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Joint research project of genetic diagnosis of papillary thyroid cancer between Semipalatinsk Medical Academy and Nagasaki University

Atsushi Kumagai [a], Hiroyuki Namba [a,*], Norisato Mitsutake [a], Akira Ohtsuru [b], Masanobu Anami [c], Tomayoshi Hayashi [c], Masahiro Ito [d], Daniyal Mussinov [e,f], Maira Espenbetova [f], Murat Teleuov [f], Shunichi Yamashita [a,b,g]

[a] *Department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[b] *Takashi Nagai Memorial International Hibakusha Medical Center, Nagasaki University Hospital, Nagasaki, Japan*
[c] *Department of Clinical Pathology, Nagasaki University Hospital, Nagasaki, Japan*
[d] *Department of Pathology, National Nagasaki Medical Center, Omura, Nagasaki, Japan*
[e] *Semipalatinsk Regional Oncology Center, Semipalatinsk, Republic of Kazakhstan*
[f] *Semipalatinsk State Medical Academy, Semipalatinsk, Republic of Kazakhstan*
[g] *Department of Public Health and Environment, WHO Headquarters, Geneva, Switzerland*

Abstract. The activating point mutation of *BRAF* gene, $BRAF^{T1799A}$, is the most common and specific genetic alteration in adult papillary thyroid carcinoma. $BRAF^{T1799A}$ seems to be associated with clinical aggressiveness of papillary thyroid carcinoma. We have established that PCR-RFLP method enables us to detect the $BRAF^{T1799A}$ mutation using DNA extracted from fine-needle aspiration biopsy samples and then applied this proprietary method not only to our clinical practice but also to international medical assistance around the Semipalatinsk Nuclear Testing Site in Kazakhstan. Seventy cases from Japan and one hundred cases from Kazakhstan were examined. There were 14 Japanese and 50 Kazakhstani cases of cytological malignant tumors in all of the examined samples, among which we detected 12 and 14 cases with $BRAF^{T1799A}$ in Japanese and Kazakhstan cases, respectively. Of these cases, we found mutations in two cytological "suspicious" cases and even two pathological "benign" cases (after surgery in Kazakhstan). All of the *BRAF* mutation-positive cases, including those four, were confirmed as papillary thyroid carcinoma by careful pathological examination including immunohistochemical analysis. In conclusion, our PCR-RFLP method for $BRAF^{T1799A}$ detection using fine-needle aspiration

* Corresponding author. Tel.: +81 95 849 7115; fax: +81 95 849.
E-mail address: namba@nagasaki-u.ac.jp (H. Namba).

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biopsy samples is useful not only for preoperative diagnosis but also for accurate pathological decision of difficult cases as a complementary diagnostic method even after surgery. The introduction of appropriate genetic evaluation together with cytological cancer diagnosis into Kazakhstan makes a novel possibility for our future joint project.

Keywords: Papillary thyroid carcinoma; *BRAF*; Mutation; Fine needle aspiration biopsy

1. Introduction

BRAF point mutation is the most common genetic alteration in adult papillary thyroid carcinomas (PTCs). The prevalence of this *BRAF* mutation in PTCs has been reported to be 29–69%. It has been reported that *BRAF* point mutation in PTCs is always observed as a thymine-to-adenine transversion at nucleotide position 1799 ($BRAF^{T1799A}$), resulting in a valine to glutamate substitution at residue 600. In thyroid, multitudes of reports demonstrated that $BRAF^{T1799A}$ is exclusively found in PTC, but not in benign thyroid tumor and follicular neoplasm [1–8]. Furthermore, although it is still controversial, clinical outcomes of PTCs with $BRAF^{T1799A}$ have been associated in several reports with advanced clinical stages [4,9–11].

Preoperative diagnosis of thyroid tumors is based on fine-needle aspiration biopsy (FNAB) cytology, though even cytology could not assure that every case would be all right. Previously, we have demonstrated the usefulness of a *BRAF* mutation analysis using FNAB samples [12], and other groups also reported that preoperative *BRAF* analysis by FNAB samples was clearly beneficial [13,14]. We have applied this *BRAF* mutational analysis using FNAB sample in clinical practice in our outpatient clinic. Mutational analyses have been used as complementary diagnosis of cytology. We report our results and illustrate an interesting case with $BRAF^{T1799A}$ that was not able to diagnose as malignant by cytology, but confirmed as poorly differentiated PTC by operation.

Additionally, we have collaborated with physicians in Kazakhstan. The purpose was to improve the quality of medical environment surrounding Hibakusha. This collaboration has been done based on the Japan–Kazakhstan joint project of adult cancer screening around the Semipalatinsk Nuclear Testing Site (SNTS). Contents of this collaboration included not only medical equipment supply but also guidance of general medical techniques such as ultrasonography, cytology and pathology. We analyzed *BRAF* status using their FNAB samples. It has been said that $BRAF^{T1799A}$ is not associated with ionizing radiation exposure [15–17]. We also found two cases with $BRAF^{T1799A}$ that were hard to detect PTC using the customary method. Our genetical findings of $BRAF^{T1799A}$ provided the opportunity to change the diagnosis.

Table 1
Parameters of subjects

	Japanese samples	Kazakhstani samples
Number of cases	70	100
Average age (minimum–maximum)	57.7 (21–84) years	52.4 (12–83) years
Female/male	60/10	94/6