Nucleic acid recovery from thyroid fine-needle cytology slides

Ácidos nucleicos extraídos de lâminas com citologia de punção de agulha fina de tiroide

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Much interest has been focused in nucleic acid isolation from tissue samples stored for long periods and their utilization on molecular biomarkers research. However, sample fixation induces chemical modifications in macromolecules (1) that result in a laborious and difficult protocol to extract nucleic acid for molecular analysis. In this context, the article recently published by Kizys and cols. (2) contributed to show efficient methods of nucleic acid recovery from archived formalin-fixed/ paraffin-embedded (FFPE) and fine-needle aspiration (FNA) samples of thyroid tissue.

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Despite the efforts, the issue that remains a challenge in the most effective protocols is to obtain better yield and quality of DNA/RNA due to the damaging effect of the fixation process, particularly for RNA analysis. In contrast with the difficulty in obtaining conserved messenger RNA from archived samples, the fraction of microRNA (miRNA) is less affected by fixation and storage time (3,4). Mature miRNA is a single-stranded noncoding small RNA of ~19-22 nucleotide length that regulates gene expression at the post-transcriptional level (5). These molecules pair to 3'UTRs of target mRNA and thereby cause their silencing or degradation. The miRNA might be a promising prognostic and diagnostic biomarker for thyroid cancer (6).

In this context, we have performed, in archived FNA-stained slides, the analysis of *BRAFT1799A* mutation (7) along with miRNAs expression, *miR-146b* (8) and *let7-f* (9), which were modulated in papillary thyroid carcinoma (PTC) (10,11). The acid guanidinium thiocyanate-phenol-chloroform method (12) using TRIzol™ enabled the RNA/DNA co-isolation from the same archived FNA, despite the reduced number of cells in the cytological slides. From the extracted RNA, we performed real-time PCR to amplify miRNA, and observed high expression of *miR-146b* in PTC compared with goiter (normalized expression *miR-146b/RNU6B*: PTC 21.55 ± 5.83* *vs.* goiter 0.18 ± 0.06, * $P < 0.05$), and no change in *let7-f* expression (normalized expression *let7-f /RNU6B*: PTC 0.30 ± 0.13 *vs.* goiter 0.07 ± 0.02, ns). In addition, DNA obtained was suitable for *BRAF* mutation analysis by RFLP-PCR (Restriction Fragment Length Polymorphism -PCR) in all FNA slides tested.

Using this approach, we observed that it is possible to access both nucleic acids, RNA and DNA, from the same archived thyroid FNA-stained slide. Furthermore, DNA was adequate to perform mutation analysis, and the quality of total RNA and its amount was satisfactory to investigate several miRNAs from the same sample. In conclusion, this procedure could be useful in detecting molecular biomarkers and drawing correlations with cytological analysis, which might improve the accuracy of FNA as a diagnostic tool of thyroid nodule.

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