Diagnostic value of GATA-3 in cytological identification of parathyroid tissues

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Abstract. Parathyroid and thyroid lesions appear morphologically similar in cytological smears, and their differentiation can be difficult. The purpose of this study was to determine the diagnostic value of T-cell-specific transcription factor GATA-3 as a marker of parathyroid differentiation in cytology specimens, and to examine the utility of liquid-based cytology (LBC). Cytology smears obtained from surgically removed parathyroid and thyroid specimens, including 15 normal parathyroid glands, 12 cases of parathyroid hyperplasia, 55 parathyroid adenomas, 2 follicular thyroid adenomas, and 3 papillary thyroid carcinomas, were examined by immunocytochemistry using antibodies against GATA-3, parathyroid hormone (PTH), chromogranin A, and thyroid transcription factor 1 (TTF-1). All normal and hyperplastic parathyroids and 98.2% of parathyroid adenomas were positive for GATA-3, while 33.3%, 66.7%, and 60.0% of them, respectively, were positive for PTH. The positive rates for chromogranin A among normal parathyroids (80.0%) and parathyroid adenomas (87.3%) were lower than those for GATA-3. At the same time, all thyroid-derived tumours were positive for TTF-1 and negative for GATA-3, PTH, and chromogranin A. LBC smears of 35 parathyroid lesions indicated that the positive rates for GATA-3, PTH, and chromogranin A were 97.1%, 97.1%, and 100%, respectively, while in conventional smears, those for PTH (25.5%) and chromogranin A (78.7%) were significantly lower (p < 0.01). Our results suggest that GATA-3 is a more reliable biomarker than PTH or chromogranin A in differentiating parathyroid from thyroid lesions in cytology smears and that LBC is useful in detecting cytoplasmic antigens such as PTH and chromogranin A.

Key words: Parathyroid, Immunocytochemistry, GATA-3, Liquid-based cytology

PARATHYROID tumours usually occur at the posterior or near the lower pole of the thyroid, where the normal parathyroid tissue is located; however, they are rarely identified within the thyroid, and may be interpreted as thyroid nodules by ultrasound examination [1]. Therefore, the increasing use of fine needle aspiration (FNA) cytology for the evaluation of thyroid nodules raises the chances to encounter unsuspected parathyroid lesions, which have very low incidence [1]; thus, we observed only three such lesions in 8,093 thyroid cases analysed by FNA in the past year. However, parathyroid and thyroid lesions are morphologically similar on cytological smears [1-4], and their differentiation is frequently required, because the distinction between thyroid and parathyroid lesions is important for selecting treatment strategies.

Immunocytochemical analysis of FNA specimens for the expression of parathyroid hormone (PTH) and thyroglobulin allows identification of parathyroid and thyroid lesions, respectively [2, 5]. Thus, Chang et al. have demonstrated that immunoperoxidase staining for PTH and thyroglobulin was useful in the differentiation between parathyroid and thyroid tissues on cytological smears [5]. Other proteins such as thyroid transcription factor 1 (TTF-1) and chromogranin A (parathyroid secretory protein 1) have also been tested for the identification of thyroid and parathyroid lesions, respectively [6]. Recently, it has been shown that GATA-3, a transcription factor expressed in the nuclei of parathyroid cells, is a very sensitive and relatively specific immunohistochemical marker of parathyroid differentiation.

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with a potential to be used for histological diagnosis [7, 8]. However, to the best of our knowledge, studies concerning the use of GATA-3 as a biomarker of parathyroid lesions in cytology specimens have not been reported. The purpose of this research was to determine the diagnostic value of GATA-3 as a marker of parathyroid differentiation in cytology specimens by comparing the specificity of immunocytochemical staining for GATA-3, PTH, chromogranin A, and TTF-1 in parathyroid and thyroid specimens. In addition, we examined the utility of liquid-based cytology (LBC) recently proposed to have many benefits over conventional technology.

**Materials and Methods**

In this study, we used specimens of parathyroid and thyroid lesions surgically resected at the Kuma Hospital between August 2013 and February 2016. The analysed samples included 15 normal parathyroid glands, 12 parathyroid hyperplasia specimens, 55 parathyroid adenomas, 2 follicular thyroid adenomas (FTAs), and 3 papillary thyroid carcinomas (PTCs). The normal parathyroid glands or metastatic lymph nodes were provided for intraoperative consultation in patients with PTC. Six of parathyroid hyperplasia cases were secondary to chronic renal disease, and the others were not associated with any causes. Among 55 parathyroid adenoma cases, 54 were sporadic, and the remaining case was associated with multiple endocrine neoplasia type 1 (MEN1). Smears of the 15 normal parathyroid glands were prepared by cutting tissue surface using the imprint method, while samples of parathyroid and thyroid lesions were obtained by aspiration using 22-gauge needles. FNA was performed ex-vivo on resected tissues immediately after resection. For 5 of 12 parathyroid hyperplasia cases and 27 of 55 parathyroid adenomas, conventional smears were prepared by depositing the aspirated material onto a glass slide and covering by a second slide, followed by immediate fixation with Cytorop (Alfresa, Tokyo, Japan); the smears were divided into four parts using the cell-transfer technique [9]. For the remaining 7 parathyroid hyperplasia specimens, 28 parathyroid adenomas, and 5 thyroid tumours, the needles were rinsed with CytoRich RED collection fluid (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and LBC smears were prepared by the SurePath hand method. Antigen retrieval methods and primary antibodies used for immunostaining are indicated in Table 1. Immunostaining was performed using the automated Leica Bond-Max system and Bond Refine detection kit (Leica Microsystems, Wetzlar, Germany) according to the manufacturer’s instructions. Only the cells with well-preserved nuclei and cytoplasm were evaluated. Statistical significance of the data was analysed using Fisher’s probability test; p < 0.05 was considered statistically significant. Informed consent was obtained from all patients, and the study was approved by the ethics committee of Kuma Hospital.

### Results

All specimens contained cell clusters sufficient for observation. However, as conventional smears tended to show naked nuclei, there were fewer evaluated cells compared to LBC smears. Immunocytochemical analysis of the investigated parathyroid and thyroid lesions is presented in Table 2. GATA-3 (Fig. 1) and TTF-1 were expressed in the nuclei, and PTH and chromogranin A in the cytoplasm. All normal and hyperplastic parathyroids and 98.2% adenomas were positive for GATA-3, while 33.3%, 66.7%, and 60.0% of them, respectively, were positive for PTH. The incidence of GATA-3-positive cases was significantly higher than that of PTH-positive ones (p < 0.001), while no significant differences were observed between parathyroid hyperplasia and adenoma cases. Similar to GATA-3, chromogranin A was expressed in all hyperplastic parathyroid specimens, but its detection rates in the normal parathyroids

| Antibody  | Clone     | Vendor           | Location        | Antigen retrieval | Dilution |
|-----------|-----------|------------------|-----------------|-------------------|----------|
| GATA-3    | L50-823   | Biocare Medical  | Concord, CA, USA| Heat (pH 9)       | 1:400    |
| PTH       | 105G7     | Novocastra       | Newcastle, UK   | (-)               | 1:300    |
| Chromogranin A | DAK-A3 | Dako             | Glostrup, Denmark| Heat (pH 9)       | 1:400    |
| TTF-1     | 8G7G3/1   | Dako             | Carpinteria, CA, USA| Heat (pH 6)       | 1:100    |

Table 1: Primary antibodies used in immunostaining and antigen retrieval methods

PTH, parathyroid hormone; TTF-1, thyroid transcription factor-1.
and adenomas (80.0% and 87.3%, respectively) were lower than those for GATA-3, although the difference was not statistically significant. One GATA-3-negative parathyroid adenoma was positive for both PTH and chromogranin A. All cells of parathyroid origin were negative for TTF-1, while all thyroid-derived tumours were TTF-1-positive, but GATA-3-, PTH-, and chromogranin A-negative. Among the 35 LBC smears of parathyroid-derived lesions, 97.1%, 97.1%, and 100% were positive for GATA-3, PTH, and chromogranin A, respectively; however, for conventional smears, the positive rates for PTH (25.5%) and chromogranin A (78.7%) were significantly lower ($p < 0.01$), while no significant difference was detected in GATA-3 reactivity. In conventional smears, all parathyroid-derived cells and even naked nuclei were strongly positive for GATA-3. Among the 47 conventional smears of parathyroid lesions, 26 demonstrated proteinaceous material in the background, which correlated with the abundance of the naked nuclei; the material was positive for PTH and/or chromogranin A in 11.5% (3/26) and 61.5% (16/26), respectively, cases (Fig. 2).

Table 2 Immunocytochemical analysis of parathyroid and thyroid lesions

| Samples (number) | GATA-3 | PTH | Chromogranin A | TTF-1 |
|------------------|--------|-----|----------------|-------|
| Parathyroid (82) | 98.8% (81) | 56.1% (46) | 87.8% (72) | 0% (0) |
| Normal – conventional (15) | 100% (15) | 33.3% (5) | 80.0% (12) | 0% (0) |
| Hyperplasia (12) | 100% (12) | 66.7% (8) | 100% (12) | 0% (0) |
| – conventional (5) | 100% (5) | 20% (1) | 100% (5) | 0% (0) |
| – LBC (7) | 100% (7) | 100% (7) | 100% (7) | 0% (0) |
| Adenoma (55) | 98.2% (54) | 60.0% (33) | 87.3% (48) | 0% (0) |
| – conventional (27) | 100% (27) | 22.2% (6) | 74.1% (20) | 0% (0) |
| – LBC (28) | 96.4% (27) | 96.4% (27) | ** | 0% (0) |
| Thyroid (5) | 0% (0) | 0% (0) | 0% (0) | 100% (5) |
| PTCs – LBC (3) | 0% (0) | 0% (0) | 0% (0) | 100% (3) |
| FTAs – LBC (2) | 0% (0) | 0% (0) | 0% (0) | 100% (2) |

PTH, parathyroid hormone; TTF-1, thyroid transcription factor 1; LBC, liquid-based cytology; PTCs, papillary thyroid carcinomas; FTAs, follicular thyroid adenomas. * $p < 0.01$, ** $p < 0.001$. 

Fig. 1 Parathyroid adenoma stained for GATA-3 using liquid-based cytology smear (SurePath). The nuclei are strongly positive for GATA-3 ($\times 400$).

Fig. 2 Parathyroid adenoma stained for PTH and chromogranin A using conventional smear. Tumour cells are weakly positive for PTH (a) and chromogranin A (b). The leaked cytoplasm in the background shows stronger staining for chromogranin A than for PTH ($\times 100$).
Discussion

GATA-3 is a member of the GATA family of zinc finger transcription factors, and is detected in human embryos from the beginning of the 4th gestation week [10]. GATA-3 plays an important role in embryogenesis, development, and cell differentiation in many organs and tissues, including the kidney, breast, nervous system, thymocytes and T lymphocytes, and hair follicles [11-13]. GATA-3 expression detected by immunohistochemistry in urothelial and breast carcinomas [11, 14, 15] has been suggested as a sensitive and specific diagnostic marker, which should be included in the initial screening panel if these carcinomas are suspected for primary tumours of unknown origin [14]. GATA-3 is also a highly reliable biomarker for detecting paraganglioma, pheochromocytoma, and neuroblast tumours [16]. Furthermore, other GATA-3-positive epithelial cancers have been reported, including basal cell carcinoma, cutaneous squamous cell carcinoma, skin adnexal tumour, choriocarcinoma, endodermal sinus tumour, renal chromophobe carcinoma, malignant mesothelioma, and salivary gland and pancreatic ductal adenocarcinomas [11]. GATA-3 is also implicated in the embryonic development of the parathyroid glands, as well as in the proliferation of adult parathyroid cells [17]. Overall, these data strongly suggest that GATA-3 can be used as a specific immunohistochemical biomarker for cells of parathyroid origin [7, 8].

Currently, the biomarkers for the parathyroid cells are PTH and chromogranin A. An anti-PTH antibody was shown to specifically react with parathyroid cells, but the sensitivity was not sufficiently high [5]. According to a previous report [18], PTH immunostaining is frequently weak or shows focal positivity, particularly in adenoma, carcinoma, and oxyphilic cells. Chromogranin A is also expressed in medullary carcinoma of the thyroid [19]. Ordóñez has reported that GATA-3 nuclear expression was revealed by immunohistochemistry in all hyperplastic parathyroid glands, as well as parathyroid adenomas and carcinomas [7]. Betts et al. considered GATA-3 as a useful marker for the distinction between thyroid and parathyroid lesions by immunohistochemistry [8]. Parathyroid tumours may invade the thyroid, and it is difficult to distinguish them from lesions of thyroid origin [1-4]. Currently, two methods are used to identify parathyroid lesions by FNA. One of them is the PTH assay, which uses the wash-out fluid of the aspiration needle [20-22]; however, the method is limited to cases when parathyroid cell proliferation has already been suspected. The other method is immunocytochemical identification of parathyroid cells using specific antibodies [2, 3, 5, 23]. Thus, Dimashkieh and Krishnamurthy performed immunocytochemical staining for PTH in 6 smears and showed distinct cytoplasmic positivity [2], while Abati et al. used a chromogranin A antibody to confirm parathyroid lesions [24]. However, to the best of our knowledge, the use of GATA-specific antibodies for cytological detection of parathyroid lesions has not been previously reported. In the present study, we demonstrated that 98.8% of parathyroid lesions were positive for GATA-3, which was significantly higher than the respective positivity rate for PTH and chromogranin A, while all thyroid lesions were GATA-3-negative. These data indicate that GATA-3 expression is specific for the cells of parathyroid origin and suggest that it is more useful than PTH in distinguishing between parathyroid and thyroid lesions. In addition, GATA-3 expressed in the nuclei presents an advantage compared to cytoplasmic PTH and chromogranin A, because its detection should not be affected by cellular damage and cytoplasmic leakage frequently observed in conventional specimens. We believe that GATA-3 presents a more reliable biomarker for differentiating parathyroid and thyroid lesions in cytology smears compared to PTH or chromogranin A. However, in clinical practice GATA-3 should be used in combination with PTH or chromogranin A, because, as evidenced by our findings, GATA-3-negative parathyroid adenomas can still be positive for both PTH and chromogranin A.

LBC is a new technique for collecting cytological samples and smearing them thinly [25]. The procedure decreases inadequate cases and achieves a diagnostic sensitivity as accurate as the conventional method, because it provides excellent cell preservation and decreases blood components masking follicular cells [26]. Furthermore, the quality of immunocytochemical reactions in LBC smears is better than that in conventional smears [27, 28]. Our results also indicated that, considering morphological details and background reactivity, the quality of immunocytochemical analysis using LBC smears was superior to that using conventional smears. We showed that in LBC smears, over 95% of parathyroid lesions were positive for GATA-3, PTH, and chromogranin A, whereas in conventional smears, the positivity rates for PTH and chromogranin...
A were significantly lower, which is consistent with a previous report that only 60% of 10 conventional parathyroid lesions were PTH-positive [5]. Cumulatively, these results strongly suggest that LBC is better than conventional cytology for the preservation of cell integrity, and, therefore, may be more useful in detecting cytoplasmic antigens such as PTH and chromogranin A. No significant difference in GATA-3 immunoreactivity was detected between LBC and conventional smears. However, the background proteinaceous material positive for PTH and/or chromogranin A was observed in 50% of conventional smears, which correlated with a significant number of naked nuclei, suggesting damaged cells and cytoplasmic leakage. Although background staining should not be considered in cytological analysis [29], we believe that the cytoplasm retains the antigenicity after leakage as evidenced by the reaction with highly specific PTH and chromogranin A antibodies, which did not recognize thyroid lesions, and, therefore, should be evaluated. Calcium-sensing receptor and chorion-specific transcription factor (GCM2) have also been studied as biomarkers for parathyroid cells [30, 31]; however, the antibodies against these factors are mostly used in research, while their clinical application is limited, and we did not use them in this study.

In conclusion, our data suggest that GATA-3 is a more reliable biomarker than PTH or chromogranin A for differentiating between parathyroid and thyroid lesions by cytology and that LBC smears are useful for detecting cytoplasmic antigens such as PTH and chromogranin A. Obviously, FNA is not a recommended diagnostic approach when a parathyroid tumour is suspected, because of a risk of tumor cell seeding, as well as massive haemorrhage or severe fibrosis, which complicate surgical resection [32]. It is important to check serum calcium levels before performing FNA of apparent thyroid nodules to minimize the chances of encountering parathyroid lesions.

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Disclosure

None of the authors has any potential conflicts of interest associated with this research.

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