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Prognostic impact of Ki-67 labeling index in minimally invasive follicular thyroid carcinoma

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Abstract. We investigated the prognostic impact of the Ki-67 labeling index (LI) in minimally invasive follicular thyroid carcinoma (FTC). We enrolled 192 patients (including four with distant metastasis at diagnosis) who were pathologically diagnosed as having minimally invasive FTC between 1998 and 2007 at Kuma Hospital. When the Ki-67 LI was higher than 5% in the hot area, we regarded it as a high Ki-67 LI. In a univariate analysis, patient age (≥45 years), high-frequent vascular invasion (≥4 in H&E specimens), and high Ki-67 LI significantly predicted the disease-free survival (DFS) of the patients. Since none of the patients <45 years old showed a recurrence, we performed a multivariate analysis of variables other than patient age. In the multivariate analysis including the presence of vascular invasion, high Ki-67 LI was an independent predictor of carcinoma recurrence. However, in the multivariate analysis including high-frequent vascular invasion, only high-frequent vascular invasion independently affected the DFS. These findings suggest that the Ki-67 LI has a rather strong prognostic value for the DFS of patients, although its impact was less than those of patient age and high-frequent vascular invasion.

Key words: Follicular carcinoma, Minimally invasive, Prognosis, Ki-67 labeling index

FOLLICULAR THYROID CARCINOMA (FTC) is the second most common malignancy originating from thyroid follicular cells. A pathological examination of surgical specimens is usually conducted to diagnose FTC based on the presence of capsular and/or vascular invasion, unless no distant metastases were detected at surgery [1]. FTCs often metastasize to distant organs such as the lung and bone, whereas lymph node metastasis from FTC is not common. Based on the degree of invasion, FTCs are divided into two categories: minimally invasive and widely invasive FTCs [2, 3]. Minimally invasive FTCs have a better prognosis than widely invasive FTCs, but the prognosis of patients with certain characteristics such as old age, large tumor size and high-frequent vascular invasion is often poor.

The Ki-67 labeling index (LI) accurately reflects cell proliferating activity because Ki-67 is a protein that is present in all cells except those in the G0 phase [4]. We showed that a high Ki-67 LI independently affected the poor prognosis of papillary thyroid carcinoma (PTC) patients [5, 6]. However, to date, no studies have been published regarding the prognostic significance of the Ki-67 LI in minimally invasive FTC, to our knowledge. In the present study, we investigated the relationship between the Ki-67 LI and the prognosis of minimally invasive FTC patients, in a series of 192 patients.

Patients and Methods

We enrolled 192 patients who underwent initial surgery for solitary or multiple FTC nodules between 1998 and 2007 at Kuma Hospital. All available H&E sections of these patients were reviewed by one of the study authors (M.H.), a thyroid pathologist. The sections were diagnosed as minimally invasive FTC. Cases with poorly differentiated lesions and other thyroid malignancies were excluded from our series. They consisted of 29 males and 163 females, and the median patient age was 46 years old (13–92 years).

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The diagnosis of FTC was based on the presence of capsular and/or vascular invasion and the absence of nuclear features of papillary thyroid carcinoma. Capsular invasion and vascular invasion were evaluated based on the categories in the textbook written by Chan [7]. Capsular invasion was diagnosed as positive when definite capsular invasion was detected or when duplication of the capsule and/or satellite nodule was observed [7]. We counted the total number of vascular invasions in all available H&E sections. We regarded cases as exhibiting the presence of vascular invasion when one or more vascular invasions were found in all available sections. We classified cases as having high-frequent vascular invasion if four or more vascular invasions were found in total by examining all available H&E sections.

Capsular invasion was positive in 186 of the 192 patients (97%). The presence of vascular invasion and high-frequent vascular invasion were observed in 81 (42%) and 12 patients (6%), respectively. Two patients were negative for capsular and vascular invasion, but had distant metastasis at diagnosis. Four patients had distant metastasis at diagnosis and were classified as M1. Thirty-six patients, including the four M1 patients, underwent total thyroidectomy, and the remaining patients underwent a more limited thyroidectomy such as lobectomy and subtotal thyroidectomy.

All patients were followed once or twice per year by ultrasound (US), chest roentogenography, and/or computed tomography (CT) to screen for recurrence in the lymph nodes and distant organs. For the M1 patients, we performed radioactive iodine (RAI) therapy after a total thyroidectomy. After the confirmation of distant recurrence during follow-up, we performed a completion total thyroidectomy and RAI therapy.

We performed Ki-67 immunostaining using 4-μm-thick, formalin-fixed, paraffin-embedded tissues and an antibody against Ki-67 (MIB1, 1:200 dilution: Dako, Carpinteria, CA, USA). We excluded the cases unsuitable for immunostaining due to calcification. The staining was carried out using Autostainer (Dako Japan, Tokyo) and the Envision Kit (Dako Japan) according to the manufacturer's recommendation. To estimate the Ki-67 LI, we counted at least 500 carcinoma cells in the hot area observed under ×400 magnification, and we calculated the percentage of nuclei that were positively stained. The Ki-67 LI was estimated by a coauthor (M.H.) who did not know the prognosis of the patients. We classified the results into three categories: Ki-67 LI <5%, 5%–10%, and >10%. We regarded cases as showing a high Ki-67 LI when the Ki-67 LI in the hot spots was ≥5%.

We repeatedly sent questionnaires to survey the patients who were postoperatively referred to other hospitals near their residences, in order to obtain data on the patients' disease-free and cause-specific survival. The median follow-up time in our series was 131 months (31–213 months). To date, eight patients showed FTC recurrence. The organs of recurrence were the lung in seven patients, bone in three patients, and mediastinal lymph node in one patient. To date, two patients, including one M1 patient, died of FTC.

We used the Chi-square test to compare variables, and the Kaplan-Meier method and log rank test to analyze time-dependent variables. We used the Cox-regression model for the multivariate analysis. A p-value <0.05 was regarded as significant. We employed Stat View 5.0 software for these analyses.

Results

We investigated the relationship between the Ki-67 LI and various clinicopathological features (Table 1). One hundred forty-six patients showed low Ki-67 LI

Table 1 Relationships between Ki-67 LI and clinicopathological features

	Ki-67 LI				p-value
	<5%	5%–10%	>10%	Total	
Age (≥45/<45 yrs)	73/73	13/11	16/6	102/90	0.1369
Gender (M/F)	22/124	4/20	3/19	29/163	0.9594
M factor (1/0)	4/142	0/24	0/20	4/188	0.5264
Tumor size (>4/≤4 cm)	83/63	17/7	8/14	108/84	0.0599
Capsular invasion (y/n)	141/5	23/1	22/0	186/6	0.6574
The presence of vascular invasion (y/n)	62/84	11/13	8/14	81/111	0.8020
High-frequent vascular invasion (y/n)	12/134	6/18	4/18	22/170	0.0329

(<5%) and the remaining 46 demonstrated high Ki-67 LI (≥5%). The Ki-67 LI was directly related to high-frequent vascular invasion but no other factors such as the presence of vascular invasion, tumor size, capsular invasion, tumor size, age, gender, and distant metastasis at diagnosis.

We then compared the Ki-67 LI data with the disease-free survival (DFS) of the patients excluding four M1 patients (188 patients in total). All of the four M1 patients were classified into low Ki-67 LI group (Table 1), and therefore, the 188 M0 patients consisted of 46 patients with high Ki-67 LI and 142 patients with low Ki-67 LI. As shown in Fig. 1a, the patients with tumors with a high Ki-67 LI showed significantly poorer prognoses (p=0.0319) compared to the patients with low Ki-67 LIs. The 10-year DFS of the patients with high and low Ki-67 LIs was 91% and 98%, respectively.

Of the other factors, age ≥45 years (10-year DFS rate, 92% *vs*. 100%, p: not calculable) and high-frequent vascular invasion (10-year DFS rate, 80% *vs*. 98%, p<0.0001) also showed a poor DFS rate in the univariate analysis (Fig. 1b, c). Regarding capsular invasion, the patients with capsular invasion had a significantly better 10-year DFS rate (97%) than those without capsular invasion (75%) (p=0.0315).

We then performed a multivariate analysis for 188 M0 patients. Patient age ≥45 years was excluded from the variables because none of the patients aged younger than 45 years showed recurrence (Fig. 1b). High Ki-67 LI independently affected the DFS of patients when the presence of vascular invasion was used (Table 2a). However, in the multivariate analysis that included high-frequent vascular invasion instead of vascular invasion, only high-frequent vascular invasion was an independent predictor of carcinoma recurrence (Table 2b).

Regarding capsular invasion, in our series, both the univariate and multivariate analyses revealed that the DFS of the cases that were negative for capsular invasion was significantly poor, indicating that positive capsular invasion has no prognostic impact in both univariate and multivariate analyses (Tables 2a, 2b).

In our series, only two patients died of FTC, and one of them was a M1 case. This M1 patient had a low Ki-67 LI, was negative for vascular invasion and capsular invasion, and was diagnosed as having minimally invasive FTC based on distant metastasis at diagnosis. The other patient had a high Ki-67 LI and was positive for high-frequent vascular invasion.

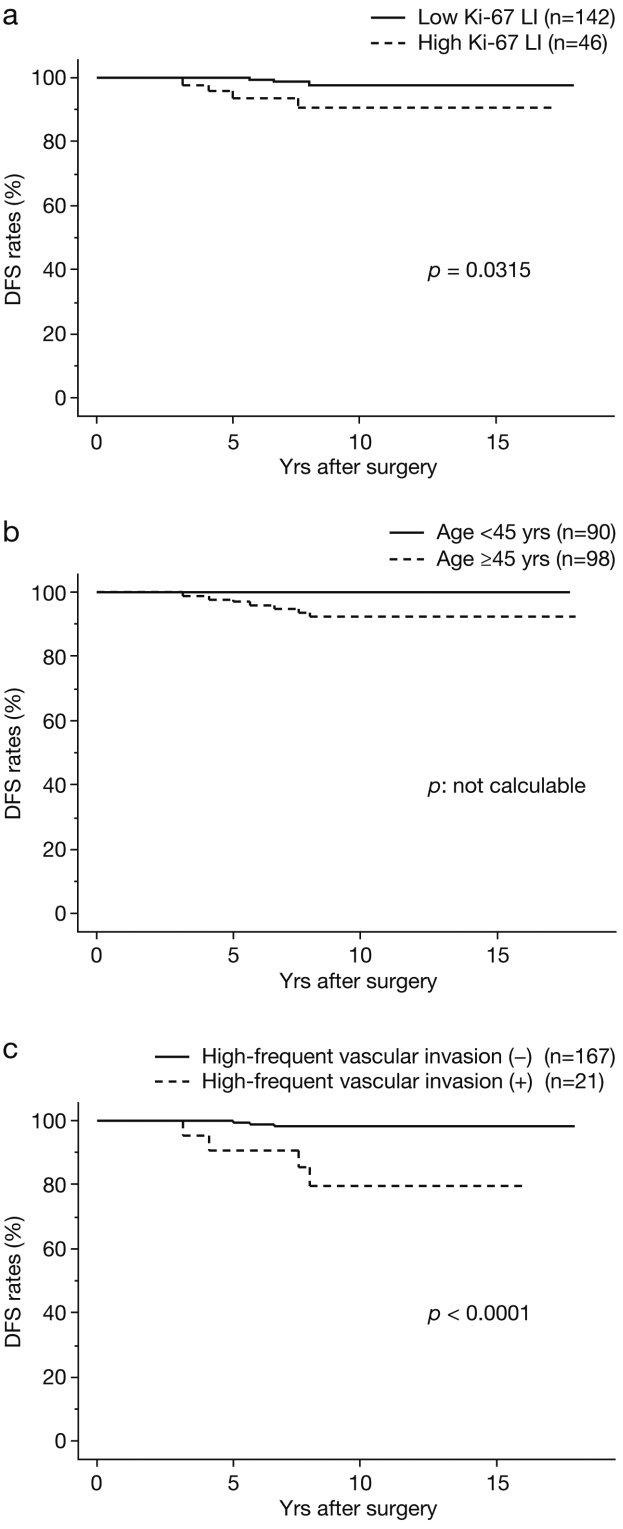

Fig. 1 **a**: The disease-free survival (DFS) of minimally invasive FTC cases with high and low Ki-67 LIs. **b**: The DFS of minimally invasive FTC patients aged ≥45 years and aged <45 years. **c**: The DFS of minimally invasive FTC cases with and without high-frequent vascular invasion.

Table 2a Univariate and multivariate analyses of clinicopathological features for DFS (1)

Variables	Univariate	Multivariate	Hazard ratio (95%CI)
Tumor size (≥4 cm)	0.1038	0.1248	5.917 (0.611–58.824)
Capsular invasion-negative *	0.0399	0.0711	8.984 (0.828–97.495)
High Ki-67 LI	0.0315	0.0243	6.061 (1.263–29.412)
The presence of vascular invasion	0.1152	0.2265	2.874 (0.520–15.873)
Female gender	0.2639	0.3341	2.342 (0.416–13.158)

* Capsular invasion-negative cases showed a poorer DFS than -positive cases.

Table 2b Univariate and multivariate analyses of clinicopathological features for DFS (2)

Variable	Univariate	Multivariate	Hazard ratio (95% CI)
Tumor size (≥4 cm)	0.1038	0.0852	8.000 (0.749–83.333)
Capsular invasion-negative *	0.0399	0.0248	17.136 (1.433–204.925)
High Ki-67 LI	0.0315	0.3373	2.487 (0.387–16.129)
High-frequent vascular invasion	<0.0001	0.0068	11.236 (1.950–13.333)
Female gender	0.2639	0.3209	2.410 (0.424–13.700)

* Capsular invasion-negative cases showed a poorer DFS than -positive cases.

Discussion

In a previous study, we demonstrated that the factors of patient age ≥45 years, high-frequent vascular invasion, and tumor size >4 cm had significant prognostic impacts in minimally invasive FTC [8]. In the present study, in addition to those factors, we investigated the prognostic value of high Ki-67 LI values.

Our analyses demonstrated that Ki-67 LI also has a prognostic impact in minimally invasive FTC. FTC is more likely to show recurrence to the distant organs such as the lung and bone than recurrence to regional lymph nodes, which was also observed in our series. For the treatment of recurred lesions, RAI therapy is the first line, which requires total thyroidectomy. The guidelines established by the Japanese Association of Thyroid Surgery/The Japan Society of Endocrine Surgeons therefore recommends a completion total thyroidectomy for widely invasive FTCs and FTCs with poorly differentiated components in the majority of carcinoma lesions [9]. The guidelines also recommend careful postoperative follow-up for minimally invasive FTCs with vascular invasion, old age, male gender, and large tumors [9].

Here we observed that a high Ki-67 LI significantly affected the DFS of patients with minimally invasive FTC, and its prognostic impact was stronger than that of the presence of vascular invasion and weaker than that of high-frequent vascular invasion. In our multivariate analysis, the Ki-67 LI was recognized as an independent prognostic factor of DFS. Therefore, patients with minimally invasive FTC and a high Ki-67 should definitely be carefully followed after surgery.

In our previous study, we proposed that a completion total thyroidectomy should be conducted for minimally invasive FTC with high-frequent vascular invasion, because the 10-year carcinoma recurrence rate was rather high at 20% [8]. Regarding the Ki-67 LI, although a high Ki-67 LI has an independent prognostic impact, it remains unclear whether minimally invasive FTCs with a high Ki-67 LI require a completion total thyroidectomy, because the 10-year recurrence rate was lower, at 9%, than that of high-frequent vascular invasion. In our series, only 4 of the 46 M0 cases (9%) with high Ki-67 LIs showed recurrence during the median 129-month follow-up period. Additionally, only one patient (0.7%) with a high Ki-67 LI died from FTC during follow-up at 131 months. Taking our past and present results together, we suspect that it might not be necessary to perform an immediate completion total thyroidectomy for minimally invasive FTC patients with a high Ki-67 LI. However, careful follow-up after surgery is recommended.

As indicated above, Ki-67 LI reflects the cell proliferating activity [4]. However, the present study showed that minimally invasive FTC having high Ki-67 LI are likely to show distant recurrence. It remains unknown whether and how Ki-67 is directly related to distant

metastasis of carcinoma cells. In fact, the exact role and overall function of Ki-67 remain unclear [10, 11], indicating the possibility that Ki-67 plays an unknown role in supporting carcinoma metastasis to distant organs. Alternatively, Ki-67 may comprehensively be related to biological aggressiveness of carcinoma, including local growth and distant metastasis. Our previous study demonstrated that high Ki-67 LI affected not only DFS but also CSS of PTC patients [5], which may support this hypothesis.

Capsular invasion is a very common event in FTC, and most FTCs are diagnosed based on capsular invasion. In our series, 97% of the cases were positive for capsular invasion. The remaining 3% were diagnosed based on vascular invasion or distant metastasis. Our series showed that the cases negative for capsular invasion had a poorer DFS than the cases that were positive for capsular invasion. Based on these findings, we can conclude that positive capsular invasion cannot be a predictor of poor prognosis in minimally invasive FTC.

Our study has some limitations. Firstly, we could not use the cases of patients who underwent surgery at an earlier time, because the paraffin blocks were not suitable for immunostaining, and we should have deleted cases with strong calcification because the blocks of these cases were decalcified and also unsuitable for immunostaining. Secondly, although the median follow-up period was not short (131 months), the number of patients showing recurrence is small, at 8 (4.2%). It remains debatable whether results in this study are statistically reliable. However, some factors such as old age, high-frequent vascular invasion, and high Ki-67 LI are still recognized as having prognostic values. Therefore, although further studies are needed for larger number of cases with longer time follow-up, at least at this moment, we should regard them as prognostic impacts of minimally invasive FTC. Thirdly, we failed to establish the prognostic value of the Ki-67 LI for CSS and/or overall survival, because only two patients died of carcinoma. It is more important to identify factors affecting CSS than DFS, which should also be studied in future.

In conclusion, a high Ki-67 LI is one of the independent prognostic factors of minimally invasive FTC, at least for disease-free survival. Even when other risk factors are lacking, patients with a high Ki-67 LI should be followed carefully.

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