Genome-wide promoter methylation analysis in cytologically indeterminate thyroid nodules

Ericka B. Trrabach, MS, PhD1, Amanda Shinzato, BS1, Chin J. Lin MD, PhD2, Suemi Marui MD, PhD1, Antonio M. Lerario, MD, PhD1

1Laboratório de Endocrinologia Celular e Molecular - LIM25, HCFMUSP, São Paulo, Brasil; 2Laboratório de Patologia Cardiovascular - LIM22, HCFMUSP, São Paulo, Brasil.

INTRODUCTION

- Thyroid nodules are extremely common in clinical practice, yet it remained a challenging to differentiate benign and malignant nodules without gland surgical resection.
- Aberrant DNA methylation is ubiquitous in human cancers, including thyroid tumors, and have been used as biomarkers providing a range of opportunities for early detection and noninvasive diagnosis.

OBJECTIVE

- To determine the genome-wide promoter methylation status of cytologically indeterminate thyroid nodules.

PATIENTS AND METHODS

- We evaluated 11 patients (10 females) with thyroid lesions: 3 classical (CV-PTC), 3 follicular variant papillary (FV-PTC), 2 follicular adenomas (FA) and 3 adenomatous goiter (AG). For all samples FNA cytology diagnosis was inconclusive or indeterminate (follicular pattern, Bethesda IV). Mean of age: 46.5 year, range 35-76; Mean of nodules size: 2.0 cm ± 0.79.
- Genomic DNA was extracted from all samples. DNA methylation fraction was enriched using methyl-DNA immunoprecipitation and interrogated on Affymetrix human promoter 1.0 array. For control, DNA from normal thyroid tissue patients' were also extracted and pooled in a single reaction.
- All array data analysis were performed using pre-defined tiling workflow in Partek® Genomics Suite™ software 6.4. P values less than 0.01 were considered statistically significant. For analysis, samples were categorized according to histopathological classification and malignancy.

RESULTS AND DISCUSSION

- Overall samples relations do not demonstrated major differences with no evidence to a pattern sample distribution (Figure 1).

Figure 1: Principal component analysis (PCA) plot of methylation data. (a) histopathological and (b) benign (BE) and malignant (MA) groups.

- Genes differentially hypermethylated were identified in each thyroid tumor subtypes compared to normal tissue: 189 in CV-PTC, 192 in FV-PTC, 313 in FA and 288 in AG (Figure 2A). Comparing, benign (FA and AG) and malignant (CV-PTC and FV-PTC) groups, 139 and 138 hypermethylated loci were exclusively observed, respectively (Figure 2B).

Figure 2: Venn diagrams for lists of differential hypermethylated genes in (a) histopathological and (b) benign (BE) and malignant (MA) groups.

- However, performing class prediction analysis no different gene clusters were observed neither comparing tumors subtypes or benign and malignant groups.
- We further found genes that thought play a role in tumorigenesis selectively hypermethylated in malignant group:

DISCUSSION AND CONCLUSIONS

- Our data suggested that DNA methylation signature of promoter regions were unable to discriminate malignant to benign thyroid tumors in cytologically indeterminate nodules, confirming previous results which demonstrated that DNA methylation profile were only distinguished between well differentiated and non-differentiated thyroid cancers(1).
- However, we could not ruled out the possibility that analysis of a larger numbers of samples may identify a significant methylation differences between these intrinsic tumors subtypes.
- Subsequent analysis with inclusions of a large numbers of patients with thyroid cancers will be required to assess the usefulness of supressor tumoral RPS6 and SLC5A4 as biomarkers.


Support by