Acta Med. Nagasaki 50: 87－89

Segmental Jumping Translocation of Ret Oncogene in Radiation-associated Thyroid Cancer

Masahiro NAKASHIMA,[1] Noboru TAKAMURA,[2] Hiroyuki NAMBA,[3] Vladimir SAENKO,[4] Tomayoshi HAYASHI,[5] Ichiro SEKINE[1,6]

[1] Tissue and Histopathology Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[2] Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[3] Department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[4] Department of International Health and Radiation Research, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[5] Department of Pathology, Nagasaki University Hospital, Nagasaki, Japan

[6] Department of Molecular Pathology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Radiation etiology is well known in thyroid carcinogenesis. Ret rearrangement is the commonest oncogenic alterations in Chernobyl-related papillary thyroid cancer (PTC). To evaluate whether there is a radiation signature, we analyzed Ret rearrangement in radiation-associated thyroid cancers with fluorescence *in situ* hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR). The FISH analysis demonstrated segmental jumping translocation (SJT) of Ret gene in radiation-associated thyroid cancers but not in sporadic well differentiated PTC. Furthermore, Ret SJT was commonly observed in anaplastic thyroid cancer (ATC) including both radiation-associated and sporadic cancers. In PTC, Ret SJT was restricted to radiation-associated or high-grade cases. Because Ret SJT was not observed in sporadic, well differentiated and low-grade cases of PTC, Ret SJT might be a molecular marker for radiation-induced and/or aggressive cases of PTC. SJTs are unbalanced translocations involving a donor chromosome arm or chromosome segment that has fused to multiple recipient chromosome, and mainly reported in treatment-related leukemias, while very rare in solid cancers. We found SJT in radiation-induced and high-grade thyroid cancers, suggesting chromosomal instability. This is the first report showing SJT in thyroid cancer, and probably the third report showing SJT in solid cancer *in vivo*.

ACTA MEDICA NAGASAKIENSIA 50: 87－89, 2005

Keywords: Radiation; Thyroid cancer; Segmental jumping translocation; Ret

Introduction

The incidence of papillary thyroid cancer (PTC) was reported to be elevated in both atomic-bomb (A-bomb) survivors in Hiroshima and Nagasaki, Japan and residents living in areas exposed to fallout from the Chernobyl accident, suggesting a radiation etiology in thyroid carcinogenesis. Ret rearrangement is a well known molecular alteration observed in PTC. A receptor tyrosine kinase, Ret oncoproteins are dimerized by ligand-binding, activated, and subsequently regulating cell growth and survival in normal tissues. During thyroid carcinogenesis, if Ret oncogenes rearranged with other genes containing coiled-coil domain, they were dimerized without ligand stimuli and, constitutively, activating cell growth and survival in thyroid follicular cells, finally promoting the occurrence of papillary carcinoma. Ret rearrangement is the commonest oncogenic alterations in Chernobyl-related PTC; four large studies have found that 50-90% of Chernobyl-related PTC show a Ret rearrangement, nearly all to Ret/PTC1 or Ret/PTC3.[1] However, specific molecular alteration has not been identified in radiation-induced PTC. To evaluate whether there is a radiation signature, we analyzed Ret rearrangement in radiation-associated PTC with FISH.

Segmental jumping translocation (SJT) is defined as unbalanced/nonreciprocal translocations involving a donor chromosome arm or chromosome segment fused to several different recipient chromosomes, suggesting chromosomal instability. In results, we found SJT, a rare cytogenetic aberration, of Ret oncogene in radiation-associated

Address correspondence: Masahiro Nakashima, M.D., Tissue and Histopathology Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523 JAPAN
TEL: +81-(0)95-849-7124, FAX: +81-(0)95-849-7130, E-mail: moemoe@net.nagasaki-u.ac.jp

Received July 26, 2005; Accepted August 1, 2005

thyroid cancers by fluorescence *in situ* hybridization (FISH). This is the first report demonstrating SJT in thyroid cancer.

Materials and Methods

Subjects

All samples were formalin-fixed paraffin-embedded tissues. The sections were used for FISH analysis and RNA preparation. Three cases of radiation-associated PTC and 9 cases of anaplastic thyroid cancer (ATC) were explored for Ret rearrangement by dual-color interphase FISH and reverse transcriptase-polymerase chain reaction (RT-PCR). The clinicopathological profiles of patients are summarized in Table 1. Three cases of radiation-associated thyroid cancers were as following: *Case 1*. A 44-year-old Japanese male, who had been exposed by A-bombing at 6 years of age, was operated under the diagnosis of thyroid cancer and treated by internal radiation with I-131 after the operation. He died of tumor recurrence at 57 years of age. Pathological diagnosis of primary cancer was well differentiated PTC (Case 1), and that of recurred tumor was PTC with anaplastic transformation (Case 13); *Case 2*. A 32-year-old Russian female received surgical treatment for thyroid cancer and histologically diagnosed as follicular variant of PTC. Four years prior to the surgery, the patient had undergone external radiation therapy (40 Gy) for primary mediastinal lymphoma. A novel tumorigenic rearrangement, Δrfp/ret which was translocation between chromosomes 6 and 10 was identified as shown in our previous report[2]; *Case 3*. The patient was 41-year-old Russian female living in a radio-contaminated area around Chernobyl-accident, aged 25 years at the time of the accident, and was operated under the diagnosis of thyroid cancer. Histological examination revealed it a follicular variant of PTC. We took 10 cases of non-radiation-associated PTC, who had neither history of A-bombing nor radiation therapy, as control.

Dual-color interphase FISH

Deparaffinized sections were heated by microwave in a 0.01 M citrate buffer (pH 6.0) and pretreated with 0.3% pepsin. Subsequently, slides were immersed in 0.1% NP-40 and denatured by heating in 70% formamide/2x SSC. The mixture containing Spectrum Green-labeled DNA probes specific for the Ret gene (BAC clone RP11-351D16: accession number AC010864, human chromosome 10q11) and Spectrum Orange-labeled DNA probes correspond to the centromere of chromosome 10 (CEP10, Vysis Inc., Downers Grove, IL, USA) was denatured and applied to the denatured tissue. The slides were covered with a coverslip, sealed with rubber cement, incubated incubated for 16hr at 42ºC in a humidified chamber. After hybridization, slides were washed, then counterstained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI-I, Vysis Inc.), and were visualized and photographed using a fluorescence microscope.

RT-PCR

Total RNA was isolated from tissues with High Pure RNA paraffin Kit (Roche, Mannheim, Germany) according to manufacturer's protocol, and examined for Ret/PTC1 and Ret/PTC3. Expression of Ret/PTC1, Ret/PTC3, and α-tubulin genes was assessed by RT-PCR using respective primers designed with the Primer Express Software (PE Applied Biosystems, Foster City, CA, USA). All reactions were performed with the SuperScript™ One-Step RT-PCR with Platinum® *Taq* System (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. Primer sequences, annealing temperature settings in the PCR reactions, and size of amplicons were listed in Table 2.

Table 1. Clinicopathological profiles of patients and summary of results

Case	Age	Sex	Pathological diagnosis	pTNM	Radiation exposure	Rearrangement	SJT
1	44	M	PTC, WD	T1N0M0	A-bomb	—	—
2	32	F	PTC, FV	T1N0M0	Radiation therapy	Δrfp/ret	<5%
3	41	F	PTC, FV	T4N1bM0	Chernobyl accident	PTC3	<5%
4	22	F	PTC, PD	T4N1aM0	—	PTC1	<5%
5	77	F	ATC	T4N0M0	A-bomb	—	15%
6	55	F	ATC	T4N0M0	Radiation therapy, A-bomb	—	25%
7	84	F	ATC	T4N0M0	A-bomb	—	10%
8	78	M	ATC	T4N0M0	—	—	10%
9	81	F	ATC	T2N0M0	—	—	10%
10	76	M	ATC	T4N0M0	—	—	ND
11	71	F	ATC	T4N1bM0	—	—	15%
12	64	M	ATC	T3N0M0	—	—	15%
13	57	M	ATC	T4N0M0	Radiation therapy, A-bomb	—	30%

PTC=Papillary thyroid cancer; ATC=Anaplastic thyroid cancer; WD=Well differentiated; FV=Follicular variant; PD=Poorly differentiated; SJT=Segmental jumping translocation; ND=Not determined

Table 2. List of primers and PCR conditions

Target	Sequence	Annealing (ºC)	Amplicon (bp)
Ret/PTC1			
Forward	GCCTGGAGGAGCTCACCAA	56	255
Reverse	CTCTGCCTTTCAGATGGAA		
Ret/PTC3			
Forward	ACCTGCCAGTGGTTATCAAGCT	58	154
Reverse	TTCGCCTTCTCCTAGAGTTTTTCC		
α-Tubulin			
Forward	AGATCATTGACCTCGTGTTGGA	56	101
Reverse	ACCAGTTCCCCCACCAAAG		

Results

All results are summarized in Table 2. The FISH analysis demonstrated nuclei showing several Ret signals and two CEP10 signals in this study, suggesting segmental jumping translocation (SJT) of Ret gene. In three cases of radiation-associated PTC, Ret SJT was observed in both Cases 2 and 3 but not in Case 1. On the other hand, in 10 cases of non-radiation-associated PTC, Ret SJT was evident in only one case (Case 4), which was poorly differentiated and high grade (pT4N1aM0) PTC from a 22-year-old Japanese female. The frequency of SJT-positive cells was approximately 5% in all of positive cases. No Ret rearrangement was identified in normal thyroid follicle surrounding tumor from all cases by FISH. Furthermore, RT-PCR analyses revealed Ret/PTC3 and Ret/PTC1 in Cases 3 and 4, respectively.

On the other hand, SJT of Ret gene was observed in all of ATC but a case which showed no positive signals with FISH. The frequency of SJT-positive cells ranged between 10 and 30% and was apparently higher in ATC than PTC. Although Ret SJT was found in ATC regardless of radiation history, its frequency was higher in Cases 6 and 13 which received radiation therapy, than others. Neither Ret/PTC1 nor PTC3 was demonstrated in all cases of ATC by RT-PCR.

Discussion

We found SJT of Ret oncogenes in radiation-associated thyroid cancer. Ret SJT was commonly observed in ATC including both radiation-associated and sporadic cancers. SJTs are unbalanced translocations involving a donor chromosome arm or chromosome segment that has fused to multiple recipient chromosome,[3] and mainly reported in treatment-related leukemias characterized by multiple copies of the ABL and/or MLL oncogenes dispersed throughout the genome and extrachromosomally. In solid cancer, although SJTs are detected in cell lines derived from various carcinomas including the bladder, prostate, breast, cervix, and pancreas,[3] only a few studies have reported the SJT in carcinoma *in vivo*, such as skin cancer from a xeroderma pigmentosum patient[4] and viral-associated hepatocellular carcinoma.[5] To our best knowledge, this is the first report showing SJT in thyroid cancer.

In thyroid cancer, Ret SJT was commonly observed in ATC including both radiation-associated and sporadic cancers. Ret SJT may be associated with anaplastic transformation of thyroid cancer. Actually, in this study, SJT of Ret was only found in recurred thyroid cancer with anaplastic transformation but not in primary well differentiated PTC. On the other hand, in PTC, Ret SJT was restricted to radiation-associated, Ret/PTC-positive and/or high-grade cases. Because SJTs of Ret were not observed in sporadic, well differentiated and low-grade cases of PTC, SJT of Ret might be a molecular marker for radiation-induced and/or aggressive cases of PTC.

In conclusion, we found SJTs, a rare cytogenetic aberration, of Ret oncogenes, in thyroid cancer. This is the first report showing SJT in thyroid cancer, and probably the third report showing SJT in solid cancer *in vivo*. The presence of Ret SJT in PTC might be a cytogenetic marker/predictor of radiation etiology/high-grade malignancy.

References

1. Williams D. Cancer after nuclear fallout: lessons from the Chernobyl accident. *Nature* 2: 543-549, 2002
2. Saenko V, Rogounovitch T, Shimizu-Yoshida Y et al. Novel tumorigenic rearrangement, Δrfp/ret, in a papillary thyroid carcinoma from externally irradiated patient. *Mutat Res* 527: 81-90, 2003
3. Padilla-Nash HM, Haselmeyer-Haddad K, Wangsa D et al. Jumping translocations are common in solid tumor cell lines and result in recurrent fusions of whole chromosome arms. *Genes Chromosomes Cancer* 30: 349-363, 2001
4. Aledo R, Aurias A, Chretien B et al. Jumping translocation of chromosome 14 in a skin squamou cell carcinoma from a xeroderma pigmentosum patient. *Cancer Genet Cytogenet* 33: 29-33, 1988
5. Wong N, Chan A, Lee SW et al. Positional mapping for amplified DNA sequences on 1q21-q22 in hepatocellular carcinoma indicates genes over-expression. *J Hepatol* 38: 298-306, 2003