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Presence of Aberrant Transcripts of *ret* Proto-oncogene in a Human Papillary Thyroid Carcinoma Cell Line

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In a papillary thyroid carcinoma cell line, TPC-1, we found transcripts hybridizing to *ret* proto-oncogene (proto-*ret*) cDNA probes. The transcripts hybridized to the probes encoding the kinase domain but not to those of the transmembrane and extracellular domains of proto-*ret*. The sizes of the main transcripts in TPC-1 were aberrant, being 2.0, 2.5, 4.0 and 5.0 kb. In the neuroblastoma cell lines, the transcripts were 3.9, 4.5, 6.0 and 7.0 kb, which have been proved to be generated by alternative splicing and polyadenylation from the non-altered proto-*ret* oncogene. All of the four transcripts in TPC-1 are about 2 kb smaller than the corresponding ones in the neuroblastoma. From the length of the transcripts, it is suspected that the transcripts in TPC-1 are a rearranged form of proto-*ret*. This is the first report describing the aberrant transcripts of proto-*ret* in a human tumor.

Key words: Aberrant transcripts — *ret* proto-oncogene — Papillary thyroid carcinoma cell line — Chromosome 10q11.2

ret proto-oncogene (proto-*ret*) has been proposed to be one of the receptor tyrosine kinases.[1,2] So far, two activated forms of proto-*ret* have been isolated from NIH3T3 cell transformants obtained by transfection assay using human T cell lymphoma DNA[3] and human colon cancer DNA.[4] However, such activation was proved to have occurred during transfection, because the rearrangement critical for the activation of proto-*ret* was not detectable in the original tumor DNAs.[5,6] We tried to detect gene alteration of proto-*ret* in a total of 106 tumors by Southern blot analysis but could not find any alterations (unpublished data). The tumors studied included 38 colon carcinomas, 3 colon polyps, 40 lung carcinomas, 2 thymomas and 11 neuroblastomas. Furthermore, over-expression of proto-*ret* was studied on more than 50 cell lines of 23 types of tumors, including 11 neuroblastoma cell lines. We found that 10 of the 11 neuroblastoma cell lines studied expressed high levels of proto-*ret*. On the other hand, other cell lines of tumors such as melanoma, pheochromocytoma, or glioblastoma did not show any signals of proto-*ret* expression. In neuroblastomas, gross changes of the proto-*ret* oncogene have not been found and we could not detect any aberrant transcripts of proto-*ret* in those tumors (unpublished data). Further, in Nagai neuroblastoma cell line,[7] the absence of any amino acid changes has been suggested.[2]

To obtain information on the clinical significance and the biological function of proto-*ret*, we determined its chromosomal localization. Human proto-*ret* was mapped to chromosome 10q11.2.[8] Interestingly, this site is near the locus responsible for multiple endocrine neoplasia 2A, in which medullary thyroid carcinoma and pheochromocytoma are induced in the same patient.[9,10] Furthermore, at the same site, an oncogene associated with human papillary thyroid carcinoma (PTC),[11] which has transforming activity in NIH3T3 cells, was also mapped.[12] Fusco *et al.* reported that the PTC oncogene is different from *ret*, judging from the restriction pattern of *Alu*-positive fragments.[11]

In this report, we describe the presence of aberrant transcripts in a PTC cell line, TPC-1,[13] which hybridize to the proto-*ret* cDNA probes and we discuss the role of the activated form of proto-*ret* in the development of the PTC.

As shown in Fig. 1, TPC-1 (lane 3) had transcripts hybridizing to the probe *z*, an *Eco*RI-*Nco*I 0.4 kb fragment which contains the region encoding the 5′ part of the proto-*ret* kinase domain (Fig. 2b). Using 10 μg of total RNA of TPC-1, two transcripts of 2.0 and 2.5 kb in size were detected as major bands (Fig. 1, lane 3). As a weakly hybridizing band, another transcript of 4.0 kb was also detected (Fig. 1, open arrow). On the other hand, one of the neuroblastoma cell lines, TGW-I-nu (TGW) had transcripts of 3.9, 4.5, 6.0 and 7.0 kb in size (lanes 4), which is compatible with our previous finding.[2] In previous work, we confirmed that these transcripts were derived from the proto-*ret* oncogene. In all neuroblastoma cell lines, four major transcripts of these

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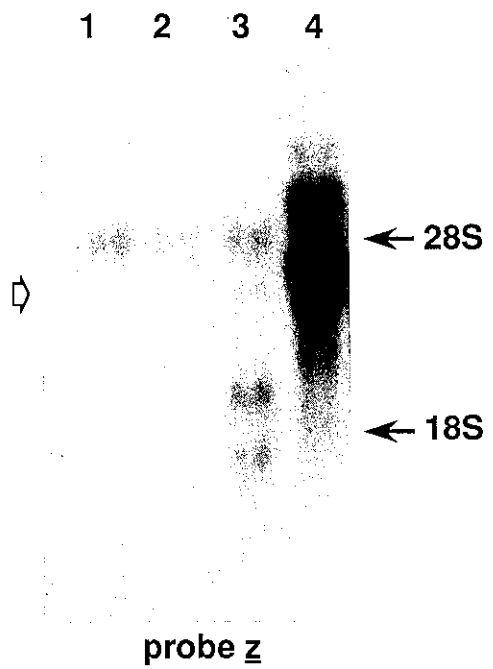

sizes were consistently observed. In TPC-1, the density of the largest transcript might be less, or it might have overlapped with 28S rRNA.

More detailed analyses on the transcripts of TPC-1 were performed on poly(A)$^+$ RNA using several parts of the cDNA fragments as probes. As shown in Fig. 2a, four species of transcripts were detected (lane 6) by probe *m*, which contains the region encoding the 3′ part of the proto-*ret* kinase domain (Fig. 2b). In another neuroblastoma cell line, NB-39-nu, (NB-39), the four

Fig. 1. Northern blot analysis for detecting transcripts hybridizing to cDNA probe of proto-*ret*. RNA extraction and northern blotting were performed according to the methods described.[15, 16] As a probe, the cDNA fragment (probe *z* in Fig. 2b) was used. Human cell lines used were as follows: (lanes 1 and 2), small cell lung carcinomas SBC-3 and -5, which are negative control cells; (lane 3), papillary thyroid carcinoma TPC-1; (lane 4), neuroblastoma TGW-I-nu (TGW). An open arrow indicates a faint band of the transcripts of TPC-1 (lane 3). Our previous work showed that TGW had no structural alteration of proto-*ret* (unpublished data). The positions of size markers, 18S and 28S rRNAs, are indicated.

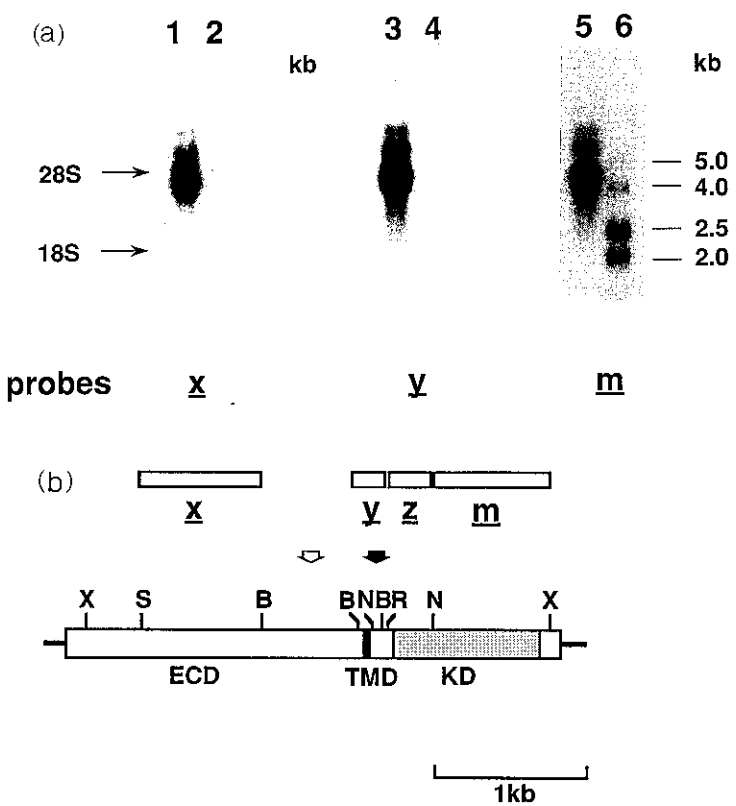

Fig. 2. Northern blot analysis of the transcripts in TPC-1 and NB-39-nu using various probes of proto-*ret* and the restriction map of proto-*ret* cDNA. (a) Five μg of poly(A)$^+$ RNA of TPC-1 and 10 μg of total RNA of NB-39-nu (NB-39) were used. Poly(A)$^+$ RNA was prepared using the fast track mRNA isolation kit (*Invitogen*) by the procedure recommended by the manufacturer. As probes, fragments *x* (*Sal*I-*Bam*HI 0.6 kb), *y* (*Bam*HI 0.2 kb) and *m* (*Nco*I-*Xho*I 0.6 kb) were used. Cell lines used were: (lanes 1, 3 and 5), NB-39; (lanes 2, 4 and 6), TPC-1. The sizes of the transcripts are indicated. The size markers of 18S and 28S rRNAs are also indicated. (b) Restriction map of proto-*ret* cDNA cloned from one of the neuroblastoma cell lines, Nagai (pN7).[2, 7] Boxes *x*, *y*, *z* and *m* indicate the cDNA fragments used as probes. An open column indicates an open reading frame of proto-*ret*. Solid and shaded areas indicate the transmembrane and the kinase domains, respectively. The rearrangement points of the activated forms of proto-*ret*, which were previously reported, are indicated by an open[3] and a solid arrow.[4] Abbreviations: B, *Bam*HI; *N*, *Nco*I; R, *Eco*RI; S, *Sal*I; X, *Xho*I; ECD, extracellular domain; TMD, transmembrane domain; KD, kinase domain.

major transcripts were also observed using 10 μg of total RNA (lane 5). The sizes of the transcripts in TPC-1 were 2.0, 2.5, 4.0 and 5.0 kb, while those in NB-39 were 3.9, 4.5, 6.0 and 7.0 kb. It is of particular importance to note that all four species of the transcripts detected in TPC-1 (Fig. 2a, lane 6) were 2 kb less in size than those of NB-39 (Fig. 2a, lane 5) and TGW (Fig. 1, lane 4).

The divergence in size of these four transcripts detected in neuroblastomas, which is one of the characteristics of proto-*ret* expression, was generated by alternative splicing and polyadenylation in the 3′ region of proto-*ret*, as reported.[2] Due to this mechanism of mRNA expression, the four major transcripts were also detected in two independent NIH3T3 cell transformants, in both of which the 5′ half of proto-*ret* was replaced by unrelated sequences.[3, 4]

We confirmed previously that probe *z* hybridized to proto-*ret* cDNA, but not to other tyrosine kinases such as *trk*, *met* or c-*src* under the conditions used in the present study (0.65 *M* NaCl, 50% formamide, hybridizing at 42°C and washing at 50°C).[4, 14] Moreover, it did not hybridize to other retrovirus DNA such as v-*ros*, v-*abl*, v-*fms*, v-*erb* B and v-*erb* A, even under much less stringent conditions (0.9 *M* NaCl, 30% formamide, hybridizing at 42°C and washing at 42°C). Further, it has been shown to detect only a single *Eco*RI fragment of proto-*ret* in human genome.[6]

These results strongly suggest that the transcripts in TPC-1 are rearranged mRNAs of proto-*ret*.

We examined whether the regions encoding the extracellular or transmembrane domains are retained in the transcripts or not. Neither of the probes *x* and *y*, which encode these domains, respectively (Fig. 2b), could detect the transcripts in TPC-1 (Fig. 2a, lanes 2 and 4), whereas they hybridized to the transcripts of NB-39 (Fig. 2a, lanes 1 and 3). These data indicate that the transcripts in TPC-1 possessed the 3′ half but not the 5′ half of the proto-*ret*. The rearrangement point would be located in the region just upsteam from the kinase domain, as one of the activated forms of proto-*ret*, *ret*-II[4] (Fig. 2b).

Because the rearranged forms of proto-*ret* have transforming activity in NIH3T3 cells,[3, 4] the presence of the rearranged form of proto-*ret* in PTC indicates that aberrant tyrosine kinase activity of the activated molecule of proto-*ret* may be required for the tumor development.

This is the first report describing truncated transcripts of proto-*ret* in a human tumor. To characterize the transcripts in TPC-1 and to screen for the involvement of proto-*ret* in other PTC cell lines, as well as in the original tumors, further studies are under way.

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