# TRIM27 expression is associated with poor prognosis in sinonasal mucosal melanoma*

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**Rhinology 61: 3,** 263 - 271, 2023
https://doi.org/10.4193/Rhin22.405

***Received for publication:**
October 18, 2022
**Accepted:** February 16, 2023

## Abstract

**Background**: Tripartite motif-containing 27 (TRIM27) has been implicated in the progression of various cancers. However, the role of TRIM27 in sinonasal mucosal melanoma (SNMM) remains poorly understood.

**Materials and Methods**: We retrospectively examined 28 patients with SNMM treated with between 2003 and 2021. We undertook immunohistochemical analysis of TRIM27, Ki-67, and p-Akt1 expression in SNMM tissues. We also investigated the relationship between TRIM27 expression and clinical characteristics, prognosis, Ki-67 as a tumor growth potential marker, and p-Akt1 as one of the prognostic factors in mucosal melanoma.

**Results**: TRIM27 expression was significantly higher in T4 disease than in T3 disease and was higher in stage IV than in stage III. Patients with high-TRIM27 SNMM had a significantly poorer prognosis in terms of overall survival (OS) and disease-free survival. There was also a significantly higher rate of distant metastasis. Univariate analysis for OS revealed that TRIM27 and T classification were significant poor prognostic factors. In addition, the Ki-67 positive score and the p-Akt1 total staining score were significantly higher in the high-TRIM27 group than in the low-TRIM27 group.

**Conclusions**: High TRIM27 expression in SNMM was associated with advanced T classification, poor prognosis and distant metastasis. We suggest that TRIM27 has potential as a novel biomarker for prognosis in SNMM.

**Key words**: TRIM27, sinonasal mucosal melanoma, p-Akt1, Ki-67

## Introduction

Mucosal melanoma is a rare disease, accounting for about 1.3% of all melanomas [1, 2], that occurs mostly in the head and neck region: 55% of all mucosal melanomas [3, 4], and about 70% of head and neck mucosal melanomas occur in the sinonasal region and 20% in the oral cavity [4, 5]. Sinonasal mucosal melanoma (SNMM) accounts for about 4% of malignant sinonasal tumors [6].

In general, mucosal melanomas differ from cutaneous melanomas in terms of biological factors and clinical behavior [7, 8]. Mucosal melanomas show more aggressive behavior, a greater tendency for metastasis and a worse prognosis than cutaneous melanomas [9, 10]. While the 5-year overall survival (OS) rate is 91.2% for cutaneous melanomas [11], it is 20-35% for all mucosal melanomas [12] and 22% in SNMM [13]. BRAF, KIT, and NRAS mutations have been reported as biomarkers for cutaneous melanoma and are used for the selection of molecular-targeted therapy [14]. However, the expression of these biomarkers is less frequently observed in mucosal melanomas than in cutaneous melanomas [15], and the establishment of novel biomarkers for prognosis and treatment selection in mucosal melanomas is required.

We focused on the Tripartite motif (TRIM) family proteins, which have been reported as biomarkers for several malignant tumors [16]. TRIM family proteins mostly demonstrate E3 ubiquitin ligase activity and function in cellular processes such as intracellular signaling, development, apoptosis, protein quality control,

innate immunity, autophagy, and carcinogenesis [16, 17]. TRIM27, a member of the TRIM family, contains the following domains: a Really Interesting New Gene (RING) finger, two B-boxes (type I and type II) [18, 19], and a RBCC motif. The RING finger domain is directly involved in E3 ubiquitin activity and ubiquitination [20]. TRIM27 was identified as a fusion partner with the RET (REarranged during transfection) proto-oncogene encoding a receptor tyrosine kinase [21]. TRIM27 demonstrates oncogenic properties in mouse embryo NIH3T3 cells when its tripartite domain is recombined with the tyrosine kinase domain of the RET proto-oncogene by DNA rearrangement [22]. TRIM27 has also been shown to be involved in various cancers such as esophageal, gastric, colorectal, lung, breast, renal and ovarian cancers [19, 21, 23-27]. Recently, it was reported that the expression of TRIM27 was correlated with prognosis in cutaneous melanomas, suggesting that TRIM27 might be a biomarker for cutaneous melanoma [28]. However, mucosal melanomas differ from cutaneous melanoma in terms of biological and clinical features, so the clinical and prognostic role of TRIM27 in SNMM remains unresolved.

In addition, it was reported that one of the mechanisms of carcinogenesis involves TRIM27 activation of phosphorylated-Akt1 (p-Akt1) in colorectal and ovarian cancers [19, 21], and that p-Akt1 overexpression is an independent prognostic factor in mucosal melanomas, with p-Akt1 expression observed to be up-regulated in mucosal melanomas [29]. Therefore, we decided to investigate the association of TRIM27 with p-Akt1 in SNMM. Moreover, it was reported that Ki-67 expression is a marker for tumor growth fraction due to its association with cell proliferation [30], and Ki-67 was shown to be related to overall survival in patients with mucosal melanomas in the head and neck, with the prognosis of patients with higher Ki-67 levels being poorer [31]. Thus, we decided to investigate the association of TRIM27 with Ki-67 expression in SNMM to evaluate TRIM27 as a potential tumor growth potential marker.

## Materials and methods

### Patients

The inclusion criteria for this study were as follows: 1) treated at Hokkaido University Hospital between May 2003 and March 2021, 2) primary site involving the nasal cavity or paranasal sinuses, 3) histologically proven malignant melanoma, and 4) treatment with curative intent. As a result, 28 patients met the inclusion criteria. We collected their clinical information such as age, sex, primary tumor site, T classification, neck lymph node metastasis, distant metastasis, clinical stage, treatment method, surgical margins and prognosis. This study was approved by the Institutional Review Board for Clinical Research of Hokkaido University Hospital, Sapporo, Japan (018-0352), and was conducted in accordance with the Declaration of Helsinki.

### Tissue specimens

Tumor tissue samples were taken from surgically removed specimens, and normal nasal mucosa tissues were taken from the same patients as a control. Normal nasal mucosa tissue was taken from the inferior or middle nasal turbinate on the same side as the tumor, but not tissue in contact with the tumor, and histologically confirmed to be free of tumor cells.

### Immunohistochemistry

Tissues were embedded in paraffin. Sections (3 μm) were then deparaffinized in xylene and rehydrated through graded ethanol, followed by antigen microwave retrieval. The slides were washed thoroughly with 1x Tris-buffered saline (TBS). Next, endogenous peroxidase was blocked with $H_2O_2$ (3%) for 10 min at room temperature, and then with 5% goat serum before staining. Sections were incubated with the following primary antibodies overnight at 4°C: anti-TRIM27 (12205-1-AP, Proteintech, Rosemont, United States), anti-p-Akt1 (66444-1-Ig, Proteintech, Rosemont, United States), and anti-Ki-67 (418071, Nichirei Biosciences, Tokyo, Japan). The slides were then incubated with HRP-conjugated anti-rabbit or mouse IgG followed by diaminobenzidine (DAB) and hematoxylin staining. The expression levels of proteins were scored based on visual grading of the staining intensity as previously reported for TRIM27 in gastric cancer and ovarian cancer [21, 27]. TRIM27 and p-Akt1 were evaluated for "intensity score" with 4 levels of staining intensity as follows: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). TRIM27, p-Akt1, and Ki-67 were evaluated for "positive score" in terms of the percentage of stained cells as follows: 0 (negative), 1 (<30%), 2 (30-60%), 3 (>60%) (Figure 1).

The total staining score for TRIM27 and p-Akt1 was calculated as the intensity score and the positive score, as previously reported for TRIM27 in gastric cancer and ovarian cancer [21, 27]. The TRIM27 total staining score was defined as the product of these two scores. Slides were scored as either low or high TRIM27 expression for scores ≤4 or >4, respectively.

Also, we evaluated "the mitotic index", which has been reported to be correlated with prognosis in SNMM [32]. We counted the number of mitoses under a microscope at 400x magnification and classified them as <10 HPF and ≥10 HPF, as in the previous study.

### Statistical analysis

We performed linear regression analysis of the correlation between IHC parameters such as TRIM27 total staining score, p-Akt1 total staining score, Ki-67 positive score, and the mitotic index. Overall survival (OS) was defined as the time between the start of initial treatment and either the date of death or the last follow-up. Disease-free survival (DFS) was defined as the time between the start of initial treatment and either the date of any recurrence, any death or the last follow-up. We used the Kaplan–

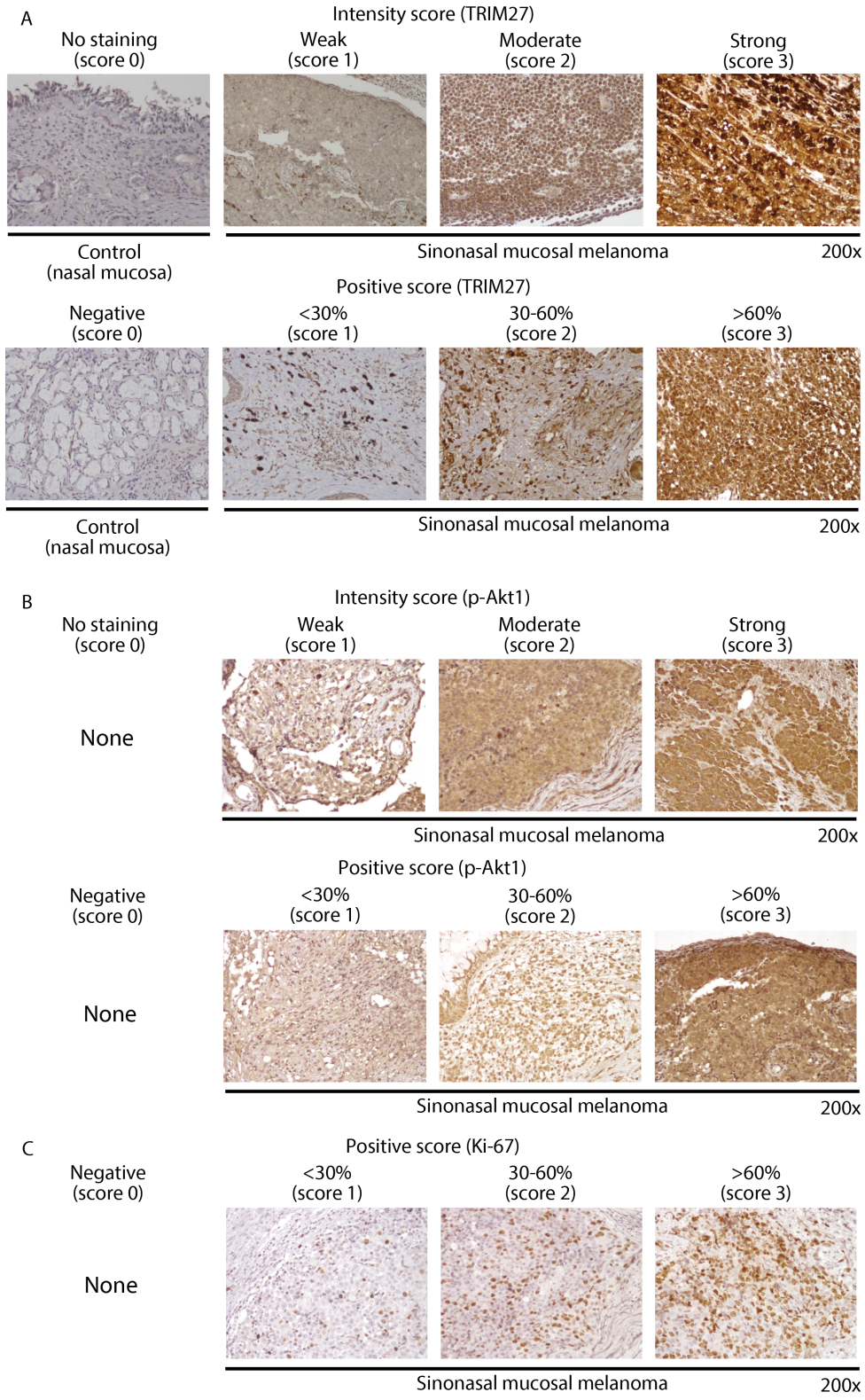



Figure 1. A) The TRIM27 intensity score and positive score after immunohistochemical analysis of SNMM and normal nasal mucosa tissues. The TRIM27 intensity score was graded as follows: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). The TRIM27 positive score was graded as follows: 0 (negative), 1 (<30%), 2 (30-60%), 3 (>60%). B) The p-Akt1 intensity score and positive score after immunohistochemical analysis of SNMM tissues. The p-Akt1 intensity score was graded as follows: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). The p-Akt1 positive score was graded as follows: 0 (negative), 1 (<30%), 2 (30-60%), 3 (>60%). C) The Ki-67 positive score after immunohistochemical analysis of SNMM tissues. The Ki-67 positive score was graded as follows: 0 (negative), 1 (<30%), 2 (30-60%), 3 (>60%).

Meier method to estimate OS and DFS. We evaluated between-group comparisons using a log-rank test that was stratified for TRIM27 expression (high vs. low) for OS and DFS. We also analyzed the time from the start of initial treatment to the first evidence of distant metastasis and neck lymph node metastasis. We performed univariate and multivariate analysis to investigate the association between prognosis and clinicopathologic parameters in SNMM. Due to the small number of cases, multivariate analysis was performed only on the explanatory variables that were found to be significantly different by univariate analysis. Ordinal variables such as T classification, intensity score and positive score for immunohistochemistry were compared using the Fisher's exact test. Cox proportional hazards models were used to compute relative risks, hazard ratios and 95% CIs. Data were expressed as the mean ± SD. A p value < 0.05 was considered statistically significant. All statistical tests were performed using the JMP® pro version 16 software program (SAS Institute Inc., Cary, NC, USA).

## Results

### Clinical characteristics of patients with SNMM

The patients with SNMM included 16 (57.1%) males and 12 (42.9%) females with a median age of 73 years (range, 45-83 years). In terms of primary tumor site, the tumor was located in the nasal cavity in 16 (57.1%) and in the paranasal sinuses in 12 (42.9%) patients. In terms of T classification, 21 (75.0%) patients were T3 and 7 (25.0%) were T4. One (3.6%) patient had neck lymph node metastasis, and none had distant metastasis. In terms of clinical stage, 20 (71.4%) patients were stage III and 8 (28.6%) were stage IV. All patients underwent surgery and postoperative radiation therapy, with 21 (75.0%) patients undergoing endoscopic sinus surgery (ESS) and 7 (25.0%) patients undergoing open surgery. The surgical margins were positive in 12 (42.9%) patients, negative in 13 (46.4%) patients, and non-evaluable in 3 (10.7%) patients. One (3.6%) patient with neck lymph node metastasis underwent neck dissection. All patients were treated with postoperative radiation therapy, with 20 (71.4%) patients irradiated by X ray and 8 (28.6%) patients irradiated by proton beams. No patients received adjuvant chemotherapy, including immune checkpoint inhibitors, after surgery or postoperative radiation therapy. However, 12 SNMM patients (5 patients in the low-TRIM27 group and 7 patients in the high-TRIM27 group) received immunotherapy, such as immune checkpoint inhibitors, for recurrence or metastasis.

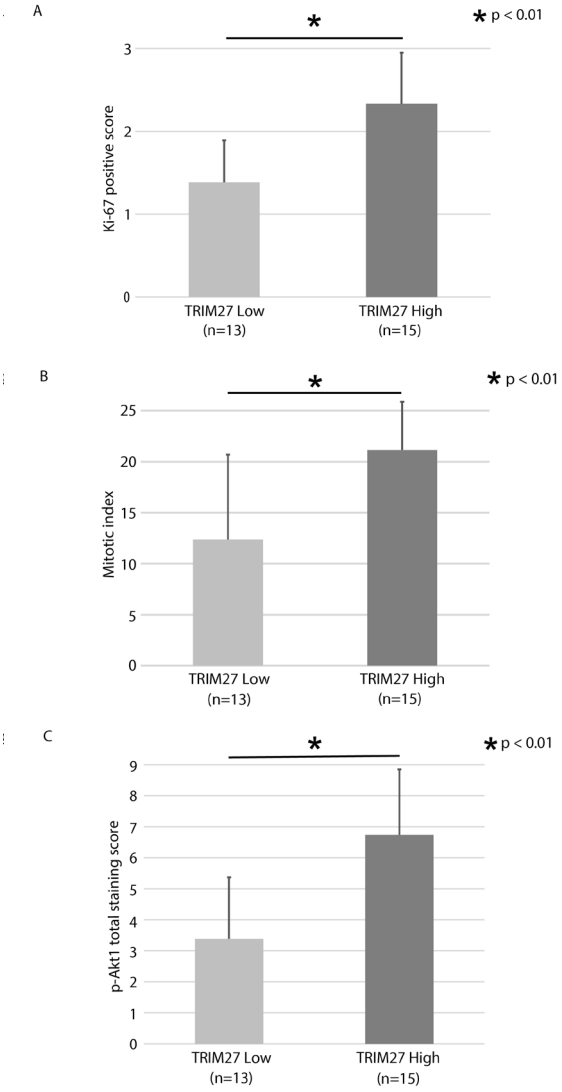



Figure 2. A) Comparison between the high-TRIM27 and low-TRIM27 group by the Ki-67 positive score in SNMM tissues. The Ki-67 positive score for the high-TRIM27 group was significantly higher than that for the low-TRIM27 group (2.33 ± 0.62 vs. 1.38 ± 0.51, p < 0.01).
B) Comparison between the high-TRIM27 and low-TRIM27 group by the mitotic index in SNMM tissues. The mitotic index for the high-TRIM27 group was significantly higher than that for the low-TRIM27 group (10.80 ± 2.43 vs. 6.38 ± 4.19, p < 0.01).
C) Comparison between the high-TRIM27 and low-TRIM27 group by the p-Akt1 total staining score in SNMM tissues. The p-Akt1 total staining score for the high-TRIM27 group was significantly higher than that for the low-TRIM27 group (6.73 ± 2.12 vs. 3.38 ± 1.98, p < 0.01).

### TRIM27 expression scores

First, immunohistochemical analysis of TRIM27 in SNMM and control nasal mucosa tissues was performed to examine TRIM27 expression (Figure 1). SNMM tissues showed significantly higher intensity and positive scores for TRIM27 than did the control (p < 0.001) (Table 2). Calculation of the total staining score from the intensity score and positive score revealed that 15 patients were classified in the high-TRIM27 group and 13 patients were classified in the low-TRIM27 group. We examined the association between TRIM27 expression and patient characteristics, with TRIM27 expression being significantly higher in those with T4 disease than in those with T3 disease (p = 0.01) and higher in

Table 1. Association of TRIM27 expression with clinical characteristics in SNMM.

| Clinical characteristics | n (%) | TRIM27 expression | | P-value |
|---|---|---|---|---|
| | | High, n (%) | Low, n (%) | |
| Sex | | | | |
| Male | 16 (57.1) | 7 (25.0) | 9 (32.1) | 0.28 |
| Female | 12 (42.9) | 8 (28.6) | 4 (14.3) | |
| Age (years) | | | | |
| ≤70 | 11 (39.3) | 6 (21.4) | 5 (17.9) | 1.00 |
| >70 | 17 (60.7) | 9 (32.1) | 8 (28.6) | |
| Primary tumor site | | | | |
| Nasal cavity | 16 (57.1) | 8 (28.6) | 8 (28.6) | 0.72 |
| Paranasal sinuses | 12 (42.9) | 7 (25.0) | 5 (17.9) | |
| T classification | | | | |
| T3 | 21 (75.0) | 8 (28.6) | 13 (46.4) | 0.01* |
| T4 | 7 (25.0) | 7 (25.0) | 0 (0.0) | |
| Neck lymph node metastasis | | | | |
| Present | 1 (3.6) | 1 (3.6) | 0 (0.0) | 1.00 |
| Absent | 27 (96.4) | 14 (50.0) | 13 (46.4) | |
| Clinical stage | | | | |
| stage III | 20 (71.4) | 8 (28.6) | 12 (42.9) | 0.04* |
| stage IV | 8 (28.6) | 7 (25.0) | 1 (3.6) | |
| Surgical margins | | | | |
| Positive | 12 (42.9) | 8 (28.6) | 4 (14.3) | 0.41 |
| Negative | 13 (46.4) | 5 (17.9) | 8 (28.6) | |
| Non-evaluable | 3 (10.7) | 2 (7.1) | 1 (3.6) | |
| Radiation therapy | | | | |
| X-ray | 20 (71.4) | 12 (42.9) | 8 (28.6) | 0.41 |
| Proton beam | 8 (28.6) | 3 (10.7) | 5 (17.9) | |

*<0.05 (Fisher's exact test); High TRIM27: staining total score >4, Low TRIM27: staining total score ≤4.

those with stage IV disease than in those with stage III disease (p = 0.04). On the other hand, there were no significant differences in sex, age, primary tumor site, neck lymph node metastasis, surgical margins, or radiation therapy between the high-TRIM27 and low-TRIM27 groups (Table 1).

### Ki-67 positive scores

In terms of the Ki-67 positive scores in the SNMM patients, 12 (42.9%) were classified as <30% (score 1), 10 (35.7%) as 30-60% (score 2) and 6 (21.4%) as >60% (score 3). The Ki-67 positive score in the high-TRIM27 group was significantly higher than that in the low-TRIM27 group (2.33±0.62 vs. 1.38±0.51, p<0.01) (Figure 2).

### Mitotic index

In terms of the mitotic index in the SNMM patients, 17 (60.7%) were classified as <10 HPF, and 11 (39.3%) as ≥10 HPF. The mitotic index in the high-TRIM27 group was significantly higher than that in the low-TRIM27 group (10.80 ± 2.43 vs. 6.38 ± 4.19, p < 0.01) (Figure 2).

### p-Akt1 expression scores

Regarding the p-Akt1 intensity scores in the SNMM patients, 9 (32.1%) were classified as weak (score 1), 12 (42.9%) as moderate (score 2), and 7 (25.0%) as strong (score 3). For the p-Akt1 positive scores in the SNMM patients, 2 (7.1%) were classified as <30% (score 1), 8 (28.6%) as 30-60% (score 2) and 18 (64.3%) as >60% (score 3). Total staining scores for p-Akt1 were calculated from the intensity score and positive score, with 19 (67.9%) classified in the high-p-Akt1 group and 9 (32.1%) in the low-p-Akt1 group (Table 2). We found that the p-Akt1 total staining score was significantly higher in the high-TRIM27 group than in the low-TRIM27 group (6.73±2.12 vs. 3.38±1.98, p<0.01) (Figure 2).

### Correlations among the IHC parameters in SNMM

Figure 3 shows the correlations among TRIM27, p-Akt1, Ki-67 and the mitotic index. All linear regression analyses showed a positive correlation, particularly strong correlations found between TRIM27 and p-Akt1, TRIM27 and Ki-67, and Ki-67 and the mitotic index (R2 = 0.50, 0.55 and 0.57, respectively).

### High-TRIM27 expression was associated with poor prognosis and distant metastasis

Patients with high-TRIM27 SNMM had a significantly poorer prognosis in terms of both OS (HR: 5.48, p = 0.01) and DFS (HR: 2.90, p = 0.02) according to log-rank test than did patients with

Table 2. A) The distribution of TRIM27 intensity scores and positive scores in SNMM and normal nasal mucosa tissues, and total staining scores in SNMM tissues. B) The distribution of p-Akt1 intensity scores, positive scores and total staining scores in SNMM tissues.

**A.**

| Intensity score (TRIM27) | No. of patients (%) | | | | |
|---|---|---|---|---|---|
| | No staining (score 0) | Weak (score 1) | Moderate (score 2) | Strong (score 3) | P value (Fisher's exact test) |
| SNMM | 0 | 12 (42.9) | 7 (25.0) | 9 (32.1) | *<0.001 |
| Control | 16 (64.0) | 9 (36.0) | 0 | 0 | |
| **Positive score (TRIM27)** | **No. of patients (%)** | | | | |
| | Negative (score 0) | <30% (score 1) | 30-60% (score 2) | >60% (score 3) | P value (Fisher's exact test) |
| SNMM | 0 | 3 (10.7) | 11 (39.3) | 14 (50.0) | *<0.001 |
| Control | 16 (64.0) | 9 (36.0) | 0 | 0 | |
| **Total staining score (TRIM27)** | **No. of patients (%)** | | | | |
| | Low (score ≤4) | High (score >4) | | | |
| SNMM | 13 (46.4) | 15 (53.6) | | | |

**B.**

| Intensity score (p-Akt1) | No. of patients (%) | | | |
|---|---|---|---|---|
| | No staining (score 0) | Weak (score 1) | Moderate (score 2) | Strong (score 3) |
| SNMM | 0 | 9 (32.1) | 12 (42.9) | 7 (25.0) |
| **Positive score (p-Akt1)** | **No. of patients (%)** | | | |
| | Negative (score 0) | <30% (score 1) | 30-60% (score 2) | >60% (score 3) |
| SNMM | 0 | 2 (7.1) | 8 (28.6) | 18 (64.3) |
| **Total staining score (p-Akt1)** | **No. of patients (%)** | | | |
| | Low (score ≤4) | High (score >4) | | |
| SNMM | 9 (32.1) | 19 (67.9) | | |

low-TRIM27 SNMM. The 2-year OS was 50.3% in the high-TRIM27 group and 100.0% in the low-TRIM27 group. The 2-year DFS was 13.3% in the high-TRIM27 group and 40.7% in the low-TRIM27 group. In addition, the cumulative incidence of distant metastasis was significantly higher in the high-TRIM27 group than in the low-TRIM27 group (HR: 3.46, p = 0.02). On the other hand, there was no significant difference in the cumulative incidence of neck lymph node metastasis (HR: 2.74, p = 0.22) between the two groups (Figure 4). Furthermore, univariate analysis for OS revealed that TRIM27 and T classification were significant poor prognostic factors, and that for DFS revealed that TRIM27, T classification and neck lymph node metastasis were significant poor prognostic factors (Table 3). However, we found no independent prognostic factors in the multivariate analysis for OS and DFS.

## Discussion

This study revealed that TRIM27 in SNMM tissues is positively correlated with T classification, clinical stage, Ki-67 and the mitotic index. These results suggest that TRIM27 is involved in cell proliferation in SNMM. Further, TRIM27 has been shown to be involved in cell proliferation in other cancers. It has been reported that TRIM27 knockdown suppresses tumorigenesis in ovarian cancer [21] and TRIM27 promotes cell proliferation by inhibiting apoptosis and regulating the cell cycle in colon cancer [26]. In addition, TRIM27 was found to be positively correlated with p-Akt1 in this study. It is suggested that TRIM27 may be involved in cell proliferation through the activation of p-Akt1 as p-Akt1 is involved in cell proliferation [33, 34] and TRIM27 activates p-Akt1 [19, 21]. These findings suggest that TRIM27 is involved in cell proliferation in SNMM.

The results of this study showed that the high-TRIM27 group had a significantly poorer prognosis in terms of both OS and DFS than did the low-TRIM27 group in SNMM. TRIM27 expression and T classification may be prognostic factors in the univariate analysis of both OS and DFS. These findings suggest that TRIM27 may be one of the prognostic factors in SNMM. Although no independent prognostic factors were found in the multivariate analysis, the reason for this is considered to be the confounding of T classification and TRIM27. The poor prognosis in the high-TRIM27 group showed similar trends to those in other studies of several cancers such as esophageal, gastric, colorectal, lung, breast, renal and ovarian cancers [19, 21, 23-27]. We also found that TRIM27 was significantly correlated with the cumulative incidence of distant metastasis associated with SNMM.

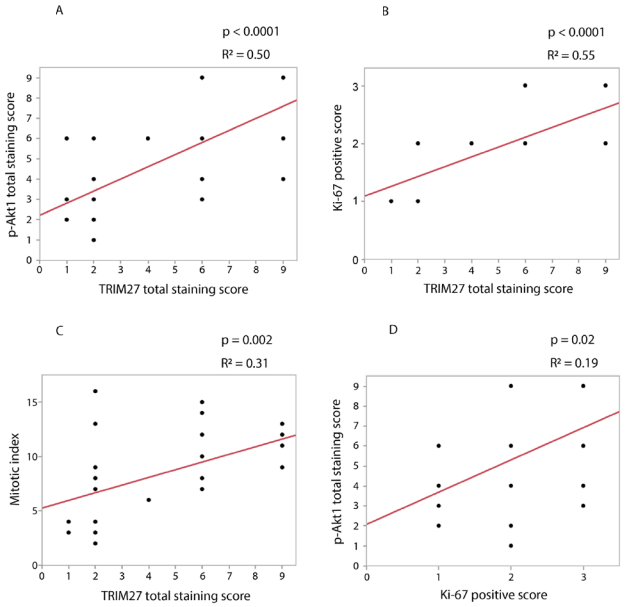
Table 3. Univariate analysis of the association between prognosis and clinicopathologic parameters in SNMM.

| Variable | HR (95% CI) | P-value | HR (95% CI) | P-value |
|---|---|---|---|---|
| Sex (Male vs. Female) | 0.91 (0.31-2.72) | 0.87 | 1.09 (0.46-2.59) | 0.84 |
| Age (>70 vs. ≤70 years) | 2.20 (0.67-7.19) | 0.19 | 1.76 (0.72-4.29) | 0.21 |
| Primary tumor site (Paranasal sinuses vs. Nasal cavity) | 1.42 (0.45-4.42) | 0.55 | 1.30 (0.55-3.11) | 0.55 |
| T classification (T4 vs. T3) | 6.48 (1.88-22.33) | <0.01* | 4.66 (1.59-13.67) | <0.01* |
| Neck lymph node metastasis (Present vs. Absent) | 1.91 (0.24-15.15) | 0.54 | 25.50 (1.59-407.66) | 0.02* |
| Surgical margins (Positive vs. Negative) | 0.96 (0.30-3.05) | 0.95 | 2.01 (0.80-5.01) | 0.14 |
| Radiation therapy (X-ray vs. Proton beam) | 4.13 (0.53-32.2) | 0.18 | 1.69 (0.61-4.68) | 0.32 |
| TRIM27 expression (High vs. Low) | 5.48 (1.21-24.84) | 0.03* | 2.90 (1.10-7.62) | 0.03* |
| Ki-67 expression (High vs. Low) | 6.64 (0.86-51.47) | 0.07 | 2.39 (0.86-6.61) | 0.09 |
| p-Akt1 expression (High vs. Low) | 7.83 (1.01-60.55) | 0.05 | 1.55 (0.59-4.09) | 0.38 |
| Mitotic index (≥10 HPF vs. <10 HPF) | 1.88 (0.60-5.95) | 0.28 | 1.80 (0.74-4.37) | 0.19 |

Table 3. Multivariate analysis of the association between prognosis and clinicopathologic parameters in SNMM.

| Variable | OS | | DFS | |
|---|---|---|---|---|
| | HR (95% CI) | P-value | HR (95% CI) | P-value |
| T classification (T4 vs. T3) | 3.78 (0.92-15.50) | 0.06 | 2.85 (0.84-9.73) | 0.09 |
| TRIM27 expression (High vs. Low) | 2.84 (0.50-15.95) | 0.24 | 2.01 (0.67-5.99) | 0.21 |
| Neck lymph node metastasis (Present vs. Absent) | | | 10.73 (0.64-179.11) | 0.10 |

HR; hazard ratio, CI; confidence interval; TRIM27 High: total staining score >4, TRIM27 Low: total staining score ≤4; Ki-67 High: positive score 2-3, Ki-67 Low: positive score 0-1; p-Akt1 High: total staining score ≥4, p-Akt1 Low: total staining score <4.



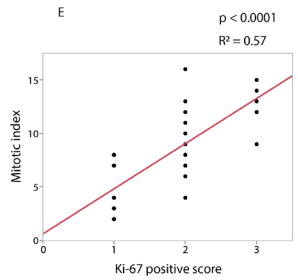




Figure 3. A) Correlation between the TRIM27 total staining score and the p-Akt1 total staining score. The TRIM27 total staining score was significantly correlated with the p-Akt1 total staining score ($p < 0.0001$, $R^2 = 0.50$). B) Correlation between the TRIM27 total staining score and the Ki-67 positive score. The TRIM27 total staining score was significantly correlated with the Ki-67 positive score ($p < 0.0001$, $R^2 = 0.55$). C) Correlation between the TRIM27 total staining score and the mitotic index. The TRIM27 total staining score was significantly correlated with the mitotic index ($p = 0.002$, $R^2 = 0.31$). D) Correlation between the p-Akt1 total staining score and the Ki-67 positive score. The p-Akt1 total staining score was significantly correlated with the Ki-67 positive score ($p = 0.02$, $R^2 = 0.19$). E) Correlation between the Ki-67 positive score and the mitotic index. The Ki-67 positive score was significantly correlated with the mitotic index ($p < 0.0001$, $R^2 = 0.57$).

A high ratio of distant metastasis along with a high rate of death resulting from distant metastasis has been reported in SNMM [35,36]. Further, it has been shown that TRIM27 is involved in cell migration and invasion [26]. Thus, it is suggested that TRIM27 is involved in cell migration and invasion in SNMM.

As mentioned above, TRIM27 was found to be positively correlated with p-Akt1 in this study, and it is known that TRIM27 activates p-Akt1 [21, 26]. Furthermore, the tumorigenic activity of p-Akt1 has also been investigated in several tumors, with Akt1 shown to upregulate cell migration and invasion [37-41]. Akt1 contributes to cellular metabolism in several cancers and p-Akt1 is associated with redox modulation in cell cycle progression [33, 34]. It has been reported that p-Akt1-nuclear expression is closely associated with a worse prognosis in breast, gastric, and

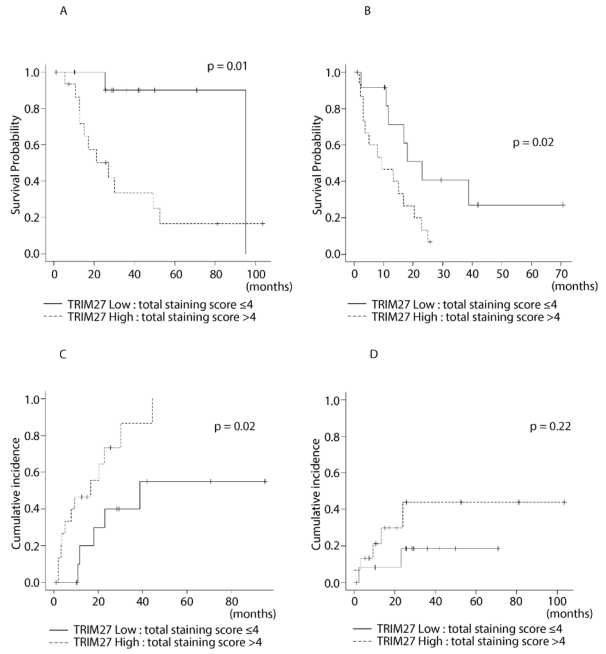



Figure 4. A) Comparison between overall survival in the high-TRIM27 group and low-TRIM27 group. The overall survival time for the high-TRIM27 group was significantly shorter than that for the low-TRIM27 group (HR: 5.48, p = 0.01). B) Comparison between disease-free survival in the high-TRIM27 group and low-TRIM27 group. The disease-free survival time for the high-TRIM27 group was significantly shorter than that for the low-TRIM27 group (HR: 2.90, p = 0.02). C) Comparison between the cumulative incidence of distant metastasis associated with SNMM in the high-TRIM27 group and low-TRIM27 group. The cumulative incidence of distant metastasis in the high-TRIM27 group was significantly higher than that for the low-TRIM27 group (HR: 3.46, p = 0.02). D) Comparison between the cumulative incidence of neck lymph node metastasis associated with SNMM in the high-TRIM27 group and low-TRIM27 group. There was no significant difference in the cumulative incidence of neck lymph node metastasis between in the high-TRIM27 group and the low-TRIM27 group (HR: 2.74, p = 0.22).

esophageal cancer [29, 42, 43]. In several malignant tumors, such as colorectal and lung cancer, Akt1 has also been implicated in the development of metastasis [44, 45]. Interestingly, it has been reported that TRIM27 knockdown induced cell cycle arrest and apoptosis in ovarian cancer cells by downregulating the expression of p-AKT [21]. According to these findings, it is suggested that TRIM27 is involved in cell migration and invasion in SNMM, and that TRIM27 may affect the migration and invasion through the activation of p-Akt1.

However, this study has several limitations as follows. The number of SNMM patients in this study was small, and further studies involving more SNMM patients are needed. Also, this study couldn't investigate the association between TRIM27 and p-Akt1 in cellular experiments. The exact association between TRIM27 and p-Akt1 in promoting the proliferation, invasion and metastasis of SNMM on the cellular level remains to be elucidated. Further in-depth investigations with a focus on the role of TRIM27 and p-Akt1 in SNMM are required.

While treatments for cutaneous malignant melanoma have improved prognosis by targeting gene mutations such as BRAF and immunotherapy, prognosis remains poor for SNMM due to death from distant metastasis. However, our findings that TRIM27 activates cell migration and invasion in SNMM, suggest that TRIM27 may be a predictor of distant metastasis in SNMM and may be used as a criterion for adjuvant chemotherapy after curative treatment. Furthermore, TRIM27 has the potential to act not only as a prognostic factor but also as a target in the development of novel therapies for SNMM. Based on these findings, we suggest that TRIM27 has potential as a novel biomarker for prognosis and treatment selection in SNMM.

## Conclusion

High TRIM27 expression in SNMM is associated with advanced T classification, poor prognosis and distant metastasis. TRIM27 could be involved in cell proliferation and affect migration and invasion in SNMM. We suggest that TRIM27 is a novel biomarker for prognosis in SNMM.

## Authorship contribution

ShK, MS, SaK and YN designed the study. ShK, MS, AyH, AN and YN performed the surgeries. AkH supervised the project. ShK, MS, YN AN, NT, SaK, MW and SH compiled the data. ShK, MS, YN, SaK and AkH wrote the manuscript. All authors provided feedback on the manuscript.

## Acknowledgement

We are grateful to Yuka Masuda, Shizuka Sugawara, Yumiko Kimura, and Ayaka Kubota for their help in preparing the manuscript.

## Conflict of interest

None of the authors have any conflicts of interest or financial disclosures that are relevant to this study.

## Funding

This study was supported by JSPS KAKENHI Grant Number 22K16923 for MS, 19K18718 for AN, 20K18301 for AyH, 19K18754 for NT, 21H02690 for MW, 22K19415 for SH and 20K09703 for YN.

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