Expression of Thyroid Hormone Receptors in Intracranial Meningiomas

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Thyroid hormone has a unique function in human organs. Many of its effects occur at the level of gene expression and are mediated by thyroid hormone receptors (TRs). We investigated the relationship between TRs and the prognosis of meningiomas. We investigated TR expression in human intracranial meningiomas using reverse transcription-polymerase chain reaction. Specimens of 25 tumors were obtained by craniotomy from various intracranial meningiomas. We found that the expression of TRs was receptor subtype- and cell type-dependent. Human TRα1 (hTRα1) was expressed in nine cases, hTRα2 was expressed in 14 cases, and both hTRα1 and hTRα2 were expressed in five cases; hTRβ1 was expressed in nine cases of recurrent or malignant tumors. The expression of hTRβ1 may be an indicator of recurrent or malignant meningiomas.

Key Words: thyroid hormone receptor, intracranial meningiomas
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Modern microsurgical techniques have made it possible to totally remove human intracranial meningiomas. Recent advances in neuroimaging studies, histopathology, and molecular biology have greatly improved our understanding of the pathophysiology of human meningiomas. We investigated the relationship between thyroid hormone receptors (TRs) and the prognosis of intracranial meningiomas.

Thyroid hormone has a unique function in human organs, regulating growth, development, differentiation, and metabolic processes. Many effects of thyroid hormone occur at the level of gene expression and are mediated by TRs. Multiple TR-encoding mRNA exists in any given series (i.e. in any cell or tissue line), and TRs are divided into α and β forms on the basis of sequence similarities and chromosomal locations [1]. Three forms of human TR (hTR)-related cDNA have been cloned: hTRα1, hTRα2, and hTRβ1 [2–4]. hTRβ2 is pituitary-specific. The genes for hTRα1 and hTRα2 are located on chromosome 17, while that for hTRβ1 is on chromosome 3 [5,6]. Some authors report that structural differences between α and β TRs can result in different target gene specifications [7,8].

The role of TRs in human neoplastic transformation is still unknown. To evaluate the potential contribution of TRs to the pathogenesis of intracranial meningiomas, we used reverse transcription coupled to the polymerase chain reaction (RT-PCR) to investigate the expression of TRs in human intracranial meningiomas, and correlated the results with their clinical behavior.

Materials and Methods

Intracranial meningioma specimens were used to examine TR gene expression. TR mRNA levels were determined using RT-PCR. hTRα1 cDNA is 1.2 kb,

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hTRα2 cDNA is 1.5 kb and hTRβ1 cDNA is 1.4 kb, as previously described [9].

Tumor tissue

Patients in our hospital diagnosed with intracranial meningioma using magnetic resonance imaging (MRI) in the last 3 years underwent craniotomy to remove the tumor. Tumor tissue was collected during surgery and a portion was fixed with 10% formaldehyde and embedded in paraffin for histopathologic verification.

RNA isolation

All reagents used for RNA isolation were molecular biology reagents from Sigma Chemicals Co. (St. Louis, MO, USA). Total RNA was prepared from each tumor specimen by the guanidinium thiocyanate method, according to the technique described by Chomczynski and Sacchi [10].

Reverse transcription-polymerase chain reaction

RT of RNA followed by PCR was carried out as described by Cook et al [11]. Briefly, 1 μg of total RNA was incubated with 0.5 μg of oligo(dt) 90, 1 mM deoxyribonucleoside triphosphates (dNTPs), 10 units RNase inhibitor, and 10 units avian myeloblastosis virus reverse transcriptase in 20 μL of 0.1 M Tris-HCl buffer pH 8.3 containing 50 mM KCl, 10 mM $MgCl_2$, and 4 mM dithiothreitol.

The cDNA products of RT were used as templates for PCR, which was carried out in 10 mM Tris-HCl/ pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, with 0.1 μg of the appropriate 5′ and 3′ primers and 0.5 units of Taq polymerase (5,000 units/mL). We used the expression of glyceraldehyde-3-phosphate dehydrogenase as an internal control.

The primers used to specifically amplify each of the three TR-isoforms were as follows: hTRα1: 5′-ATGGAACAGAAGCCAAGCAA and 3′-TTAGACTTCCTGATCCTCAA; hTRα2: 5′-ATGGAACAGAAGCCAAGCAA and 3′-TCAGGGAGAGGCTGCATTGC; and hTRβ1: 5′-ATGACAGAAAATGGCCTTAC and 3′-CTAATCCTCGAACACTTCCA.

cDNAs were amplified using 34 cycles of PCR. Each cycle consisted of denaturation at 94°C for 1 minute, annealing at 54°C for 2 minutes, and extension at 72°C for 2 minutes. After amplification, the products were examined by agarose gel electrophoresis and amplified fragments were visualized using ethidium bromide staining.

Southern hybridization

We used Southern blotting to identify the bands, as previously described [12]. PCR products were blotted by capillary transfer onto nitrocellulose membrane and hybridized with the isoform-specific probes.

RESULTS

In this series, 25 cases of intracranial meningioma were confirmed by histopathology and included 16 meningotheliomatous cases, two cases of transitional type, three cases of atypical type, and four cases of recurrent meningioma (of which two were atypical, one was transitional, and one was meningiomatous). Patient age ranged from 40 to 80 years (mean, 62 years), while tumor diameters ranged from 2 to 12 cm. TR expression in the different types of meningiomas is shown in the Table.

The expression of TRs is selectively active or inactive in different types of intracranial meningiomas. hTRα1 was expressed in nine cases, hTRα2 was expressed in 14 cases, and both hTRα1 and hTRα2 were expressed in five cases. hTRβ1 was selectively active in nine cases of recurrent or malignant meningioma.

These results demonstrated, for the first time, that the presence of a meningioma-specific form of TR could be important for the prognosis of human intracranial meningiomas, and was correlated with clinical behavior.

DISCUSSION

Thyroid hormone has a unique function in the human body. It is important for all human tissues and has profound effects on metabolic rate and oxygen consumption. Many effects of thyroid hormone in human tissues are mediated through binding to TRs and are related to the *c-erb-A* oncogene family. The existence of several receptor isoforms suggests that different functions are mediated by specific isoforms and raises the possibility of functional differences. TRs are derived from two genes [6]. hTRα is 86% identical to hTRβ in the DNA- and T3-binding domains. Each gene gives two isoforms (hTRα1, hTRα2, and

Table. Expression of human thyroid hormone receptors (hTRs) in human intracranial meningiomas

Case no.	hTRβ1 (1.4 kb)	hTRα1 (1.2 kb)	hTRα2 (1.5 kb)
Recurrent meningioma			
1	+	–	–
2	+	–	+
3	+	–	–
4	+	+	–
Atypical meningioma			
5	+	–	–
6	+	–	–
7	+	–	–
Transitional meningioma			
8	+	–	+
9	+	–	+
Meningotheliomatous meningioma			
10	–	–	–
11	–	+	+
12	–	–	+
13	–	+	–
14	–	+	+
15	–	–	+
16	–	+	+
17	–	–	+
18	–	–	–
19	–	+	+
20	–	+	+
21	–	+	–
22	–	–	+
23	–	+	–
24	–	–	+
25	–	–	+

hTRβ1, hTRβ2) due to alternative splicing of the primary transcripts [6]. hTRα and hTRβ isoforms are products of two distinct genes, while the β1 and β2 isoforms are splice variants of the same genes. hTRα1 mRNA is down-regulated by T3 in multiple tissues including heart, kidney, and pituitary, but not in brain [9]. hTRα1 and hTRβ1 are widely expressed, whereas the hTRβ2 isoform is mainly limited to the pituitary [11].

The pathophysiologic state of malignancy involves alterations in normal cell proliferation and differentiation, and specific genetic sequences (oncogenes) are implicated in the genesis of the phenotype. The appropriate expression of oncogenes appears to involve cell proliferation, differentiation, and metabolic regulation, while inappropriate expression leads to malignant phenotypes. Some actions of thyroid hormone and cellular oncogenes appear similar and interrelated, suggesting that thyroid hormone may influence carcinogenesis.

To define the role of TRs in human tumorigenesis, we previously characterized TRs in human pituitary tumor cells and found that hTRβ1 is over-expressed in invasive human pituitary tumor cells [13]. Other authors have characterized TRs in hepatoma cell lines [14,15]. The degree of differentiation was found to be inversely correlated with the levels of hTRβ1. Tumors with invasive potential have high levels of hTRβ1, and cells are poorly differentiated. Cells with low levels of hTRβ1 are well differentiated [15].

Meningiomas are common intracranial neoplasms. They are typically benign, although the high recurrence rate, incidence of growth at inoperable sites, and perineural growth complicate surgical interventions. Recently, characterization and identification of genes differentially expressed in human meningiomas have been observed and reported.

In the present study, human intracranial meningiomas shown to be recurrent, invasive, or with malignant potential by both imaging studies (computerized tomography and MRI) and histopathology were found to have hTRβ1 over-expression (Table; Cases 1–4). This finding is compatible with characteristics of both pituitary tumors and hepatoma cell lines, and may be an indicator for tumor recurrence or malignant transformation.

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