ABSTRACT

Papillary thyroid carcinoma (PTC) is the most frequent thyroid malignant neoplasia. Oncogene activation occurs in more than 70% of the cases. *BRAF* mutations occur in about 40% of PTCs, whereas RET rearrangements (RET/PTC oncogenes) are present in about 20% of cases. Finally, RAS mutations and TRK and PPARG rearrangements account for about 5% each of these malignancies.

However, despite the presence of tumor-initiating driver events, cancer results from the progressive accumulation of mutations in genes that confer growth advantage over surrounding cells. A better understanding of molecular alterations of PTC will provide important insights into cancer etiology. It will also lead to advance in their diagnosis, possibly opening the way for developing novel molecular therapies.

Thus, the aim of this PhD project is to deeply explore the transcriptome of PTC in order to identify new driver events in this type of cancer.

In the first part of this study, we used RNA-Sequencing in a discovery cohort of 18 patients with papillary thyroid carcinoma to identify fusion transcripts and expressed mutations in cancer driver genes. Furthermore, we used targeted sequencing on the DNA of these same patients to validate identified mutations. We extended the screening to thyroids of 50 PTC patients and of 30 healthy individuals. Using this approach we identified new somatic mutations in *CBL*, *NOTCH1*, *PIK3R4* and *SMARCA4* genes. We also found mutations in *DICER, MET* and *VHL* genes, previously found mutated in other tumors, but not described yet in PTC. We also identified a new chimeric transcript generated by the fusion of lysine deficient protein kinase 1 (*WNK1*) and beta-1,4-N-acetyl-galactosaminyl transferase 3 (*B4GALNT3*) genes and correlated with an overexpression of *B4GALNT3* gene.

Moreover, although protein coding genes play a leading role in cancer genetics, in recent years, many studies focused on a novel class of non coding RNAs, long non coding RNAs (lncRNAs), which regulate the expression levels of protein coding genes. Since deregulated expression of lncRNAs has been reported in many cancers, it suggests that that they may act as potential oncogene or tumor-suppressor.

Thus, to assess if lncRNAs can exert a tumorigenic role in thyroid, in the second part of my PhD project I systematically quantified lncRNAs' expression in PTC vs

healthy thyroids using our RNA-Seq data. Combining *ab initio* reconstruction to a custom computational pipeline we found that novel and known lncRNAs are significantly altered in PTC, and some of them are possibly associated with cancer driver genes. Then we extensively focused on an unannotated lncRNA transcribed antisense to *MET* oncogene, named in this study *MET-AS*. Both genes are significantly up-regulated in a sub-class of PTCs - i.e. patients with BRAF gene mutations and RET gene rearrangements, compared to other PTCs and "non-tumor" thyroid biopsies. Preliminary data indicate that *MET-AS* knockdown induces down-regulation of *MET*, and induces a changes in cell cycle in a PTC cell line, suggesting the novel lncRNA might be a new *MET* regulator. Further studies should be conducted to demonstrate detailed mechanism of our findings.

Finally, our data confirmed the genetic heterogeneity of papillary thyroid carcinoma revealing that gene expression correlates more with the mutation pattern than with tumor staging. Overall, this study provides new information about PTC genetic alterations, suggesting potential pharmacological adjuvant therapies in PTC.