METHYLATION PROFILE OF FOLLICULAR CELL DERIVED THYROID LESIONS

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BACKGROUND: Papillary thyroid carcinoma (PTC) comprises 80-85% of the diagnosed thyroid cancers, followed by follicular (FTC), poorly differentiated (PDTC), and anaplastic carcinomas (ATC). Cancer cells typically exhibit aberrant DNA methylation patterns that can drive malignant transformation, but the involvement of epigenetic alterations is not well understood in TC subtypes. The pathway analysis to study the genome wide methylation data has the potential to highlight relevant biological processes and point out new drivers involved in the thyroid carcinogenesis.

HYPOTHESIS: A differential methylation profile involved in the thyroid lesions pathogenesis has the potential to reveal mechanisms underlying the evolution of each tumor type and provide new epigenetic markers.

METHODS: Differential methylation analyses were performed using the Infinium® Human Methylation450 BeadChip (Illumina) in 50 non-neoplastic thyroid tissues (NT), 17 benign lesions (BTL) (8 adenomas, 6 goiters and 3 thyroiditis) and 74 thyroid carcinomas (60 PTC, 8 follicular - FTC, 2 Hurthle carcinomas - HCC, 1 poorly differentiated - PDTC and 3 anaplastic carcinomas - ATC). 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 0.20 and adjusted p-value <0.05. Unsupervised hierarchical clustering analysis was performed using complete linkage and one-minus-correlation distance parameters, comprising the most variably methylated probes (interquartile range (IR) >0.2) with BRB Array Tools v. 4.4.0 software. *In silico* pathway analysis was conducted with Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) and KOBAS (v. 2.0; http://kobas.cbi.pku.edu.cn/home.do) software. Only differentially methylated probes mapped in the promoter region were considered in this analysis.

RESULTS: The hierarchical clustering analysis using 8,016 probes revealed 4 main clusters. The cluster 1 was enriched by follicular adenomas, nodular goiter and minimally invasive follicular carcinomas, the cluster 2 by NT, cluster 3 by PTC and cluster 4 by ATC/PDTC, FTC and lymphocytic chronic thyroiditis. The differential methylation analysis revealed more hypermethylated CpG in BTL (531 probes) and FTC/HCC (4,100 probes) compared to NT, whereas hypomethylation was prevalent in PTC (2,773 probes) and ATC/PDTC (28,252 probes). Hypermethylated CpGs were enriched in CpG island regions in all comparisons, whereas hypomethylation was more frequently observed outside of CpG islands. In total, 620 hypermethylated probes were exclusively found in BTL, 113 in PTC, 2,244 in FTC/HCC and 5,411 in PDTC/ATC. Similarly, 95 hypomethylated probes were found in BTL, 1,996 in PTC, 465 in FTC/HCC and 26,734 in PDTC/ATC. A total of 117 pathways was identified by IPA and 129 using KOBAS (Fisher exact test p<0.005). In BTL, 26 pathways using IPA and one using KOBAS were found. In PTC and FTC, vitamin D (VDR) and retinoid X (RXR) were predicted as activated pathways among the 28 and 10 pathways (IPA) and 26 and 28 pathways (KOBAS), respectively. In PDTC and ATC samples, 53 pathways and 73 pathways KOBAS were identified using IPA, among them the Gαi Signaling, a pathway involved in proliferation, survival and cell migration.

CONCLUSION: Together, these data provide a better understanding of epigenetic changes leading to tumor TC development and progression.