INTEGRATIVE ANALYSIS OF EXPRESSION AND DNA METHYLATION DATA REVEAL PUTATIVE MOLECULAR DRIVERS IN PAPILLARY THYROID CANCER

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BACKGROUND: The worldwide incidence of thyroid cancer has increased along the last decade. Papillary thyroid carcinoma (PTC) is the most common thyroid cancer subtype characterized by *BRAF*V600E mutation in 45% of the cases. Epigenetic alterations have been reported in PTC development and some of them were associated with *BRAF* mutation. The goal of this study is to evaluate methylation profile of PTC versus normal thyroid (NT) samples and according to *BRAF*V600E mutation. The methylation data were integrated with expression profile aiming to identify potential genes involved in PTC development and progression, which are regulated by methylation.

HYPOTHESIS: DNA methylation regulates genes associated with PTC development and has the potential to point out molecular drivers.

PATIENTS AND METHODS*:* The methylation profile of 41 PTC versus NT samples and 28 *BRAF*V600E PTC versus 13 *BRAF*WT PTC were investigated using the Methylation 450 Human Infinium®BeadChip platform (Illumina) following to the manufacturer's recommendations. To normalize and analyze the data the SVA, wateRmelon and LIMMA packages were used. To identify differentially methylated probes, paired analysis was performed using delta beta 0.15 and p-value <0.05 and for the analysis according *BRAF* mutation. The delta beta used was 0.10 and the p-value <0.05. Differential expressed genes (Sure Print G3 8x60K; Agilent Technologies) in the same set of PTC were assessed from a previous study (Barros-Filho et al. 2015). The integrative analysis was performed using 34 PTC cases with both DNA methylation and expression data (r- and p<0.05). Six genes were selected to be confirmed using pyrosequencing and qRT-PCR in 94 PTC and 50 NT samples. Results were compared with The Cancer Genome Atlas (TCGA) database. Genes potentially regulated by methylation were inputted to *in silico* pathway analysis using Ingenuity Pathway Analysis (IPA) and KEGG Orthology Based Annotation System (KOBAS) software.

RESULTS*:* Methylation analyses revealed 6,070 CpG differentially methylated (5,425 hypomethylated and 645 hypermethylated) in PTC and 4,563 CpG differentially methylated (3,312 hypomethylated and 1,251 hypermethylated) in *BRAF*V600E cases. Integrative analyses revealed 214 genes (PTC *versus* normal) and 69 genes (*BRAF*V600E positive *versus* negative) with negative correlation between DNA methylation and gene expression. Using TCGA database, 86% and 52% (PTC and *BRAF* analysis, respectively) of methylation data were confirmed. Methylation and expression levels of *ERBB3, FGF1, FGFR2, GABRB2, HMGA2* and *RDH5* were confirmed suggesting their involvement in PTC development. Moreover, FGF signaling pathway showed aberrant activation in this tumor type.

CONCLUSION: A differential methylation profile was detected in the comparison of PTC and NT samples as well as with BRAF mutation. We highlighted the involvement of genes regulated by methylation in PTC and associated with the tumor development.

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