

SUPPLEMENTAL METHODS

Ultrasound-guided core-needle biopsy

Following local anesthesia with 2% lidocaine, patients underwent ultrasound-guided core-needle biopsy (CNB) utilizing 18-gauge disposable needles with a double-action spring-activated mechanism (1.1- or 1.6-cm excursion; TSK Ace-cut, Create Medic, Yokohama, Japan) [1]. The core-needle was advanced towards the target nodule using a freehand technique, starting from the isthmus of the thyroid. Once the needle tip reached the periphery of the nodule, the stylet and cutting cannula of the needle were sequentially activated. After the CNB, the biopsy site received firm, localized compression for a duration of 10 to 20 minutes [2].

Immunohistochemical staining of the HBME-1 and galectin-3

The CNB samples underwent standard paraffin embedding and subsequent hematoxylin and eosin (H&E) staining. For immunohistochemistry, representative paraffin blocks containing both tumor and normal thyroid tissue were selected. Galectin-3 (Gal-3, dilution 1:200, BenchMark XT auto-immunostainer, Novo, Ventana, AZ, USA) and Hector Battifora mesothelial-1 (HBME-1, dilution 1:200; BenchMark XT auto-immunostainer, Dako, Carpinteria, CA, USA) were used for immunohistochemical staining. Galectin-3 exhibited expression in the cytoplasm and/or nucleus, while HBME-1 showed cytoplasmic and cell membrane expression. The expression of these markers was assessed based on the following criteria: negative (0), focal positive (1, less than 25% of cells), and positive (2, more than 25% of cells), considering the positive expression of tumor cells. In the statistical analysis, cases showing any level of positive staining were categorized as positive [3].

DNA isolation and detection of the *BRAF*^{V600E} and *RAS* mutation

Genomic DNA was isolated from formalin-fixed, paraffin-embedded tissue sections representing each archival tissue block. The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was

utilized for DNA purification. The purified DNA was directly subjected to polymerase chain reaction (PCR) with primers designed to detect each mutation site. The amplification targeted exon 15 of the *BRAF* gene, which contains *V600E* mutation and exons 2 and 3 of the *NRAS*, *HRAS*, and *KRAS* genes. The amplification protocol consisted of initial denaturation at 95°C for 15 minutes, followed by 45 cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C to 65°C for 30 seconds, primer extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The PCR reaction was then cooled to 4°C [4]. The final PCR products were verified through electrophoresis. To determine any genetic mutation, DNA sequencing was carried out employing the antisense primers. The DNA sequences were subsequently analyzed using a DNA analyzer (Bioedit v7.2.0, Ibis Biosciences, Carlsbad, CA, USA). To confirm the presence of *BRAF* and *RAS* mutations, each DNA sample was analyzed at least twice.

SUPPLEMENTAL REFERENCES

1. Yeon JS, Baek JH, Lim HK, Ha EJ, Kim JK, Song DE, et al. Thyroid nodules with initially nondiagnostic cytologic results: the role of core-needle biopsy. *Radiology* 2013;268:274-80.
2. Na DG, Kim JH, Sung JY, Baek JH, Jung KC, Lee H, et al. Core-needle biopsy is more useful than repeat fine-needle aspiration in thyroid nodules read as nondiagnostic or atypia of undetermined significance by the Bethesda System for Reporting Thyroid Cytopathology. *Thyroid* 2012;22:468-75.
3. Park YJ, Kwak SH, Kim DC, Kim H, Choe G, Park DJ, et al. Diagnostic value of galectin-3, HBME-1, cytokeratin 19, high molecular weight cytokeratin, cyclin D1 and p27(kip1) in the differential diagnosis of thyroid nodules. *J Korean Med Sci* 2007;22:621-8.
4. Kim M, Jeon MJ, Oh HS, Park S, Kim TY, Shong YK, et al. BRAF and RAS mutational status in noninvasive follicular thyroid neoplasm with papillary-like nuclear features and invasive subtype of encapsulated follicular variant of papillary thyroid carcinoma in Korea. *Thyroid* 2018;28:504-10.