Semi-quantitative analysis of P53 binding-protein 1 (53BP1) expression in thyroid cytology: a novel method for preoperative diagnosis of follicular tumors

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INTRODUCTION

- The preoperative diagnosis of thyroid follicular carcinomas (FCs) by fine-needle aspiration cytology (FNAC) is almost impossible, because the criteria for distinguishing these lesions are based on histological evidence, and not on cytologic features, as is the case for papillary carcinoma (Fig. 1).
- We have reported that the expression of 53BP1 nuclear foci (NF), reflecting DNA damage response (DDR) (Fig. 2), by immunofluorescence (IF) is useful to estimate the genomic instability (GIN) during diverse tumorigenesis including thyroid carcinoma.
- Our retrospective study demonstrated that IF analysis for type of 53BP1 expression analysis the incidence of high DDR-type 53BP1 immunoreactivity in follicular tumors (FTs) could be an attractive candidate biomarker to distinguish FC from follicular adenoma (FA) (Fig. 3). Indeed, when we adopted 3.1% as a cut-off value for the incidence of high DDR-type, this test could differentiate FC or FA among 69 FFPE FT samples with a sensitivity of 90.5% and a specificity of 77.8% (Thyroid in press).
- Thus, IF analysis of 53BP1 expression will not only be an auxiliary histologic technique to accurately diagnose FTs but also a novel technique to make preoperative diagnoses with FNAC from FTs.

AIM

To prospectively clarify the impact of 53BP1 expression by IF as a biomarker to differentiate FTs with liquid based cytology (LBC).

MATERIALS AND METHODS

A total of 183 consecutive obtained-LBC samples, which were clinically and cytologically suspected as FT before surgery in Kuma Hospital, were available in this study. All of LBC samples were obtained from surgically resected tumors by FNA. The type of 53BP1 immunoreactivity in LBC was preoperatively classified as showing in Fig. 4.

![Fig. 4. Type of 53BP1 expression in LBC samples by IF.](image)

Table 1. The mean incidence (%) of each type of 53BP1 expression in LBC samples from FTs by IF.

<table>
<thead>
<tr>
<th>Type</th>
<th>Total n</th>
<th>Stable</th>
<th>Low DDR (LODDR)</th>
<th>High DDR (HODDR)</th>
<th>Large NF (LF)</th>
<th>Abnormal</th>
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<tbody>
<tr>
<td>FA</td>
<td>60</td>
<td>47295</td>
<td>65.4</td>
<td>14.3</td>
<td>10.4</td>
<td>7.4</td>
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<tr>
<td>FT-UMP</td>
<td>18</td>
<td>8029</td>
<td>56.7</td>
<td>15.4</td>
<td>6.0</td>
<td>7.9</td>
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<tr>
<td>FC</td>
<td>28</td>
<td>13774</td>
<td>58.3</td>
<td>12.3</td>
<td>11.7</td>
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<th>total n</th>
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Jokncheere-Verstraet’s test revealed the significant (P-value < 0.0001) differences in type of 53BP1 expression in LBC samples among these types of FTs.

The mean incidence of abnormal type of 53BP1 expression significantly increased with malignant potential of FTs, such as FA < FT-UMP < FC.

RESULTS

- Total n = 183
- 53BP1 immunofluorescence
- Include n = 166
- Exclude n = 17
- Follicular adenoma n = 50
- FT-UMP n = 18
- Follicular carcinoma n = 28
- Adenomatous nodule n = 30
- WDT-UMP n = 10
- WDC-ND n = 4
- papillary carcinoma n = 32
- Poor differentiated carcinoma n = 2

Fig. 5. Flow diagram for analyzing the type of 53BP1 expression in LBC samples with final pathological diagnosis after surgery. Seventeen cases were excluded because of unfavorable staining for evaluation. Among other 166 cases, 60, 18, and 28 cases were diagnosed as FA, FT-uncertain malignant potential (UMP), and FC, respectively. Total of 106 FT cases were post-operatively collared with the type of 53BP1 immunoreactivity in LBC by IF.

Fig. 6. Representative images for type of 53BP1 expression in LBC samples from FTs by IF.

- Upper panels, FA and FT-UMP, occasionally showed stable or DDR types of 53BP1 expression.
- Lower panels, FT-UMP and FC, frequently showed LF or abnormal types of 53BP1 expression.

Fig. 7. Comparison of the Abnormal rate (%) in follicular adenoma (FA) and follicular carcinoma (FC).

Table 2. Test performance of 53BP1 abnormal expression in follicular tumors (n=88)

<table>
<thead>
<tr>
<th>53BP1 abnormal expression</th>
<th>FA</th>
<th>FC</th>
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<tbody>
<tr>
<td>*4.3%&lt; , n</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>&lt; *4.3% , n</td>
<td>50</td>
<td>3</td>
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Test performance

<table>
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<tr>
<th>sensitivity</th>
<th>specificty</th>
<th>PPV</th>
<th>NPV</th>
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<tr>
<td>89.3</td>
<td>83.3</td>
<td>71.4</td>
<td>94.3</td>
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*Abnormal > 4.3% % AUC 0.9012 95% CI: 0.8318-0.9706

SUMMARY AND CONCLUSION

- In summary, this prospective study suggests that the incidence of abnormal-type 53BP1 immunoreactivity in FTs could be an attractive candidate biomarker to distinguish FC from FA. Indeed, when we adopted 4.3% as a cut-off value for the incidence of abnormal-type, this test could differentiate FC or FA among 88 LBC samples from FTs with a sensitivity of 89.3% and a specificity of 83.3%.
- Interestingly, the mean incidence of abnormal-type 53BP1 immunoreactivity in FT-UMP was found as intermediate ratio between FA and FC, suggesting intermediated malignant potency as FT5.
- This study suggests that IF analysis of 53BP1 expression is a novel diagnostic method to estimate the malignant potential of FTs with LBCs.
- We hypothesize that the type of 53BP1 expression can be a hallmark of GIN in tumor cells. IF analysis of 53BP1 expression will be not only an auxiliary histologic technique to accurately diagnose FTs but also a novel technique to make preoperative diagnosis with FNAC.
- A limitation of this study is that LBC samples are obtained from surgically resected tumors but not from patients’ thyroids, preoperatively.