NUAK2 is over-expressed and DNA copy number of NUAK2 is increased in acral melanomas: its significance on the survival of patients

Kohei Nojima¹⁾, Masahiro Hayashi²⁾, Masato Funazumi¹⁾, Masashi Ishikawa³⁾, Yasuhiko Kaneko⁴⁾, Masakazu Kawaguchi²⁾, Tamio Suzuki²⁾, Atsushi Tanemura⁵⁾, Ichiro Katayama⁵⁾, Taisuke Mori⁶⁾, Naoya Yamazaki⁷⁾, Hiroo Yokozeki¹⁾, Vincent J Hearing⁸⁾ Takeshi Namiki¹⁾,

1) Department of Dermatology, Tokyo Medical and Dental University
2) Department of Dermatology, Yamagata University
3) Department of Dermatology, Saitama Cancer Center
4) Research Institute for Clinical Oncology, Saitama Cancer Center
5) Department of Dermatology, Osaka University
6) Department of Pathology, National Cancer Center Hospital
7) Department of Dermatologic Oncology, National Cancer Center Hospital
8) Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health

Introduction

Recent analysis using array-CGH data demonstrated that NUAK2 is an important gene that regulates cell cycle progression and cell migration in melanoma cells. The fact that high expression of NUAK2 has an impact on the survival of patients with acral melanomas also shows that NUAK2 plays an important role in melanomagenesis.

In the previous study, PTEN deficiency is coupled tightly with genomic amplification encompassing the NUAK2 locus. Survival analyses also revealed that over-expression both of NUAK2 and of p-Akt(S473) has a significant impact on relapse-free survival of acral melanoma patients. Here, we validated the significance of over-expression both of NUAK2 and of p-Akt(S473) on patient's survival of melanomas with an increased number of cases.

Methods

Tumor specimens: 153 paraffin-embedded specimens of primary melanomas were obtained from 4 institutions. 114 were classified as acral melanomas and 39 as non-CSD melanomas.

Immunohistochemistry: Formalin-fixed, paraffin-embedded tissues on slides were deparaffinized and rehydrated. Antigen retrieval was performed. After blocking with normal horse serum, the tissues were incubated with a rabbit polyclonal anti-NUAK2 antibody or a rabbit monoclonal anti-p-Akt(S473) antibody at 4 °C overnight.

The tissue were then incubated with a biotin-labeled secondary antibody and then with avidin-peroxidase for 30 min each. The slides were then developed with DAB and were counterstained with Giemsa. Data evaluation: Immunostaining was scored from 0 to +3, depending on the percentages of cells. The basal expression group (negative staining group) includes specimens with a 0 score, and the overexpression group (positive staining group) includes specimens with +1, +2, or +3 scores. Immunostaining of NUAK2 were validated by positive cells in eccrine and in sebaceous glands, and negative cells in the epidermis.

Results

The correlation between tumor thickness and NUAK2 expression was analyzed by Mann–Whitney U test. In 113 acral melanoma patients, there was a significant difference between them. ($P<0.001$) (Table 1). But, in 39 non-CSD melanoma patients, there was no significant difference. ($P=0.989$)

The relapse-free survival of acral melanoma patients differed significantly between both NUAK2 and p-Akt(S473) positive group, and either NUAK2 or p-Akt(S473) alone ($P=0.001$) (Figure 2A). The overall survival of acral melanoma patients had slight difference between those groups ($P=0.023$) (Figure 2B). In 39 Non-CSD melanoma patients, there is no significant difference between those groups both in the relapse-free survival($P=0.712$) and in the overall survival($P=0.816$) .

	NUAK2 positive	NUAK2 negative	Correlation between NUAK2 and tumor thickness (<i>P</i> value)
acral	69	44	$P<0.001$
non-CSD	18	21	$P=0.989$ *Mann-Whitney U test

Table 1. The correlation between tumor thickness and NUAK2 expression was analyzed by Mann–Whitney U test.

To validate the previous results, we analyzed in an increased number of cases as 114 acral melanomas and 39 Non-CSD melanomas. The relapse-free survival of acral melanoma patients differed significantly between the group scored +3 and the group scored 0($P<0.001$) (Figure 1A). The overall survival of acral melanoma patients had a slight difference between those groups ($P=0.048$)(Figure 1B). In 39 Non-CSD melanoma patients, there is no significant difference between those groups both in the relapse-free survival($P=0.454$) and in the overall survival($P=0.624$) .

Figure 1. Kaplan-Meier curves of acral melanoma patients. (A) Relapse-free survival time of acral melanoma patients. **(B)** Overall survival time of acral melanoma patients. X axis indicates time (months). Y axis indicates cumulative survival rate. Blue line: scored 0, Green line: scored 1+, Yellow line: scored 2+, Purple line: scored 3+

Figure 2. Kaplan-Meier curves of acral melanoma patients. (A) Relapse-free survival time. **(B)** Overall survival time. Blue, green and yellow lines indicate “neither NUAK2 nor p-Akt positive”, “either NUAK2 or p-Akt positive” and “both NUAK2 and p-Akt positive”, respectively.

Multivariate Cox regression analysis showed that acral melanoma patients with high expression levels of NUAK2 expression were more likely to relapse than were acral melanoma patients with low levels of NUAK2 expression (hazard ratio = 3.52; 95% confidence interval = 1.65-7.55; $P=0.0012$) (Table 2).

Variable*	Hazard ratio	95% confidence interval	<i>P</i> value
NUAK2	3.52	1.65–7.55	0.0012
Sex	0.77	0.38–1.58	0.48
Age	1.42	0.63–3.20	0.39

Table 2. Multivariate Cox regression analysis of high expression levels of NUAK2 in relapse free survival of acral melanoma patients. Coding of variables: High expression of NUAK2 was coded as 1 = negative; 2 = positive. Sex was coded as 1 = male; 2 = female. Age was coded as 1 < 60y; 2 => 60y.

Conclusions

- NUAK2 might be involved in the tumor growth of acral melanomas.
- Relapse-free survival time of patients with expression of both NUAK2 and p-Akt(S473) was dramatically shorter than patients without both those expressions ($P=0.001$) in acral melanomas.
- Multivariate Cox regression analysis revealed acral melanoma patients with high expression of NUAK2 were more likely to relapse than those without them ($P=0.0012$).
- This study could successfully validated our previous significant results with an increased number of cases.