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Original contribution

# Aberrant expression of thyroid transcription factor–1 in schwannomas☆

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**Summary** Aberrant expression of thyroid transcription factor-1 (TTF-1) has been observed in tumors arising in locations other than thyroid gland, lung and ventral forebrain. However, TTF-1 expression in schwannomas has not yet been studied. Meanwhile, a few inconsistent changes in protein expression have been identified between schwannomas and other peripheral nerve sheath tumors. We evaluated TTF-1 expression in 161 schwannomas and 43 other peripheral nervous system lesions, including ganglioneuromas (n = 8), malignant peripheral nerve sheath tumors (MPNSTs) (n = 11), neurofibromas (n = 24), and traumatic neuromas (n = 9), using immunohistochemistry and verified it using quantitative real-time reverse-transcription polymerase chain reaction (qPCR) to explore TTF-1 expression in peripheral nervous system lesions. Formalin-fixed paraffin-embedded (FFPE) tissues were obtained for both analyses. In this study, we observed nuclear TTF-1 staining in 109 (67.7%) schwannomas, including 102 of 131 (77.9%) conventional, 1 of 20 (5.0%) cellular and 6 of 10 (60.0%) plexiform schwannomas. Nuclear staining was not observed in normal peripheral nerves and non-schwannoma lesions. qPCR verified the aberrant expression and revealed a correlation between TTF-1 protein and mRNA levels ($r = 0.633$, $P = .003$). In conclusion, the data from our study show that TTF-1 is selectively expressed in the majority of schwannomas, particularly the conventional variants. Based on this observation, the TTF-1 protein and mRNA are specifically expressed in schwannomas. This highly aberrant expression of varying amounts of TTF-1 may provide new clues to reveal the pathogenesis of schwannoma.


## 1. Introduction

Thyroid transcription factor-1 (TTF-1), a homeodomain transcription factor named Nkx2.1, plays a crucial role in the embryonic development of the thyroid, lung and ventral forebrain [1]. Accordingly, TTF-1 expression is observed in these normal tissues and tumors derived from these organs. Although a number of studies have revealed the aberrant expression of TTF-1 in other tumors from the gastrointestinal tract and female reproductive systems [2,3], the reason for the aberrant expression is still unclear. Interestingly, TTF-1 has been shown to play both oncogenic and tumor suppressive roles [4-6]. This hypothesis suggested that TTF-1 might participate in tumorigenesis and could explain the aberrant expression.

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In the nervous system, TTF-1 is expressed in tumors located in the sellar region (such as granular cell tumors, pituicytoma and spindle cell oncocytoma) and third ventricle (such as chordoid glioma, and subependymal giant cell astrocytoma), which are derived from the ventral forebrain during development [7-11]. Meanwhile, a number of studies have described its aberrant expression in other brain tumors, such as anaplastic astrocytoma, anaplastic oligodendroglioma and glioblastoma [12,13]. However, the expression of TTF-1 in the peripheral nervous system has not yet been studied; we do not know whether TTF-1 is expressed in schwannomas and other Schwann cell neoplasms.

Schwannoma, like neurofibroma, ganglioneuroma, and malignant peripheral nerve sheath tumors (MPNSTs), is characterized by neoplastic proliferation and Schwann cell differentiation. The main subtypes of schwannoma include conventional, cellular and plexiform schwannomas [14]. Although these various peripheral nervous tumors and schwannoma variants show distinct histological features, a few inconsistent protein alterations have been identified among them [15,16].

We examined the expression of the TTF-1 protein in schwannomas, other peripheral nervous tumors and traumatic neuromas using immunohistochemistry and verified its expression at the transcriptional level using qPCR to determine whether TTF-1 is expressed in peripheral nervous system lesions.

## 2. Materials and methods

### 2.1. Case selection

Institutional ethical guidelines were followed in this retrospective study. All archived formalin-fixed, paraffin-embedded (FFPE) blocks were obtained from our institution between the years 2012 and 2016. One hundred sixty-one schwannomas were classified as conventional schwannomas (n = 131), cellular schwannomas (n = 20), or plexiform schwannomas (n = 10) [17]. For comparison, 9 traumatic neuromas and other peripheral nervous tumors were selected, including ganglioneuromas (n = 8), MPNSTs (n = 11) and neurofibromas (n = 24). For multiple or recurrent cases, only one site and primary lesion were included in this study. Of all patients with schwannoma, 6 had neurofibromatosis type 2 (NF2), based on the Manchester clinical diagnostic criteria [18], and 2 had schwannomatosis, based on the clinical diagnostic criteria in the literature [19]. Prior to this study, 3 pathologists reviewed all cases together with the available materials to confirm and reach a consensus diagnosis.

### 2.2. Immunohistochemistry and scoring

Immunohistochemistry was performed on 4-μm sections of FFPE blocks. All sections were deparaffinized with xylene and rehydrated through a graded ethanol series. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min. Slides were incubated with the mouse anti-human TTF-1 monoclonal antibody clone SPT24 (Novocastra, UK, 1/100 dilution; EDTA at pH 9.0 for antigen retrieval) in a pressure cooker for 4 min. Immunoreactivity was detected using the Dako EnVision method (Dako, Glostrup, Denmark), according to the manufacturer's recommended procedure.

We obtained a combined score to evaluate the intensity and distribution of nuclear staining. The intensity of nuclear staining was assigned a score of 0 (absent), 1 (faint), 2 (visible at low magnification), or 3 (bright). The distribution was graded as follows: no discernible staining = 0, 0%-1% = 1, 1%-5% = 2, 5%-25% = 3, 25%-50% = 4, 50%-75% = 5, or >75% = 6. The 2 scores were added up to yield an overall score of 0 to 9. Scores of 0 and 1 were considered negative, scores of 2 to 5 were weak, scores of 6 to 7 were medium, and scores of 8 to 9 were strong. The immunohistochemical staining was independently assessed by 3 authors in a blinded manner. In cases with discrepancies in scores among observers, a final score was recorded, and a consensus assessment was achieved using a multi-head microscope.

### 2.3. RNA isolation from FFPE blocks, reverse-transcription, and quantitative real-time PCR

The expression of the TTF-1 mRNA was verified using qPCR and compared with the protein level to confirm TTF-1 expression in schwannomas. According to the immunohistochemical results and the most recent surgical date, we selected 5 cases from each group of schwannomas and grouped them into strong, medium, weak, or negative staining groups. Four non-schwannoma cases of ganglioneuroma, MPNST, neurofibroma and traumatic neuroma were selected for comparison. In addition, 5 cases of normal thyroid tissues served as positive controls. All cases were FFPE samples.

Total RNA was extracted using an RNeasy FFPE Kit (QIAGEN, Germany). Total RNA (1 μg) was reverse-transcribed into cDNAs using a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Inc, Lithuania). TTF-1 was amplified in a 20-$\mu$L reaction volume that contained 2 $\mu$L of cDNAs, 1 $\mu$L of primer (10 μmol/L) and 10 $\mu$L of PowerUp SYBR Green Master Mix (2×) (Life Technologies, Austin, TX). β-Actin (ACTB) served as the reference gene and its expression in experimental and positive control groups was tested using the $2^{-\Delta\Delta Ct}$ method [20-23]. The primer sequences used to amplify TTF-1 and ACTB were the following:

TTF1-F (Forward): AGGACACCATGAGGAACAGC
TTF1-R (Reverse): GCCATGTTCTTGCTCACGTC
ACTB-F (Forward): GAGCACAGAGCCTCGCCTTT
ACTB-R (Reverse): TCATCATCCATGGTGAGCTGGC

qPCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, Singapore) using the following