RET/PTC Rearrangements Preferentially Occurred in Papillary Thyroid Cancer among Atomic Bomb Survivors Exposed to High Radiation Dose

Kiyohiro Hamatani,[1] Hidetaka Eguchi,[1,8] Reiko Ito,[1] Mayumi Mukai,[1] Keiko Takahashi,[1] Masataka Taga,[1] Kazue Imai,[1] John Cologne,[2] Midori Soda,[3] Koji Arihiro,[4] Megumu Fujihara,[5] Kuniko Abe,[9] Tomayoshi Hayashi,[9] Masahiro Nakashima,[10] Ichiro Sekine,[10] Wataru Yasui,[6] Yuzo Hayashi,[7] and Kei Nakachi[1]

Departments of [1]Radiobiology/Molecular Epidemiology, [2]Statistics, and [3]Epidemiology (Nagasaki), Radiation Effects Research Foundation, [4]Department of Pathology, Hiroshima University Hospital, [5]Department of Pathology, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, [6]Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences, and [7]Geriatric Health Service Facility Hidamari, Hiroshima, Japan; [8]Translational Research Center, Saitama University, International Medical Center, Saitama, Japan; and [9]Department of Pathology, Nagasaki University Hospital, and [10]Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Abstract

A major early event in papillary thyroid carcinogenesis is constitutive activation of the mitogen-activated protein kinase signaling pathway caused by alterations of a single gene, typically rearrangements of the *RET* and *NTRK1* genes or point mutations in the *BRAF* and *RAS* genes. In childhood papillary thyroid cancer, regardless of history of radiation exposure, *RET/PTC* rearrangements are a major event. Conversely, in adult-onset papillary thyroid cancer among the general population, the most common molecular event is $BRAF^{V600E}$ point mutation, not *RET/PTC* rearrangements. To clarify which gene alteration, chromosome aberration, or point mutation preferentially occurs in radiation-associated adult-onset papillary thyroid cancer, we have performed molecular analyses on *RET/PTC* rearrangements and $BRAF^{V600E}$ mutation in 71 papillary thyroid cancer cases among atomic bomb survivors (including 21 cases not exposed to atomic bomb radiation), in relation to radiation dose as well as time elapsed since atomic bomb radiation exposure. *RET/PTC* rearrangements showed significantly increased frequency with increased radiation dose ($P_{trend} = 0.002$). In contrast, $BRAF^{V600E}$ mutation was less frequent in cases exposed to higher radiation dose ($P_{trend} < 0.001$). Papillary thyroid cancer subjects harboring *RET/PTC* rearrangements developed this cancer earlier than did cases with $BRAF^{V600E}$ mutation ($P = 0.03$). These findings were confirmed by multivariate logistic regression analysis. These results suggest that *RET/PTC* rearrangements play an important role in radiation-associated thyroid carcinogenesis. [Cancer Res 2008;68(17):7176–82]

Introduction

Thyroid cancer is, as is well-known, associated with exposure to external or internal ionizing radiation, such as from the atomic bombings (1) or the Chernobyl nuclear power plant accident (2, 3). The excess relative risk of thyroid cancer per Gy weighted thyroid dose was 1.15 in the Life Span Study (LSS) of atomic bomb (A-bomb) survivors (4), and a strong relationship between thyroid cancer and radiation exposure was indicated from the data of the Chernobyl accident (3). A histopathologic study has revealed that the thyroid cancers found in A-bomb survivors were largely conventional papillary in nature, and this is also the case of spontaneous thyroid cancer in the Japanese population at large. Solid variant papillary thyroid cancer (PTC) has not been found in A-bomb survivors yet, although this cancer has been frequently observed among post-Chernobyl children (5, 6).

Gene alterations that lead to constitutive activation of the mitogen-activated protein kinase (MAPK)-signaling pathway are frequently found in PTC. These alterations are mutually exclusive, nonoverlapping events that involve rearrangements of the *RET* and *neutrotrophic tyrosine kinase receptor 1* (*NTRK-1*) genes and point mutations in the *RAS* and *BRAF* genes (7–9). Alteration of one of these genes can be detected in >70% of PTC, suggesting that the constitutive activation of the MAPK-signaling pathway is a major early event in papillary thyroid carcinogenesis.

RET proto-oncogene is normally expressed in a subset of cells derived from the neural crest as well as from the kidney and the enteric nervous system (10, 11). In PTC, the *RET* proto-oncogene is activated by fusion of the *RET* TK domain with the 5′ terminal sequence of one of different heterologous genes via rearrangements that generate a series of chimeric-transforming oncogenes collectively described as *RET/PTCs*. To date, at least 12 rearranged forms of the *RET* gene have been isolated, of which *RET/PTC1* and *RET/PTC3* are by far the most common (12). *RET/PTC* rearrangements were commonly found in childhood PTC regardless of radiation history (13–15). Among the childhood PTC from areas contaminated by the Chernobyl nuclear accident in 1986, *RET/PTC3* rearrangement seemed to be strongly associated with solid variant-type PTC and with a short latency period after exposure (15, 16).

On the other hand, in the Japanese general adult population, typical frequency of *RET/PTC* seems to be of the magnitude of 10% to 40%, although a wide variation, ranging from 2.6% to 70%, has been observed in different geographic areas (17–19). *RET/PTC* rearrangements, especially *RET/PTC1*, was reported as being detected at higher frequency in PTC from adult patients with a

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Requests for reprints: Kiyohiro Hamatani, Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima-shi, Hiroshima 732-0815, Japan. Phone: 81-82-261-3169; Fax: 81-82-261-3170; E-mail: hamatani@rerf.or.jp.

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history of radiotherapy than in those without radiation history (20), but another report disputed such findings (21). Interestingly, we found that *RET/PTC1* rearrangements were induced in human thyroid cells by X-irradiation *in vitro* and *in vivo* as tissue transplants in severe combined immunodeficient mice (22). These findings may provide supporting evidence that activation of the *RET* oncogene via rearrangements plays a crucial role in radiation-associated papillary thyroid carcinogenesis.

The *BRAF* gene encodes a serine/threonine kinase responsible for transduction of signals in the MAP-kinase cascade (23). *BRAF* somatic mutations were first discovered in several types of human cancers, including malignant melanomas (24). Except for very rare instances, the *BRAF* mutation identified in thyroid cancer is thus far almost exclusively thymine-to-adenine transversion at nucleotide 1799, resulting in substitution of glutamate with valine at residue 600 (V600E; ref. 25). The V600E substitution is thought to convert BRAF inactive conformation into its active form by disrupting the residue-residue interaction between the activation loop and the ATP binding site (26).

BRAF^{V600E} mutation has thus far been described as occurring with frequency ranging from 29% to 83% in PTC among an adult general population (25). Regarding the relationship with radiation exposure, the *BRAF*^{V600E} gene mutation was studied in post-Chernobyl PTC, which is believed to have developed in those exposed to radiation in childhood. A very low frequency of *BRAF*^{V600E} mutations in this PTC has been reported (range, 0–12%; refs. 27–31). However, prevalence of *BRAF*^{V600E} mutation was originally low (range, 0–6%) in PTC among children, unrelated to their history of radiation exposure (27, 28, 31). Therefore, it may be difficult to assess the relationship between radiation exposure and childhood PTC in terms of *BRAF*^{V600E} mutation. On the other hand, in adult-onset PTC among A-bomb survivors, we have previously reported that prevalence of *BRAF*^{V600E} mutation was very low in adult-onset PTC among A-bomb survivors exposed to high radiation dose (>0.5 Gy), in contrast to high prevalence in nonexposed survivors or in the general population (32).

These findings lead us to a hypothesis that *RET/PTC* rearrangements in the MAPK-signaling pathway might play a major role in development of adult-onset radiation-associated PTC among A-bomb survivors. Therefore, to examine this hypothesis, this article analyzed pathologic and epidemiologic characteristics of adult-onset PTC in A-bomb survivors in terms of *RET/PTC* rearrangements and *BRAF*^{V600E} mutation.

Materials and Methods

Patients and tissue specimens. Study patients comprised 71 adult-onset PTC cases diagnosed from 1956 to 1993, consisting of 50 exposed and 21 nonexposed patients found among A-bomb survivors in Hiroshima and Nagasaki; 54 of these 71 cases were those used in our previous study on *BRAF*^{V600E} mutation (32). In the LSS (4), a total of about 250 PTC cases were identified in a cohort of LSS among A-bomb survivors during the aforementioned period. To date, we have obtained thyroid tissue specimens from 90 cases of these pathologically confirmed 250 cases, This number covered only about 36% of PTC found in the LSS cohort among A-bomb survivors during 1958 to 1993. After examining quality of RNA, 71 cases were analyzable for both *RET/PTC* and *BRAF*^{V600E} in this study.

Classification of histology was done by one of the authors (T.H.) according to histopathologic typing established by the WHO (33). All study materials were formalin-fixed and paraffin-embedded PTC tissue specimens surgically resected during 1956 to 1993. This study was conducted under approval of the Human Investigation Committee and the Ethics Committee for Genome Research at the Radiation Effects Research Foundation (RERF).

RNA preparation and cDNA synthesis. RNA was extracted from microdissected noncancerous or cancerous regions using the High Pure RNA Paraffin kit (Roche Diagnostics GmbH), as described previously (34). Reverse transcription was performed with random primers (9 mer) using 100 ng total RNA as template, as described previously (34).

Identification of *RET/PTC* rearrangements and *BRAF*^{V600E} mutation. Reverse transcription-PCR (RT-PCR) with *BCR* as internal control was

Table 1. Pathologic and epidemiologic characteristics of patients by radiation exposure status

		Exposed (dose > 0 mGy; n = 50)	Nonexposed* (n = 21)	P
Gender	Male (n)	6	2	1 †
	Female (n)	44	19	
Histologic subtype	Conventional PTC (n)	47	21	0.6 †
	Follicular variant (n)	3	0	
Median age ATB ‡ (y, range)		22 (1–47)	20 (0–50)	0.3§
Median age at diagnosis (y, range)		50 (18–89)	48 (24–84)	0.9§
Median time after exposure (y, range)		24 (11–46)	—	—
Median radiation dose (mGy, range)		203 (0.4–2,758)	0	—
RET/PTC rearrangement	Absence (n)	39	20	0.09 †
	Presence (n)	11	1	
	Frequency (%)	22	5	
BRAF^{V600E} mutation	Absence (n)	22	4	0.06 †
	Presence (n)	28	17	
	Frequency (%)	56	81	

*The nonexposed patients were either those with radiation dose estimated to be 0 mGy or those who were not in the city of Hiroshima or Nagasaki at the time of bombing.
† Fisher's exact test.
‡ ATB: at the time of atomic bombing.
§ Mann-Whitney's U test.

Table 2. Pathologic and epidemiologic characteristics of patients by *RET/PTC* rearrangement status

		All patients			Exposed patients (>0 mGy)		
		RET/PTC (*n* = 12)	Wild-type *RET* (*n* = 59)	*P*	*RET/PTC* (*n* = 11)	Wild-type *RET* (*n* = 39)	*P*
Gender	Male (*n*)	1	7	0.6*	1	5	0.6*
	Female (*n*)	11	52		10	34	
Histology	Conventional PTC (*n*)	11	57	0.9*	10	37	0.9*
	Follicular variant (*n*)	1	2		1	2	
Median age ATB †	Years	15	21	0.2 ‡	13	26	0.1 ‡
	Range	(3–41)	(0–52)		(3–41)	(1–47)	—
Median age at diagnosis	Years	39	51	0.1 ‡	39	54	0.05 ‡
	Range	(21–59)	(18–89)	—	(21–59)	(18–89)	—
Median time after exposure	Years	—	—	—	20	24	0.3 ‡
	Range	—	—	—	(15–36)	(11–46)	—
Median radiation dose	mGy	943	12	0.001 ‡	960	151	0.005 ‡
	Range	(0–2,304)	(0–2,758)	—	(67–2,304)	(0.4–2,758)	—

* Fisher's exact test.
† ATB: at the time of atomic bombing.
‡ Mann-Whitney's *U* test.

conducted to confirm whether RNA extracted from archival tissue specimens was available for RT-PCR. The samples were examined for expression of *RET* TK domain by RT-PCR. RNA with detectable expression of the TK domain was further analyzed for determination of rearrangement types. cDNA derived from 10 ng of total RNA was used as an RT-PCR template. RT-PCR was performed with 0.5 U of Platinum Taq DNA polymerase (Invitrogen) for BCR, the TK domain, *RET/PTC1* and *RET/PTC3*, or 0.5 U of Platinum Taq DNA polymerase High Fidelity (Invitrogen) for *TRK-T2* and novel *RET/PTC* in 25 μL volume containing 1× PCR buffer, 200 μmol/L each of deoxynucleotide triphosphate mixture, and 0.4 μmol/L of each primer. RT-PCR conditions consisted of initial denaturation (95°C for 3 min), followed by 40 cycles (36 cycles for TK domain of *RET*) of denaturation at 95°C for 30 s, annealing for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Primer sets, oligonucleotides, annealing temperature, and Mg^{2+} concentration are summarized in Supplementary Table S1.

For samples that showed expression of *RET* gene TK domain but not assigned as *RET/PTC1* or *RET/PTC3*, rearrangement types were examined by an improved SMART RACE method, which was developed by us.[11] Briefly, after completion of cDNA synthesis, the reaction solution was further incubated at 42°C for 60 min in the presence of SMART adaptor. This SMART-PCR was conducted using FastStart High Fidelity PCR system (Roche Diagnostics GmbH), and primers RET-Ex12PR9 and S-RACE 1, followed by nested RT-PCR using primer RET-Ex12A4 and SMART adaptor. SMART-PCR conditions were as described above, except for the cycle numbers (45 cycles for 1st PCR and 25 cycles for nested PCR). All target bands in RT-PCR were confirmed by digestion of restriction enzyme, *BamH* I (TaKaRa) for *RET/PTC1* and *RET/PTC3, Alu* I for *BCR*, and *Hae* III for the TK domain, which existed within each amplified target fragment. Other *RET/PTC* rearrangement types identified by improved SMART RACE were confirmed by sequencing using a CEQ8000 DNA sequencer (Beckman Coulter, Inc.).

BRAF gene mutation causing amino acid substitution of glutamic acid for valine at codon 600 ($BRAF^{V600E}$) was determined by RFLP using *TspR* I (New England Biolabs) and direct sequencing, as described previously (32).

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Statistical analysis. Mann-Whitney's *U* test was used for nonparametric two-sample comparisons of continuous variables. Fisher's exact test was used for categorical variables. The Cochran-Armitage test was used for nonparametric trend analysis. Logistic regression analysis was carried out among 39 A-bomb survivor exposed patients who had either *RET/PTC* rearrangement or $BRAF^{V600E}$ mutation, to assess differences between PTC patients with *RET/PTC* rearrangement and those with $BRAF^{V600E}$ mutation, in terms of pathologic and epidemiologic variables, including radiation dose, histology, gender, and time-related factors [Note that age at diagnosis = age at the time of A-bombing (ATB) + the time since exposure]. All statistical analyses were performed with SPSS software (version 12.0).

Radiation dose. A-bomb radiation doses used in this analysis were shielded organ dose to the thyroid estimated by the recently implemented DS02 system (35).

Results

Pathologic and epidemiologic characteristics of PTC among A-bomb survivors. Pathologic and epidemiologic characteristics of study patients are shown in Table 1. All tumors were well-differentiated PTC including three cases of follicular variant. When comparing exposed and nonexposed patients, no differences were found based in gender, histologic subtypes, age ATB, and age at diagnosis.

Of 71 patients, we detected *RET/PTC* rearrangements in 12 patients: 9 with only *RET/PTC1*, 1 with both *RET/PTC1* and *RET/PTC3*, 1 with *RET/PTC8*, and 1 with a novel *RET* rearrangement. This novel *RET/PTC* (*RET/PTCX*) was regarded as one *RET* rearrangement, whose partner gene, acyl-CoA binding domain containing 5 (*ACBD5*, located on chromosome 10p12.1), had at least one coiled-coil domain, expression of which was confirmed by RT-PCR (Supplementary Fig. S1). Although the exposed patients showed a higher frequency of *RET/PTC* rearrangements than did nonexposed ones, this difference was not statistically significant (Table 1). On the other hand, frequency of $BRAF^{V600E}$ mutation was marginally lower in exposed patients than that in nonexposed ones (P = 0.06; Table 1).

Pathologic and epidemiologic characteristics by *RET/PTC* rearrangement status. Pathologic and epidemiologic characteristics of study patients were shown by *RET/PTC* rearrangement status in Table 2, where nonexposed patients (0 mGy) were excluded ("exposed patients") or included ("all patients"). Significant difference was found in radiation dose between all patients with and without *RET/PTC* rearrangement ($P = 0.001$; median dose, 943 versus 12 mGy), and also between exposed patients with and without *RET/PTC* rearrangement ($P = 0.005$; median dose, 960 versus 151 mGy; Table 2). Presence or absence of *RET/PTC* rearrangement revealed marginal association with age at diagnosis in exposed patients ($P = 0.05$), although no significant association was found in all patients ($P = 0.1$). No significant relationship was observed between *RET/PTC* rearrangement status and age ATB, histologic subtype, or gender in both all patients and only exposed patients. Furthermore, no significant association was found in exposed patients with time elapsed since A-bomb exposure to diagnosis.

Pathologic and epidemiologic characteristics by *BRAF*^{V600E} mutation. Pathologic and epidemiologic characteristics of study patients were shown by *BRAF*^{V600E} mutation status (Table 3). Close association of *BRAF*^{V600E} mutation status with radiation dose and time since exposure remained unchanged from our previous results (32): PTC patients with *BRAF*^{V600E} mutation showed significantly lower radiation dose ($P = 0.0001$ or 0.0002 in all patients or exposed patients, respectively) and significantly longer time since exposure ($P = 0.0003$ in exposed patients), compared with those without *BRAF* mutation. Age at diagnosis was found to be significantly older in patients with *BRAF*^{V600E} mutation than those without *BRAF*^{V600E} mutation ($P = 0.001$ or 0.0002 in all patients or exposed patients, respectively), although this association did not reach significance in our previous study (32) based on a smaller number of patients. In addition, in only exposed patients, *BRAF*^{V600E} mutation status revealed a significant association with age ATB, but this was not significant in all patients. Furthermore, no significant association was found between *BRAF* mutation status and histology or gender as was also the case in our previous study (32).

Increased *RET/PTC* rearrangements and decreased *BRAF*^{V600E} mutation frequency with increased radiation dose. To examine the relationship between *RET/PTC* and *BRAF*^{V600E} mutation and radiation dose, exposed PTC patients were divided into three groups by dose tertiles. *RET/PTC* rearrangements were more frequently found in patients with increased radiation dose ($P_{trend} = 0.002$; Fig. 1*A*). Specifically, *RET/PTC* rearrangements were found in 50% (8 of 16) of PTC patients who were exposed to high doses (>0.5 Gy) in Fig. 1*A*: 5 with only *RET/PTC1*, 1 with both *RET/PTC1* and *RET/PTC3* (2.2 Gy), 1 with *RET/PTC8* (2.3 Gy), and 1 with *RET/PTCX* (1.5Gy).

On the other hand, prevalence of *BRAF*^{V600E} mutation significantly decreased with radiation dose ($P_{trend} = 0.00006$). In addition, PTC patients having wild-type *RET* and *BRAF* showed a marginally significant increasing trend with radiation dose ($P = 0.08$; Fig. 1*A*).

Frequency of *RET/PTC* and *BRAF*^{V600E} alterations in PTC patients grouped by time elapsed since atomic radiation exposure. *RET/PTC* rearrangements and *BRAF*^{V600E} mutation were further studied in relation to time since radiation exposure (Fig. 1*B*). *BRAF*^{V600E} mutation significantly increased with increased time since exposure ($P_{trend} = 0.001$), whereas unidentified alterations in PTC having wild-type *RET* and *BRAF* significantly decreased with increased time since exposure ($P_{trend} = 0.001$). In contrast, *RET/PTC* rearrangements showed a peak at time since exposure 18 to 27 years, suggesting that unidentified alterations other than *RET/PTC* may also play an important role in PTC occurred in relatively short time since the exposure.

Radiation-related factors underlying occurrence of *RET/PTC* rearrangements versus *BRAF*^{V600E} mutation. As was the case for PTC among the general population (7–9), *RET/PTC* rearrangements and *BRAF*^{V600E} mutation were found to be mutually exclusive among exposed PTC patients (Supplementary Table S2). On the basis of this result, pathologic and epidemiologic characteristics were compared between 11 PTC patients having

Table 3. Pathologic and epidemiologic characteristics of patients by *BRAF*^{V600E} mutation status

		All patients			Exposed patients (>0 mGy)		
		BRAF^{V600E} ($n = 45$)	Wild-type *BRAF* ($n = 26$)	*P*	BRAF^{V600E} ($n = 28$)	Wild-type BRAF ($n = 22$)	*P*
Gender	Male (n)	5	3	0.7*	3	3	0.5*
	Female (n)	40	23		25	19	
Histology	Conventional PTC (n)	44	24	0.3*	27	20	0.4*
	Follicular variant (n)	1	2		1	2	
Median age ATB †	Years	22	17	0.09 ‡	31	17	0.04 ‡
	Range	(0–52)	(1–47)	—	(1–47)	(1–47)	—
Median age at diagnosis	Years	54	39	0.001 ‡	55	38	0.0002 ‡
	Range	(20–89)	(18–62)	—	(20–89)	(18–59)	—
Median time after exposure	Years	—	—	—	29	18	0.0003 ‡
	Range	—	—	—	(15–46)	(11–36)	—
Median radiation dose	mGy	8	538	0.0001 ‡	69	859	0.0002 ‡
	Range	(0–2,758)	(0–2,304)		(0.4–2,758)	(12–2,304)	—

*Fisher's exact test.
†ATB: at the time of atomic bombing.
‡Mann-Whitney's *U* test.

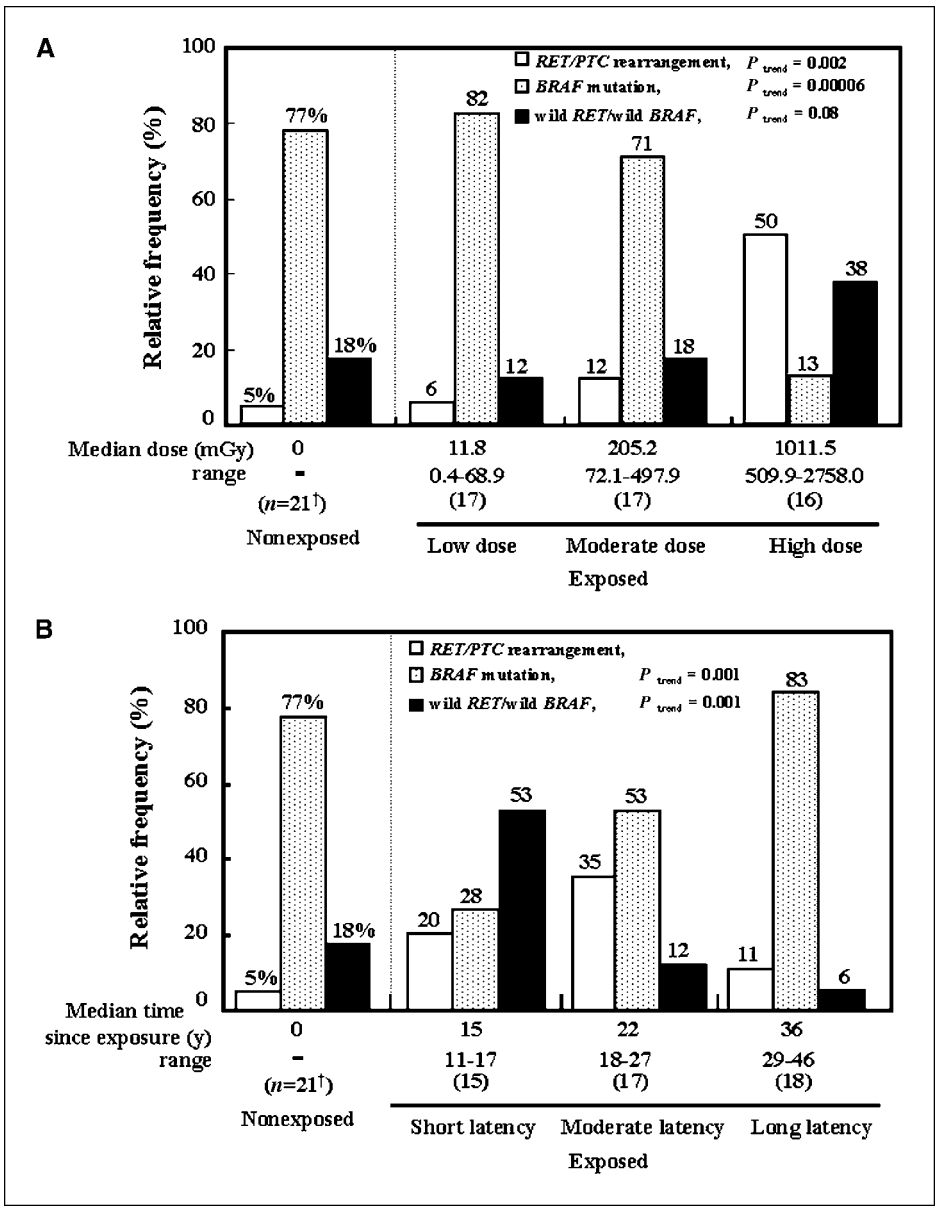

Figure 1. *A,* relative frequency of *RET/PTC* and *BRAF*^{V600E} alterations in PTC patients grouped by radiation exposure dose levels (nonexposed and dose tertiles). Exposed PTC patients were divided into three groups by dose tertiles. *B,* relative frequency of *RET/PTC* and *BRAF*^{V600E} alterations in PTC patients grouped by time elapsed since atomic radiation exposure (nonexposed and tertiles of time since exposure). Exposed PTC patients were divided into three groups by tertiles of time since exposure. †, one case in the nonexposed group had both *RET/PTC* and *BRAF*^{V600E}. Relative frequency of genes in the nonexposed group was calculated by using 22 for number of gene alterations. PTC with *RET/PTC* rearrangement (*open bars*), with *BRAF*^{V600E} mutation (*dotted bars*), or with other unknown alterations (*closed bars*), respectively, are shown.

RET/PTC rearrangements and 28 patients having *BRAF*^{V600E} mutation. PTC patients with *RET/PTC* rearrangements revealed past exposure to significantly higher radiation dose (P = 0.001; Fig. 2*A*), shorter time elapsed since radiation exposure (P = 0.03; Fig. 2*B*), and younger age ATB (P = 0.06; Fig. 2*C*), compared with the patients with *BRAF*^{V600E} mutation.

Subsequent logistic regression analysis for mutually exclusive occurrence of *RET/PTC* rearrangements or *BRAF*^{V600E} mutation confirmed these findings, using "age ATB" and "time since exposure" as independent time-related explanatory variables (Note that "age at diagnosis" = "age at exposure" + "time since exposure"). Radiation dose, age at exposure, and time elapsed since exposure were significantly associated with which alteration type of *RET/PTC* rearrangements or *BRAF*^{V600E} mutation occurred in the development of PTC among A-bomb survivors (P = 0.012, 0.031, and 0.034, respectively; Table 4).

Rearrangements of *NTRK1* and *BRAF* genes. *NTRK1* rearrangements and the *AKAP9-BRAF* fusion gene were also examined in the 71 cases. The *TRK-T2* gene was detected in only one exposed case with wild-type *RET* and *BRAF*. However, five *NTRK1*-derived nucleotides were deleted in this amplified fragment. On the other hand, no *AKAP9-BRAF* fusion gene was detected in these 71 cases.

Discussion

In papillary thyroid carcinogenesis, constitutive activation of the MAPK–signaling pathway, namely rearrangements of *RET* and *NTRK* genes and mutations in *RAS* and *BRAF* oncogenes, seems to be required for transformation (36). Recent *in vitro* and *in vivo* experiments have also shown the requirement of activation of the RET/PTC-RAS-BRAF-MAPK pathway in thyroid tumorigenesis (37–39). Interestingly, mutual exclusion of these genetic alterations in the MAPK-signaling pathway was reported; one event among *BRAF* mutation, *RAS* mutations, and *RET/PTC* rearrangements (7, 8, 29) or one among *BRAF* mutation, *RET/PTC*

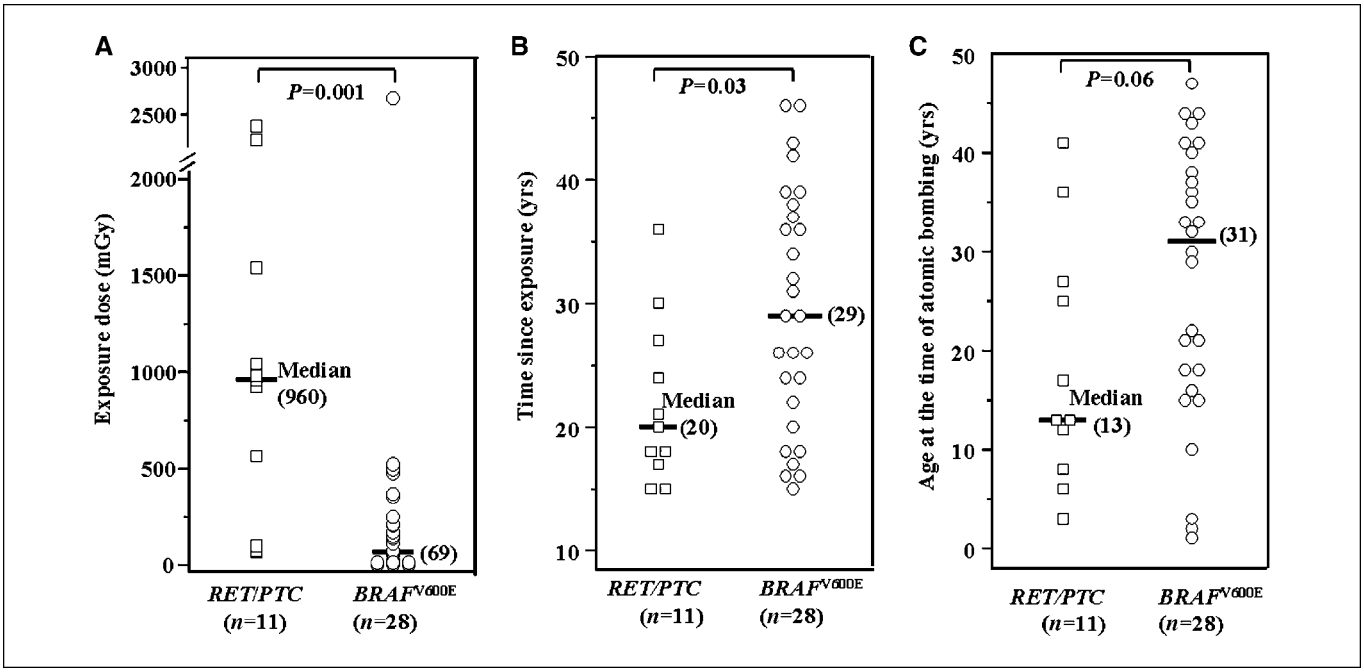

Figure 2. Comparison of *RET/PTC* and *BRAF*^{V600E} alterations in PTC patients. *A,* radiation dose; *B,* time since exposure; *C,* age at the time of atomic bombing. *RET/PTC PTC* rearrangement (□) and *BRAF*^{V600E} mutation (○), respectively, are shown.

rearrangements, and *NTRK1* rearrangements (9) was singularly found, indicating that one such gene alteration is an important early event in development of PTC. Furthermore, a recently identified *AKAP9-BRAF* rearrangement did not coexist with *BRAF* mutation in radiation-associated PTC (30). These data suggest that a single genetic event in the MAPK-signaling pathway may be sufficient for thyroid cell transformation and tumorigenesis.

In this study, pathologic and epidemiologic characteristics, specifically radiation-related ones, of PTC having *RET/PTC* rearrangements contrasted clearly with those of PTC having *BRAF*^{V600E} mutation. Noting that 17 (81%) and 1 (5%) of 21 nonexposed PTC patients having *BRAF*^{V600E} mutation and *RET/PTC* rearrangement in this study, respectively, are in agreement with other data on nonexposed adult-onset Japanese PTC (18, 25, 40–42), we for the first time have shown that the frequency of *RET/PTC* rearrangements significantly increased with increased radiation dose as well as shorter time elapsed since radiation exposure and younger age at the time of bombing (Figs. 1*A* and 2; Table 4). *RET/PTC* rearrangements were detected in 50% (8 of 16) of adult-onset PTC patients who were exposed to radiation dose of >0.5 Gy, although this frequency was somehow lower than that (about 80%) reported for French thyroid cancer patients who had received external radiotherapy (18). This difference in frequency of *RET/PTC* rearrangements may be due to the different radiation conditions (i.e., single or repeated irradiation and dose). On the other hand, *BRAF*^{V600E} mutation significantly decreased frequency with increased radiation dose (Fig. 1*A*). This finding seems to be consistent with our parallel observations, shorter time elapsed since exposure, and younger age at the time of bombing in PTC patients with *RET/PTC*, compared with those in the patients with *BRAF*^{V600E} (Fig. 2; Table 4). Taken together, our findings imply that *RET/PTC* rearrangements, not *BRAF*^{V600E} mutation, are closely associated with radiation-associated adult-onset PTC.

The existence of a molecular mechanism other than *RET/PTC* rearrangement is suggested from Fig. 1*B*: *RET/PTC* rearrangements showed a peak at 20 to 30 years since radiation exposure and relatively low frequency of 20% in <20 years since exposure, in contrast to 53% of unidentified alterations other than *RET/PTC* and *BRAF*^{V600E}. Because *RET/PTC* and *BRAF*^{V600E} account for 82% of nonexposed PTC and about 60% to 70% of PTC in the Japanese general population (18, 25, 40–42), this increase of unidentified alterations in <20 years is thought to be caused by radiation. This unidentified mechanism may be involved in radiation-associated PTC, which occurred earlier after radiation exposure than did PTC having *RET/PTC*. However, regarding *NTRK1* rearrangements and the *BRAF* fusion gene, the *TRK-T2* gene lacking five nucleotides was

Table 4. Logistic regression analysis of 39 exposed PTC patients with *RET/PTC* rearrangements or *BRAF*^{V600E} mutation

Variables	β*	*P*
Radiation dose (mGy)	0.002	0.012
Age at the time of atomic bombing (y)	−0.113	0.031
Year since exposure (y)	−0.192	0.034
Gender, male vs. female	2.674	0.204
Histology, conventional vs. follicular variant	0.157	0.927

NOTE: A dependent variable was defined as follows: rearranged *RET* and wild *BRAF* = 1; wild *RET* and mutated *BRAF* = 0.
*Regression coefficients in the logistic regression model.

detected in only one exposed case. Therefore, the unidentified alterations may be involved in pathways other than the MAPK-signaling pathway.

We need to confirm our findings with an increased number of study patients, given that the present study covered only about 36% of PTC found in the LSS cohort among A-bomb survivors during 1958 to 1993 for whom tissue specimens could be obtained. Toward this end, an efficient system to collect archival specimens from A-bomb survivors, which are dispersed over a number of hospitals in Hiroshima and Nagasaki, will be necessary in cooperation with the institutions concerned (it took 3 years to collect 90 PTC specimens, 71 of which were used in the present study). Because the specimens deteriorate as time goes by, it is urgent that our collection and analyses be conducted soon to increase our knowledge, which in turn might lead to improved treatment and prevention of radiation-associated cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Kiyohiro Hamatani, Hidetaka Eguchi, Reiko Ito, et al.

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