Endocrine, *vol. 13, no. 3, 341–344, December 2000* 0969–711X/00/13:341–344/$11.00

Low Frequency of Rearrangement of TRK Protooncogene in Chinese Thyroid Tumors

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The TRK protooncogene (*NTRK1*) encodes a cell-surface transmembrane tyrosine kinase (TK) acting as a receptor for nerve growth factor. Oncogenic potential in thyrocytes results from replacing the 5' portion by regulatory parts of other genes, leading to constitutive TK expression. In Italy, human papillary thyroid carcinoma (PTC) shows a frequent activation (50%) of the TK receptor genes *NTRK1* and *RET*. Both genes undergo oncogenic rearrangements by the same mechanism. We previously reported high frequency (6/11) of rearrangement of the RET protooncogene in Chinese PTCs. Wide differences in the frequency (0–10.9%) of the NTRK1 rearrangement in PTCs have been reported in different populations. To investigate the frequency of TRK protooncogene rearrangement in Chinese thyroid tumors, we performed reverse transcriptase polymerase chain reaction to amplify specific TRK rearrangement transcripts. We examined thyroid tumors of 40 patients, including 14 papillary carcinomas, 4 follicular carcinomas, 1 Hurthle cell carcinoma, 1 insular carcinoma, and 20 nodular goiters. NF874 NIH3T3, NF723 NIH3T3, NF861 NIH3T3, and NF881 NIH3T3 were used as controls for TRK-T3, TRK-T2, TRK-T1, and TRK, respectively. No known TRK protooncogene rearrangements were detected among the 40 thyroid tumors in our studies. We suggest that the TK receptor NTRK1 activation seems less important than RET activation in PTCs in the Chinese population.

Key Words: Transmembrane tyrosine kinase; papillary thyroid cancers; TRK

Introduction

The TRK protooncogene (*NTRK1*) encodes a cell-surface transmembrane tyrosine kinase (TK) acting as a receptor for nerve growth factor *(1)*. The expression of *NTRK1* is tightly controlled, being restricted to the peripheral nervous ganglia *(2)*. Oncogenic potential in thyrocytes results from replacing the 5' portion by regulatory parts of other genes, leading to constitutive TK expression *(3)*. The *NTRK1* oncogenic rearrangement involves at least three different genes *(4)*: the activating 5' portion is provided by a tropomyosin gene, *TPM3 (5)*, as in the original TRK oncogene *(6)*; the activation is owing to fusion with TFG (TRK fusing gene), a novel gene on chromosome 3 *(7)*, named *TRK-T3* oncogene; and the TPR gene is involved and responsible for TRK-T1 and TRK-T2 *(8)*. In the TRK-T2 rearrangement, the TPR part is longer by 737 amino acids than in TRK-T1, where the TPR part consists merely of 192 amino acids *(3)*.

In Italy, human papillary thyroid carcinoma shows a frequent activation (50%) of the TK receptor genes *NTRK1* and *RET (9)*. Both genes undergo oncogenic rearrangements by the same mechanism. We previously reported high frequency (6/11) of rearrangement of the RET protooncogene in Chinese papillary throid cancers (PTCs) *(10)*. Wide differences in the frequency (0–10.9%) of the NTRK1 rearrangement in PTCs have been reported in different populations *(11–14)*. We describe herein oncogenic NTRK1 rearrangements in Chinese thyroid tumors.

Results

Figure 1 shows positive controls for specific TRK oncogene transcripts. There were 258-, 1302-, 396-, and 199-bp fragments corresponding to TRK, TRK-T1, TRK-T2, and TRK-T3 positive controls, respectively (Fig. 1A,B).

All 40 thyroid tumors were screened for TRK, TRK-T1, TRK-T2, and TRK-T3 rearrangement by reverse transcriptase polymerase chain reaction (RT-PCR). No NTRK1 rearrangement was found in any of the papillary, follicular, and benign thyroid tumors that we tested. Figure 2 shows some negative results from papillary, follicular, and benign thyroid tumors. As seen in Fig. 2, there was no band corresponding to any of the NTRK1 rearrangements. To confirm the adequate procedure for RNA extraction and RT-PCR, we used β-actin as controls. We can therefore see a clear band corresponding to β-actin in each of the thyroid tumors (Fig. 2A,B).

Received June 23, 2000; Accepted July 18, 2000; To Humana: July 27, 2000.
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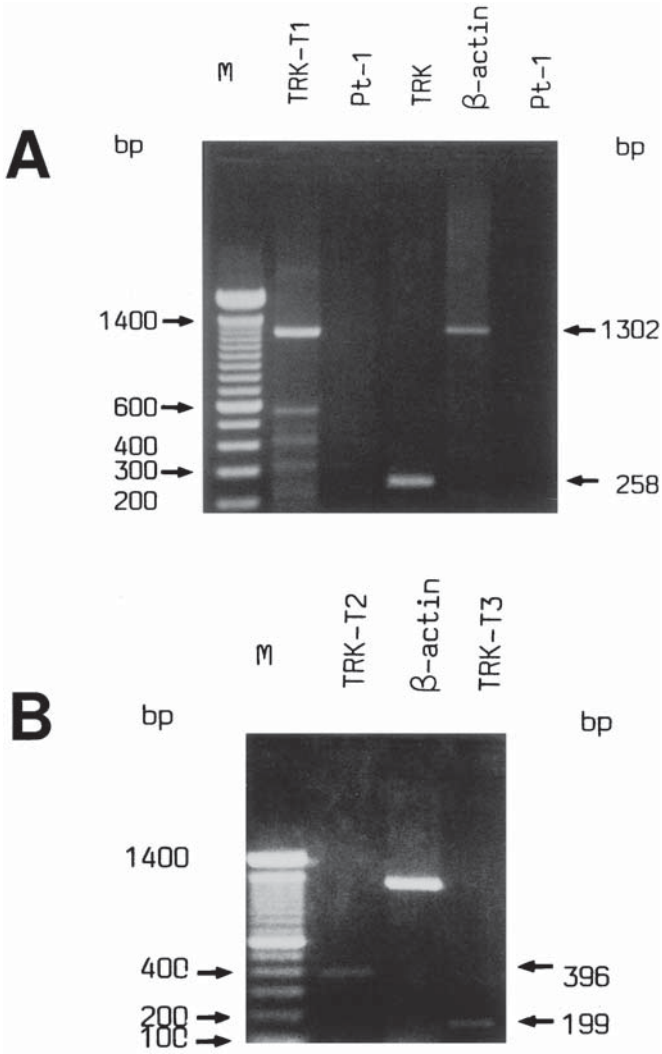

Fig. 1. Detection of specific TRK oncogene transcripts. **(A)** Positive controls of TRK and TRK-T1. Pt-1 = patient-1, a patient with PTC. **(B)** Positive controls of TRK-T2 and TRK-T3. PCR products were analyzed on a 4% agarose gel and visualized after ethidium bromide staining. β-actin was used as an adequate procedure control.

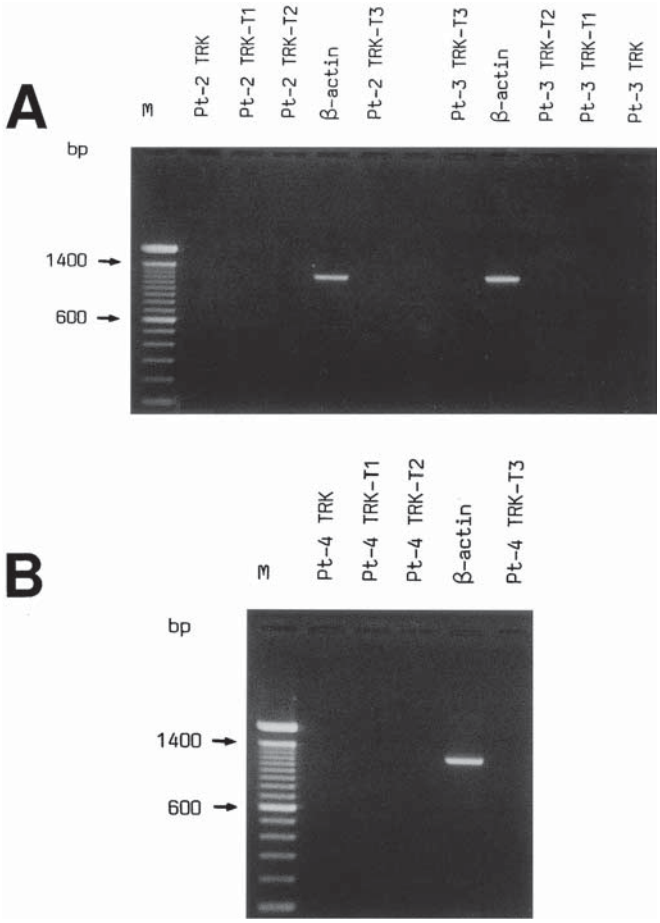

Fig. 2. Detection of NTRK1 rearrangements in thyroid tumors. Negative results from **(A)** Pt-2, a patient with PTC; Pt-3, a patient with follicular thyroid carcinoma; and **(B)** Pt-4, a patient with benign thyroid tumor.

Discussion

The frequency of NTRK1 rearrangement in human PTCs varies widely from 0 to 10.9% among patient series from different geographic regions (*see* Table 1), and it has been suggested that different genetic or environmental factors are involved in determining the frequency of NTRK1 rearrangement in PTCs. The low frequency of NTRK1 rearrangement in the PTCs of Chinese populations suggests that NTRK1 activation seems not to play an important role in the tumorigenesis of PTCs in the Chinese population. In addition to PTCs, other thyroid tumors such as follicular carcinomas, insular thyroid cancer, and benign thyroid tumors were not found in NTRK1 rearrangements in our study.

High frequency of activation (50%) of the TK receptor genes (representing the sum of NTRK1 and RET) in human PTC was reported in Italy *(9,15)*. Although both genes undergo oncogenic rearrangements by the same mechanism, RET rearrangements in PTCs have been found in the Chinese population (55%) and in other series (2.5–67%) *(10)*. There was a high prevalence (67%) of RET rearrangements *(16)* but only a 7.4% prevalence of NTRK1 rearrangements in PTCs of children from Belarus after the Chernobyl reactor accident *(14)*.

We suggest that the TK receptor NTRK1 activation seems less important than RET activation in PTCs in the Chinese population.

Materials and Methods

Tumors

All thyroid tissues were obtained from surgery at Veterans General Hospital, Taipei, Taiwan. They were frozen in liquid nitrogen immediately after surgery and stored at –70°C until analysis. Forty thyroid tumors were stud-

Table 1
Prevalence of NTRK1 Rearrangements in PTCs

Country	Total number of tested tumors	NTRK1 rearrangement-positive tumors	Percentage	Reference
Japan	38	2	5.3	*11*
France	16	0	0	*12*
Italy	92	10	10.9	*13*
Belarus	81	6	7.4	*14*
China (Taiwan)	14[a]	0	0	This study

[a]Fourteen PTCs of 40 total thyroid tumors tested.

Table 2
Primers Used in PCR of TRK, TRK-T1, TRK-T2, and TRK-T3

Gene	Primer	Sequence
TRK	1	5'-TGAGGAAGAAATCAAGATTC-3'
TRK	2	5'-AACTTGTTTCTCCGTCCACA-3'
TRK-T1	3	5'-TGTTGCAGCAAGTCCTGGAG-3'
TRK-T1	4	5'-GCTGGTACCAGGGCTGCTT-3'
TRK-T2 (4)	5	5'-TAATATGGAAGTCCAAGTT-3'
TRK-T2	6	5'-CACTTGAGCACGATGTC-3'
TRK-T3 (14)	7	5'-ATGGCAGCAAGTATGTCTGC-3'
TRK-T3	8	5'-GGAAGAGGCAGGCAAAGAC-3'

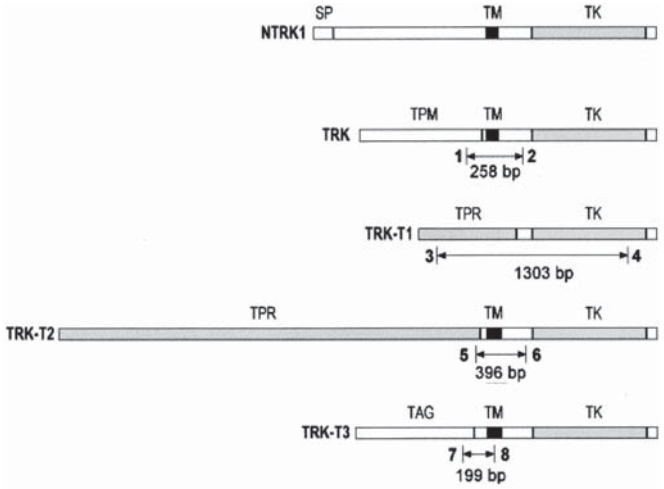

Fig. 3. Schematic representation of the human TRK protooncogene and various TRK oncogenes. The putative signal peptide (SP), transmembrane (TM), and tyrosine kinase (TK) domains are indicated. The location of primers for the corresponding genes and size of PCR products are indicated. The primers are labeled as numbers in Table 2.

ied: 14 papillary carcinomas, 5 follicular carcinomas (including 1 Hurthle cell carcinoma), 1 insular carcinoma, and 20 nodular goiters. NF874 NIH3T3, NF723 NIH3T3, NF861 NIH3T3, and NF881 NIH3T3 were used as controls for TRK-T3, TRK-T2, TRK-T1, and TRK, respectively.

RNA Extraction

Tissues were crushed in liquid nitrogen. RNA was then immediately extracted according to the RNAzol B (TEL-TEST) method.

Reverse Transcription

Reverse transcription was performed on 1 μg of RNA from each sample. RNA was mixed with diethyl pyrocarbonate–treated water and 1.5 μ*M* oligo(dT) primer in a thermal cycler for 2 min at 70°C and then in ice. The reaction mixture (Advantage RT-for-PCR kit; Clontech) contained 3 m*M* $MgCl_2$, 0.5 m*M* deoxynucleotide triphosphate (dNTP), 1 μ*M* oligo (dT) primer, 1 U/μL of ribonuclease inhibitor, and 10 U/μL of Moloney murine leukemia virus RT. Reverse transcription was performed in a thermal cycler for 1 h at 42°C, followed by 5 min of denaturation at 94°C.

Polymerase Chain Reaction (PCR)

PCR was carried out in a 50-μL reaction mixture containing 10 μL of cDNA, 1.5 m*M* $MgCl_2$, 0.2 m*M* of each primer, 0.2 m*M* dNTP, and 2.5 U of Taq polymerase (Viogene). The primers used are listed in Table 2 and illustrated in Fig. 3. One drop of mineral oil (M-5904; Sigma) was added over each PCR well. The PCR was performed with a thermal cycler (Techne) according to the following protocol: initial denaturation at 94°C for 1 min followed by 21 cycles (1 min/cycle) of 0.5°C touchdown from 65 to 55°C, and then 72°C for 1 min. Nineteen cycles at 94°C for 1 min, 55°C for 1 min, and 72°C 1 min; and an extension at 72°C for 5 min.

Acknowledgment

We thank Dr. Angela Greco at Divisions of Experimental Oncology A, Istituto Nazionale Tumori, Milan, Italy, for kindly providing the RNAs from NIH3T3 cell lines.

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