighted several genomic loci associated with increased risk of DTCs. Relatively strong associations were detected for germline variants located in *disrupted in renal carcinoma 3* (*DIRC3*), a poorly characterized long non-coding RNA gene. This PhD thesis is the first to investigate the functional role of *DIRC3* in thyroid carcinogenesis. Using clinical material and bioinformatic data I have established that *DIRC3* is downregulated in DTCs. *DIRC3* expression level in malignant tissue appeared to influence the risk of DTC recurrence. *DIRC3* was found to be strongly co-expressed with *insulin-like growth factor binding protein 5* (*IGFBP5*), a gene known to regulate cellular response to insulin-like growth factor 1 (IGF-1). A set of comprehensive *in vitro* experiments demonstrated that *DIRC3* transcripts are enriched in the nucleus, where they promote expression of *IGFBP5*. Silencing of *DIRC3* in thyroid cancer cell lines produced a phenotypic dichotomy: it boosted migration and invasiveness, decreased the starvation-induced apoptosis, but also abrogated the MTT reduction rate (the indirect indicator of cell viability and proliferation). Gene rescue experiments indicated that this pro-migratory phenotype was related to the alterations in expression of *IGFBP5*. In contrast, the influence of *DIRC3* on the results of MTT assays appeared be at least partially independent from *IGFBP5*. Transcriptomic profiling of thyroid cancer cells experiencing silencing of *DIRC3* or *IGFBP5* showed a significant redundancy in the activities of both genes. Gene ontology analysis indicated that terms significantly enriched in the response to silencing of *DIRC3* were involved in biological processes related to the cellular migratory potential. I also demonstrated that downregulation of *DIRC3* enhanced the susceptibility of cancer cells to the stimulation with IGF-1, what consequently promoted the oncogenic AKT signaling pathway. Overexpression experiments that utilized *CRISPR activation* (CRISPRa) successfully upregulated *DIRC3*. While this did not elicit changes in expression of *IGFBP5*, the MTT reduction rate was consequently augmented in thyroid cancer cells. Finally, I utilized CRISPR/Cas9 to edit one of the top germline DTC

susceptibility variants in *DIRC3*, rs11693806. Modification of a heterozygotic rs11693806[C/G] thyroid cancer cell line into monoallelic [G/-] or homozygotic [G/G] derivatives produced a marked downregulation of *DIRC3* and *IGFBP5*. Furthermore, these genomic modifications phenocopied the pro-migratory effects observed after silencing of *DIRC3*. I also confirmed that these genomic modifications resulted in global transcriptomic alterations, often affecting genes involved in different aspects of carcinogenesis.

In conclusion, *DIRC3* emerges as a lncRNA gene functionally implicated in DTCs. Its downregulation stimulates cancer invasiveness, but on the other hand it may produce inhibitory effects in MTT assays. Mechanically, *DIRC3* regulates expression of *IGFBP5*, thus contributing to the altered sensitivity of cancer cells to IGF-1. Accordingly, I propose an interplay between the germline cancer risk variants, *DIRC3* expression and IGF-1 signaling as a mechanism that jointly orchestrates thyroid carcinogenesis.