Supplementary materials and methods

Ultrasound-guided fine-needle aspiration. Using 22~23 G fine-needle aspiration, rapid extraction of samples was performed under ultrasound guidance in the thyroid lesions. The extraction process was performed within a few seconds, and the needle aspirates were evenly spread on the slides. The slides were fixed in 95% ethanol for 5 minutes at room temperature immediately after preparation, and hematoxylin and eosin (H&E) staining were performed after 5 min of fixation as described below.

For the preparation of the cell block, first, the cells punctured by a fine needle were placed directly on the slide, and the tip of the needle was used to gather the cells into cell clusters. The slide was slightly tilted and immersed in 95% ethanol for 1-2 min. Then, the slides were immersed in a 3.7% neutral formaldehyde solution and fixed for 30-60 min at room temperature. The slide was removed, and the cell clusters were scraped off with surgical blades and wrapped with embedding paper (L5-0016A Jinhua Lisheng). Next, they were placed into a 3.7% neutral formaldehyde solution and fixed for 2-4 h at room temperature, after which, the cell block was routinely dehydrated and sectioned(1). The cells were then fixed in a 3.7% neutral formaldehyde solution for 2-4 h, routinely dehydrated, embedded, and sectioned into 4-μm-thick sections.

H&E. For H&E staining, the tissues were first fixed in a 10% formaldehyde solution for a period of 24 h at room temperature. After that, they were subjected to a dehydration process and subsequently embedded in paraffin. Then, they were cut into 4 μm-thick sections. These sections underwent a dewaxing procedure utilizing xylene and ethanol before being thoroughly rinsed with water. The prepared sections were then sequentially stained with hematoxylin and eosin dyes.

Antibodies used for immunohistochemistry. The primary antibodies used in the present study were CK19 (cat. no. CCM-1101), HBME1 (cat. no. CHM-0110), galectin-3 (cat. no. CGR-0101), TTF-1 (cat. no. CTM-0261), TG (cat. no. CTM-0150), CK7 (cat. no. CCR-0990), CK20 (cat. no. CCR-1111),CEA(cat.no.CCM-0831),Syn(cat.no.CSM-0250), CgA (cat. no. CCM-0851), CDX-2 (cat. no. CCR-0821), EMA (cat. no. CEM-0041), ER (cat. no. CEM-0081), calcitonin (CCR-0190), and Ki-67 (cat. no. CKM-0032). All antibodies were purchased from CELNOVTE. Tissue sections were cut into 4-μm-thick sections and mounted onto slides. After deparaffinization (cat. no. 230105/54B Fujian Qisheng), rehydration, and blocking endogenous peroxidase activity with 3% hydrogen peroxide for 10 minutes, the slides were incubated with the appropriate antibody (1:100) for 12 h at 4˚C in a humidified chamber.

References

1. liu; z, jiao; q and liu d: Expert consensus on the cytopathological diagnosis of thyroid fine needle aspiration(version 2023). Zhonghua bing li xue za zhi = Chinese journal of pathology 52: 441-446, 2023.

Figure S1. The metastatic gland showed positivity for CK-19, EMA, and galectin-3 immunoreactivity and negativity for HBME1, CgA, and CK7. Magnification, x100; scale bar, 100 μm.

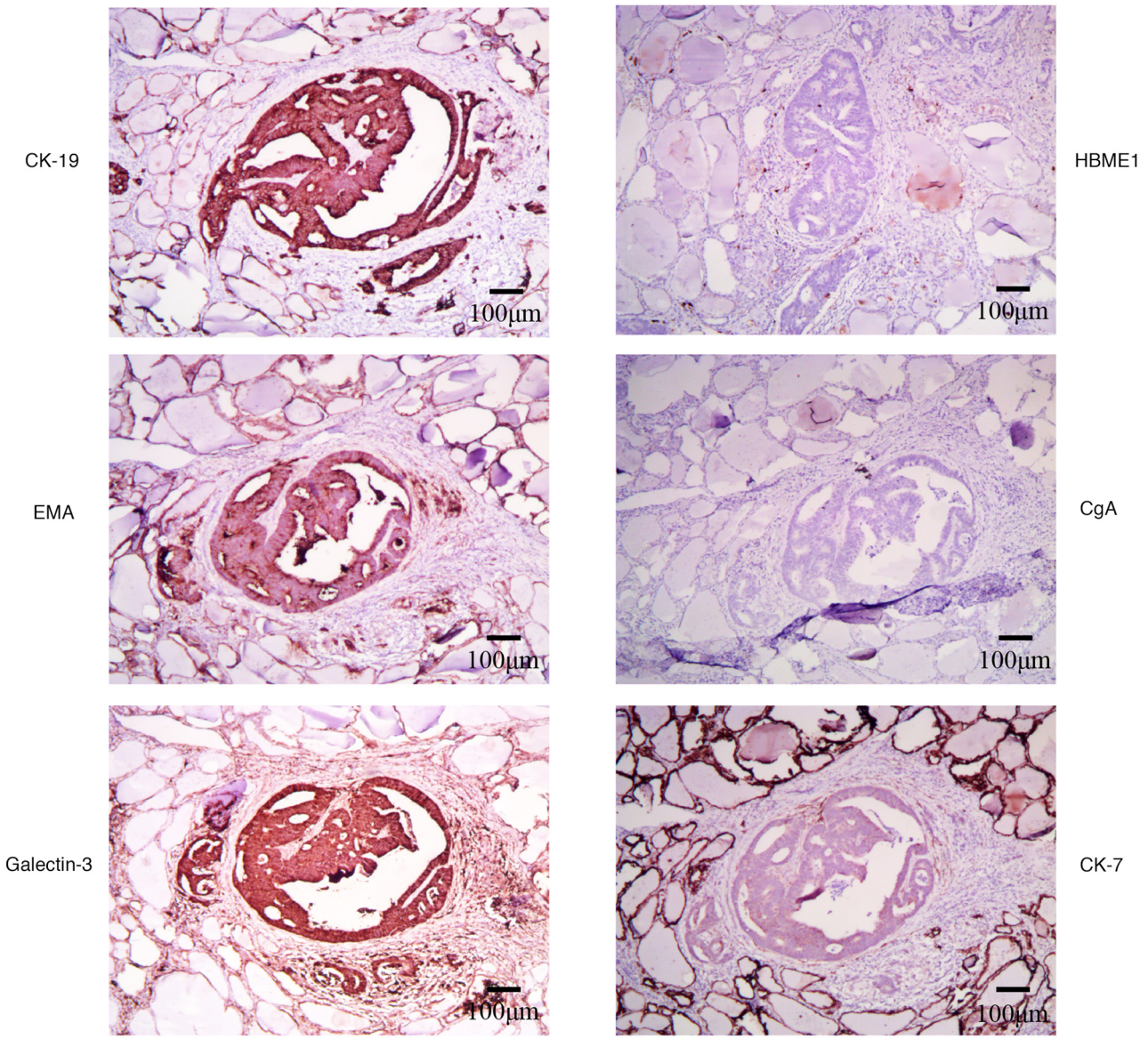
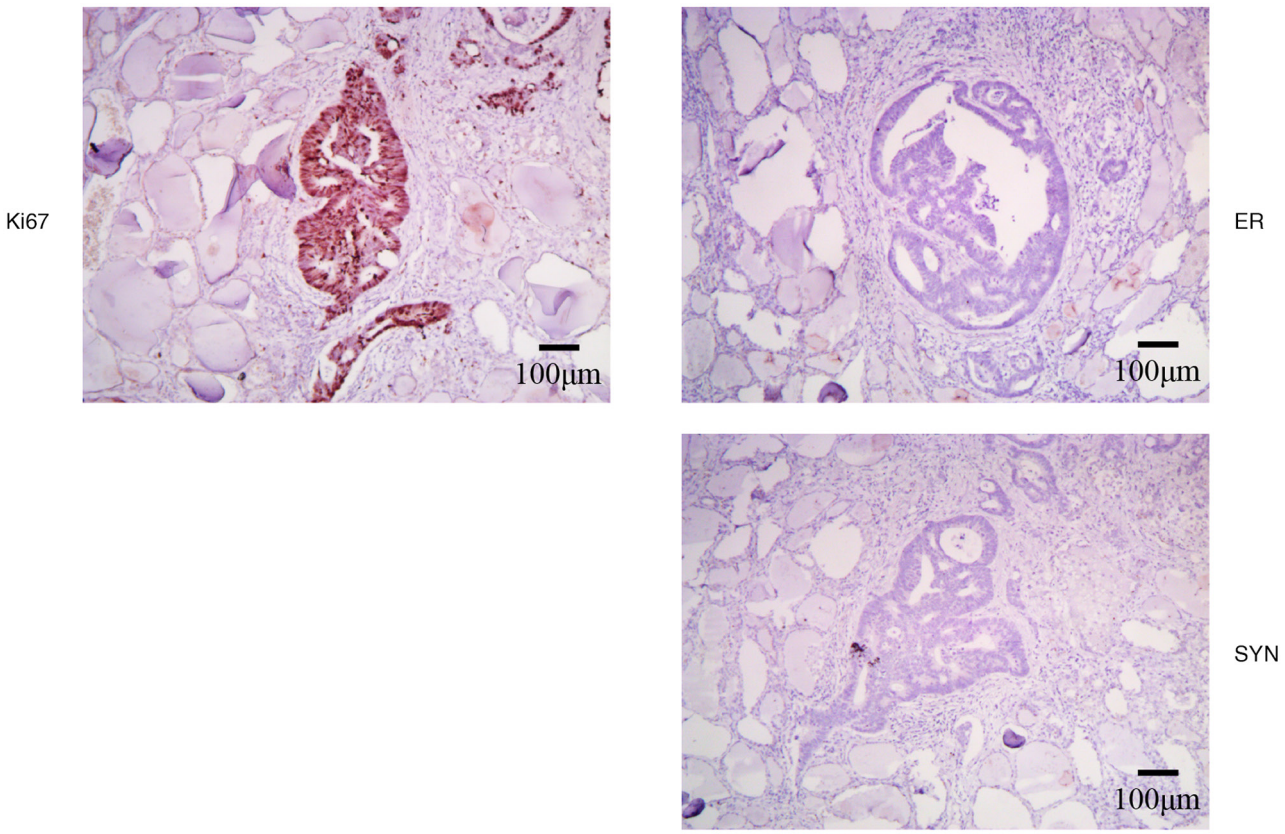
Figure S2. The metastatic gland exhibited a Ki-67 proliferation index of >50% and was negative for SYN and ER immunoreactivity. Magnification, x100; scale bar, 100 μm.