Thyroid Cancer: Molecular Characteristics of Radiation-Associated Papillary Thyroid Cancer, with a Special Reference to of Atomic Radiation Exposure

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Abstract

Among atomic-bomb (A-bomb) survivors of Hiroshima and , incidence of thyroid cancer significantly increased after exposure to nuclear radiation. This review will focus on the initiating gene alterations in the development of adult-onset papillary thyroid cancer (PTC) among A-bomb survivors. The effects of A-bomb radiation on chromosomal rearrangements (RET and NTRK1 rearrangements) and point mutations (BRAF and RAS mutations) after exposure were different. In contrast to PTC cases with point mutations, PTC cases with chromosomal rearrangements were observed more frequently among those exposed to high radiation doses compared to low doses, and these cases developed cancer earlier after exposure than did cases with point mutations. Interestingly, PTC cases with non-detected gene alterations were found more frequently among patients who were exposed to high radiation doses and who developed cancer earlier after radiation exposure than did the cases with BRAF point mutation. This suggests that heretofore non-detected gene alterations may also be involved in adult-onset PTC among A-bomb survivors.

Keywords Mutations; Anaplastic lymphoma kinase; Nuclear radiation; Thyroid cancer

Introduction

Thyroid cancer is one of the malignancies most closely associated with radiation exposure. External radiation exposure is related to papillary thyroid cancer (PTC) based on data from atomic-bomb (A-bomb) survivors in Hiroshima and , and also among people exposed to medical radiation sources. Epidemiological studies on the Life Span Study (LSS) cohort of A-bomb survivors have revealed that the excess relative risk (ERR) of thyroid cancer was significantly high and that it linearly increased with radiation dose [1,2]. The patients who received external radiation therapy for either benign or malignant diseases e.g. tinea capitis (Israel), enlarged thymus gland, benign head and neck conditions, lymphoid hyperplasia, childhood cancer, and cervical cancer (USA) showed an increased incidence of thyroid cancer [3,4]. Those radiation-associated thyroid cancers also showed a tendency toward a higher ERR associated with younger age at the time of exposure [1,4]. In addition, cohort studies on subjects who were exposed to ionizing radiation after the Chernobyl nuclear accident in 1986 indicate a very strong association between radiation exposure in childhood or adolescence and the development of thyroid cancer in heavily contaminated areas in Belarus, Northern Ukraine, and [5-7].

Histologically, thyroid cancer among cohorts exposed externally or internally to ionizing radiation is mainly papillary type much like sporadic thyroid cancer. However, there are differences in subtypes of PTC between A-bomb survivors and post-Chernobyl children. Among A-bomb survivors, the thyroid cancers were largely conventional papillary in nature [8], which is also the case for sporadic thyroid cancer in the general Japanese population. In addition, adult-onset PTC among A-bomb survivors included infrequent follicular variants and no solid variants, which are subtypes of PTC. For children internally exposed in Chernobyl, however, malignant thyroid tumors are principally PTC, and include frequent follicular variants and solid variants [9-11], but these morphologic characteristics may have been related to low dietary iodine levels and childhood cancer types [12].

Radiation types and PTC Gene Alterations

Both sporadic PTC and radiation-associated PTC are characterized by the constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway. The major factors involved in the activation of this signaling pathway are RET/PTC rearrangements and BRAF point mutation [13-17]. It is well known that RET/PTC rearrangements were frequently found in PTC among children from areas contaminated by [11,14,18,19]. However, since sporadic childhood PTC with no radiation history shows a high incidence of RET/PTC rearrangements, it is difficult to distinguish whether the high prevalence of RET/PTC rearrangements in PTC from post-Chernobyl children is due to internal radiation exposure or childhood cancer. On the other hand, some reports have found a higher frequency of RET/PTC rearrangements in PTC from adult patients who had received external radiotherapy than in those without any radiation history [23,24]; other reports have disputed such findings [22,25]. As seen above, radiation effects on molecular events at an early stage of papillary thyroid carcinogenesis remain undefined. This ambiguity may be due to the different radiation conditions, namely whether internal exposure or external exposure, and whether single exposure or repeated exposures. In addition, radiation effects may differ depending on age at exposure and/or age at onset of PTC. Such differences make comparative analysis difficult and prevent the deepening of our understanding of radiation effects on initiating molecular events in PTC. On the other hand, A-bomb survivors were exposed externally to A-bomb radiation. Cases of PTC developing among LSS cohort members of A-bomb survivors are derived from adult patients with known radiation exposure. Therefore, we believe
that adult-onset PTC among LSS cohort members is a good model for examination of the relationship between radiation dose and gene alterations at early stages of papillary thyroid carcinogenesis. This review will focus on characteristics of early molecular events in pathogenesis of adult-onset PTC among A-bomb survivors (Table 1).

<table>
<thead>
<tr>
<th>Radiation-associated PTC</th>
<th>Chromosomal rearrangements</th>
<th>Point mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-bomb survivors (Our study)</td>
<td>RET/PTC 4%</td>
<td>BRAFV600E 70%</td>
</tr>
<tr>
<td>Non-exposed</td>
<td>TRK &amp; TRK-T1,2,3 0%</td>
<td>K, H, N-RAS 4%</td>
</tr>
<tr>
<td>Exposed</td>
<td>18%</td>
<td>2%</td>
</tr>
<tr>
<td>Post-Chernobyl</td>
<td>34-87% [27,52,53,66,67]</td>
<td>11% [27,28,61,65-67]</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>51-84% [22-25]</td>
<td>19% [56]</td>
</tr>
<tr>
<td>Sporadic PTC</td>
<td>3-61%</td>
<td>28-83% [15-17,26,64,67]</td>
</tr>
<tr>
<td>Adult-onset</td>
<td>6-12% [20,26,56,57]</td>
<td>1% [28]</td>
</tr>
<tr>
<td>Childhood</td>
<td>0-6% [66-67]</td>
<td>0-7% [65,72]</td>
</tr>
</tbody>
</table>

Table 1: Gene alterations in radiation-associated and sporadic PTC (*detected only in PTC developed 5-6 years after radiation exposure).

**Constitutive Activation of MAPK Signaling Pathway**

A major early molecular event in the development of PTC is believed to be the constitutive activation of the MAPK signaling pathway, which is caused by gene alterations including rearrangement of RET, NTRK, and BRAF genes, and point mutation of BRAF and RAS genes. Furthermore, those gene alterations are well known to occur in a mutually exclusive manner, and they were found in more than 70% of PTC [16,17,26-28]. Specific activation of RET/PTC1 or RET/PTC3 (types of RET rearrangements), TRK-T1 (one type of NTRK1 rearrangements), c-Ha-Ras, or BRAFV600E in transgenic mice produced thyroid cancer with characteristic papillary features [29-34]. In addition, a part of microscopic PTC (micronodular) is known to harbor RET/PTC rearrangements, BRAFV600E point mutation, or NTRK1 rearrangement [35-41], which suggests that a single alteration of these genes involved in the MAPK signaling pathway may be the most important initiating event and may play a causative role in the pathogenesis of PTC. In addition to the MAPK signaling pathway, activation of the phophatydylinositol 3-kinase/AKT pathway through alterations of PIK3CA and PTEN genes was reported to be implicated in the development of not only follicular carcinoma but also some PTC [42-45].

**Chromosomal Rearrangements**

Gene rearrangements reported so far in PTC are RET/PTC, NTRK1, and BRAF/AKAP9 rearrangements. Among those, RET rearrangements are the most common, especially in PTC developed in subjects with a radiation exposure history, which is supported by several studies indicating the induction of RET/PTC1 and RET/PTC3 rearrangements in human thyroid cells by X-ray or γ-ray irradiation, both in vitro and in vivo, as tissue transplants in severe combined immunodeficient mice [46-49].

**RET/PTC rearrangements**

RET/PTC rearrangements are formed by the fusion of part of the intracellular tyrosine kinase domain with the 5'-end of other genes. RET/PTC fusion protein is constitutively expressed by promoter activity of a partner gene, and is then activated by constitutive dimerization. To date, at least 15 different types of RET/PTC rearrangements resulting from RET fusion to 12 various partner genes have been isolated, of which RET/PTC1 and RET/PTC3 are by far the most common [14,50]. RET/PTC rearrangements have frequently been found in childhood PTC with and without a radiation exposure history [11,14,18,22,51-53]. In post-Chernobyl children with PTC, RET/PTC3 rearrangement seemed to be strongly associated with solid variant PTC and/or with a short latent period after exposure, while RET/PTC1 rearrangement was mainly found in conventional PTC with a long latent period after exposure [11,18,19,53]. In contrast, the frequency of RET/PTC rearrangements in adult-onset PTC in the general population was not as high as that in childhood PTC [13,14,54,55] (Table 1). In PTC from patients exposed to therapeutic irradiation, the frequency of RET/PTC rearrangements was higher than in PTC from non-exposed patients [23,24], although several papers have reported that no significant difference was detected in the frequency of RET/PTC rearrangement for adult-onset PTC with and without a history of radiotherapy [22,25].
NTRK1 and BRAF rearrangements

Rearrangements of the neurotrophic receptor-tyrosine kinase NTRK have been observed in a small number of PTC cases in the general population [20,56,57] (Table 1). NTRK1 rearrangements were also found in a small number of PTC from post-Chernobyl children [19] and patients with a radiotherapy history [56]. Rearrangement of the BRAF gene (AKAP9-BRAF) was identified in post-Chernobyl childhood PTC [28]: AKAP9-BRAF rearrangement was reported to be related to post-Chernobyl PTC that developed shortly after exposure [28].

Point Mutations

BRAF point mutation

Another major early event in the development of PTC is point mutation of the BRAF gene. The BRAF point mutation identified in PTC so far is almost exclusively in the thymine-to-adenine rearrangements and a NTRK1 rearrangement were detected in 11 PTC adolescents with no radiation history [65-67] (Table 1). In addition, BRAF point mutation in PTC among the general populations, BRAF V600E mutation has so far been reported as occurring at a high frequency [61-64], although very low frequencies of BRAF V600E mutation were found in PTC among children and adolescents with no radiation history [65-67] (Table 1). In addition, radiation-associated PTC showed a very low frequency of BRAF V600E mutation regardless of the age of patients [27,28,65-68] (Table 1).

RAS point mutations

The RAS point mutations are not restricted to PTC, unlike RET/PTC rearrangements and BRAF point mutation, and have been found with a wide range of frequency in follicular adenomas, follicular thyroid carcinomas (FTC), PTC, and anaplastic carcinomas (ATC). The prevalence of RAS point mutations in PTC among the general populations is not as high as that in FTC and ATC [54,64,69-72]. Furthermore, no RAS point mutations (codons 12, 13, 61) have been observed in post-Chernobyl children PTC [65,73,74]. Some PTC from patients with a radiotherapy history are reported to have RAS mutations [75,76] (Table 1).

Gene Alterations in A-bomb Survivors

To clarify the relationship between radiation exposure and development of PTC, we attempted to identify preferentially occurring gene alterations in radiation-associated PTC. Toward this end, we analyzed RET/PTC, NTRK1, and BRAF rearrangements and BRAF and RAS point mutations in 73 cases of adult-onset PTC (52 exposed patients and 21 non-exposed patients) among A-bomb survivors. The gene alterations detected in the exposed PTC cases were mutually exclusive, although one non-exposed PTC case had both RET/PTC1 rearrangement and BRAF point mutation.

Chromosomal rearrangements in PTC among A-bomb survivors

Only one non-exposed PTC case showed RET/PTC1 rearrangement, but among exposed PTC cases, RET/PTC1 rearrangements and a NTRK1 rearrangement were detected in 11 PTC cases and one case, respectively. In addition to eight PTC cases with only RET/PTC1 and one with both RET/PTC1 and RET/PTC3, a novel type of RET/PTC rearrangement as well as a rare RET/PTC8 was identified in A-bomb survivors exposed to high radiation doses (1,500 mGy and 2,000 mGy, respectively) [77,78]. The frequency of chromosomal rearrangements composed of RET and NTRK1 rearrangements among exposed subjects was higher than among non-exposed patients, although the significance of this difference was only marginal (Fisher’s exact test, P=0.09) (Figure 1A). And, no AKAP9-BRAF rearrangement was detected in adult-onset PTC among A-bomb survivors [77].
more frequent with increased radiation dose (Figure 1B), suggesting that in addition to RET and NTRK1 rearrangements, radiation-associated gene alterations other than rearrangements of RET, NTRK1, and BRAF might be involved in adult-onset PTC cases among A-bomb survivors exposed to high radiation doses.

**Relationship between years elapsed since exposure and gene alterations**

Three groups also showed different responses to time from exposure to diagnosis (three categories: short, 11–20 years; medium, 21–30; long, 31–46) as shown in Figure 2A. Point mutations increased with increased time since exposure, while non-detected gene alterations tended to decrease with increased time since exposure (Figure 2A). On the other hand, chromosomal rearrangements showed a peak around 21-30 years after exposure (Figure 2A). Furthermore, PTC cases with chromosomal rearrangements or non-detected gene alterations developed cancer sooner following exposure than did the cases with point mutations (modified from ref. 77). No AKAP9-BRAF rearrangement was detected in adult-onset PTC among A-bomb survivors exposed to high radiation doses. This might be due to the difference in the time from exposure to diagnosis between post-Chernobyl childhood and among A-bomb survivors' PTC (since all tissue specimens were derived from PTC that developed more than 10 years since A-bomb radiation exposure). Therefore, it remains unclear whether AKAP9-BRAF rearrangement is involved in adult-onset radiation-associated papillary thyroid carcinogenesis.

**Relationship between age at the time of bombing and gene alteration**

Groups with different types of gene alterations also revealed different responses based on age at the time of the bombings (age ATB) (three categories: childhood/adolescence, 0–19; young adult, 20–39; middle age, 40–47), as shown in Figure 2B. Prevalence of PTC cases with point mutations increased with age ATB, while chromosomal rearrangements showed a small decrease with age ATB (Figure 2B). However, the PTC cases with chromosomal rearrangements showed younger age ATB than did those with point mutations (modified from ref. 77). PTC cases with no detected gene alterations showed no association with age ATB.

**Implications from findings in PTC among A-bomb survivors**

Thus, more than 70% of all radiation-exposed PTC cases with RET/PTC rearrangements were in the group with >500 mGy, and a RET/PTC8 rearrangement and a novel type of RET/PTC rearrangement were also identified besides RET/PTC1 in these high-radiation dose-exposed cases [77]. One NTRK1 rearrangement was also found in a survivor with a high radiation dose. Interestingly, many RET/PTC rearrangements were observed in PTC cases having a relatively short time since radiation exposure. Those findings strongly suggest that chromosomal rearrangements, especially RET/PTC rearrangements that were possibly caused by radiation exposure, are strongly involved in adult-onset radiation-associated papillary thyroid carcinogenesis.

All initiating gene alterations occurring in PTC cannot be categorized with only the rearrangements of RET, NTRK1, and BRAF genes, and point mutations of BRAF and RAS genes. Interestingly, adult-onset PTC without any gene alteration of RET, NTRK1, BRAF, or RAS among A-bomb survivors was marginally more frequent in cases who were exposed to high radiation dose (>500 mGy) and in the cases with shorter time since exposure (<20 years), compared with

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non-exposed cases. Those results raise the possibility that there are radiation-related gene alterations other than rearrangements of \( RET, NTRK1, \) and \( BRAF \) genes in radiation-associated PTC. To understand the mechanism of adult-onset radiation-associated PTC, it is essential to identify gene alterations occurring in such PTC cases. Figure 3 indicates a model of initiating molecular events in radiation-associated adult-onset papillary thyroid carcinogenesis in A-bomb survivors exposed to high radiation doses. Recently, echinoderm microtubule-associated protein-like 4 (\( EML4 \))- anaplastic lymphoma kinase (\( ALK \)) fusion gene was discovered in some PTC cases among atomic bomb survivors that carried no alterations in \( RET, NTRK1, BRAF, \) and \( RAS \) genes [80].

**Future Prospects**

The molecular oncology study of PTC in A-bomb survivors suggests that, in addition to the important roles of \( RET/PTC \) and \( NTRK1 \) rearrangements in adult-onset radiation-associated papillary thyroid carcinogenesis, gene alterations other than \( RET/PTC, NTRK1 \) and \( AKAP9-BRAF \) rearrangements are involved in development of some radiation-associated PTC of adult patients who were exposed to high radiation or whose cancer developed in a relatively short time since exposure. \( EML4-ALK \) fusion gene may be one of candidates. Identification of gene alterations in PTC besides \( RET, NTRK1, BRAF, \) and \( RAS \) genes is crucial for understanding the mechanisms of the development of PTC, not only among A-bomb survivors but also for other adult patients who were externally exposed to radiation. If the molecular analysis of adult-onset PTC in patients exposed in childhood to Chernobyl is conducted and integrated with the analyses of A-bomb survivors’ PTC, the mechanism of radiation-associated adult-onset papillary thyroid carcinogenesis should become clearer.

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