Expression of neurotrophin receptors in surgically resected thymic epithelial tumors

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Received 12 June 2005; accepted 22 June 2005; Available online 25 August 2005

Abstract

Objective: Neurotrophins are known to exert a variety of pleiotropic responses in different target tissues, but little is known about their effect on thymic epithelial tumors. Therefore, we analyzed the expression of neurotrophin receptors in surgically resected thymic epithelial tumors and evaluated their clinical relevance. Methods: The expression of neurotrophin receptors (Trk-A, Trk-B, Trk-C and p75\textsuperscript{NTR}) in thymic epithelial tumors was evaluated in 99 consecutive patients based on immunohistochemical staining. The pattern of expression was analyzed according to the WHO classification, and survival outcomes were estimated. Results: Thymic tumors were classified as type A (n = 6), AB (n = 21), B1 (n = 15), B2 (n = 24), B3 (n = 22) or C (n = 11). All tumors, except one type C thymoma, demonstrated cytoplasmic Trk-A immunostaining, and no thymic tumors showed Trk-B or Trk-C immunoreactivity. p75\textsuperscript{NTR} immunostaining demonstrated characteristic patterns according to the WHO subtypes of thymomas. All type A and type AB thymomas showed p75\textsuperscript{NTR} immunoreactivity, except one type A tumor. The expression of p75\textsuperscript{NTR} was negative in 6 patients (40%) with type B1 thymomas, 19 patients (79.2%) with type B2 thymomas, 17 patients (77.3%) with type B thymomas and 10 patients (90.9%) with type C thymomas. Tumor-related survival at 5 and 10 years was 95.5 and 89.5%, respectively, in p75\textsuperscript{NTR}-negative thymomas, positive thymomas and 82.8 and 77.2%, respectively, in p75\textsuperscript{NTR}-negative thymomas; however, the differences were not statistically significant (P = 0.14). Conclusions: Among the neurotrophin receptors examined, the pattern of p75\textsuperscript{NTR} expression closely correlated with the WHO subtypes of thymomas. Further study of p75\textsuperscript{NTR} expression may aid in understanding the biology of thymic epithelial tumors.

Keywords: Thymoma; Surgery; Receptors; Pathology; Prognosis

1. Introduction

Neurotrophins are a family of secreted growth factors consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) [1,2]. The neurotrophins are capable of promoting neuronal cell survival or death, depending on the neuronal environment [3]. The pleiotrophic actions of neurotrophins are mediated by two structurally unrelated classes of receptors—the Trk receptors and the common neurotrophin receptor p75\textsuperscript{NTR}. The Trk family of receptors possesses tyrosine kinase activity and is made up of three different neurotrophin receptors, each with a different specificity: Trk-A binds NGF, Trk-B binds BDNF and Nt-4/5, and Trk-C binds NT-3 [4]. p75\textsuperscript{NTR} is a member of the tumor necrosis factor receptor family and is able to bind all neurotrophins [1,4].

Although originally isolated from neural tissues, neurotrophins may play a role in tumorigenesis in non-neural tissues [5-8]. Elevated Trk-A,C and reduced Trk-B expression are associated with tumor progression in medullary carcinoma of the thyroid [5], and phosphorylation of Trk-A is a predictor of poor outcomes in solid ovarian carcinoma [6]. Conversely, the expression of Trk-A is associated with a better prognosis in neuroblastoma [7]. A recent large study evaluated the expression of p75\textsuperscript{NTR} receptors in a variety of human tumors. Frequent expression was observed in some soft tissue tumors, while most carcinomas and mesotheliomas were negative for p75\textsuperscript{NTR} expression [8].

In normal thymuses, the epithelial cells can be divided into two main subtypes based on their immunohistochemical characteristics and possible on their origin—the subcapsular and medullary epithelial cells on one side and the cortical ones on the other. The first subtype of epithelial cells shows mainly Trk-A immunoreactivity [9], and it has been suggested that they are derived from the neural crest [10]. p75\textsuperscript{NTR} immunoreactivity has been detected in interdigitated reticular cells, follicular dendritic cells and epithelial Hassal’s bodies [11]. The expression of neurotrophin receptors in thymic epithelial tumors had not previously been studied in a large number of patients with detailed clinical profiles.

The objective of this study was to investigate the expression of neurotrophin receptors (Trk-A, B, C and p75\textsuperscript{NTR}) in thymic epithelial tumors. Having a large sample
of patients allowed us to evaluate the relationship between expression status and clinicopathologic features.

2. Materials and methods

2.1. Patients

One hundred and twenty-six patients with thymoma underwent surgery at the Department of Thoracic and Cardiovascular Surgery of Yonsei University College of Medicine during the period from 1992 to 2002. Patients who were initially treated elsewhere (n=6), patients who received preoperative chemotherapy or radiotherapy (n=4), patients who died of sepsis following surgery (n=1) and patients who underwent a open biopsy alone (n=7) were excluded from the study. Among the remaining 108 patients, paraffin-embedded tissue blocks were unavailable in 9 patients, leaving a total of 99 cases which were considered for immunohistochemical analysis.

Clinical records were reviewed to determine the demographic of patients, the type of surgery, the completeness of resection and the survival outcome. Goals of the surgical resection included the complete removal of all thymic tissues and the exploration of both pleural cavities to determine if satellite lesions were present. We preferred a median sternotomy because complete surgical exploration of the chest could then be conducted. Extended thymectomy was defined as the resection of the entire thymus and mediastinal fat tissue between both phrenic nerves; thymectomy was defined as the resection of thymoma leaving residual thymic tissue; debulking was defined as removal of the tumor to the extent possible, usually at least two-thirds of the tumor or more. Invasiveness of tumors was judged on the basis of surgical findings and pathology reports, and clinical staging was conducted according to the Masaoka classification system [12].

2.2. Pathology review

All specimens underwent morphological evaluation, and histologic typing was done according to the WHO classification system, as follows [13]: type A thymomas are composed mainly of epithelial cells, i.e. lymphocytes are rare throughout all sections. The epithelial cells in most cases are spindle- or oval shaped, without nuclear atypia. Type AB thymomas exhibit tumor foci with features of type A thymomas, in addition to lymphocyte-rich areas. The segregation between the two components is usually sharp. Type B1 thymomas are lymphocyte-predominant tumors that resemble the normal functional thymus in that they combine large expanses with an appearance practically indistinguishable from that of normal thymic cortex, with areas resembling thymic medulla. Type B2 thymomas are characterized by pale and polygonal neoplastic epithelial cells scattered individually or in small clusters among immature lymphocytes. Perivascular spaces are common, and the tumor cells exhibit palisading around the spaces. Type B3 thymomas are composed predominantly of epithelial cells with mixture of minor components of immature lymphocytes. Tumor cells have a clear or eosinophilic cytoplasm and well-defined cytoplasmic margins and exhibit mild atypia. Foci of squamous metaplasia and perivascular spaces are common. Type C thymomas exhibit clear-cut cytologic atypia and a set of cytoarchitectural features not specific to the thymus, but analogous to those seen in carcinomas of other organs.

Tissue sections were initially examined by one of the authors (W.I.Y) in a blinded manner, and another pathologist (S.H.K) independently reviewed them. Disagreements in histologic typing occurred in few cases (mainly between the subtypes of type B thymomas), and this problem was resolved by additional reviews of more tissue sections until mutual agreement was achieved. In some cases of mixed thymomas (especially B2 plus B3 histology), the WHO subtype was assigned to the more abundant histologic type (area > 50% of the total area). As a result, we were able to assign each thymoma to one of the WHO subtypes (A, AB, B1, B2, B3, C) for further analysis.

2.3. Immunohistochemical analysis

Immunohistochemical staining of 4 µm-thick sections obtained from formalin-fixed and paraffin-embedded blocks was performed with the DAKO EnVision™ system (DakoCyto- tomation, Glostrup, Denmark) using 3',3'-diaminobenzidine (DAB) as a chromogen. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, and non-specific staining was blocked with a protein-blocking reagent (DakoCyto- tomation). Microwave-based antigen retrieval (800 W, 15 min) using a citric acid buffer (pH 6.0, 0.1 M) was employed for all primary antibodies. Phosphate-buffered saline (pH 7.6) was used in place of primary antibodies as a negative control. Paraffin-embedded tissue sections from human nerve plexuses served as positive controls for all primary antibodies Primary antibodies included a monoclonal mouse anti-p75NTR (1:50 dilution, DakoCyto- tomation), polyclonal rabbit anti- Trk-A (1:200 dilution, sc-118, SantaCruz Biotechnology, Inc., Santa Cruz, CA, USA), polyclonal rabbit anti-Trk-B (1:200 dilution, sc-12, SantaCruz Biotechnology, Inc.), and polyclonal rabbit anti Trk-C (1:200 dilution, sc-117, SantaCruz Biotechnology, Inc.). Incubation with primary antibodies was performed in a 4 °C refrigerator overnight.

Immunohistochemical staining results were evaluated without knowledge of the clinical data. Cytoplasmic immunoreactivity with Trk-A, trk-B and Trk-C and membranous immunoreactivity with p75NTR were considered as positive. For Trk immunohistochemical staining, the intensity of cytoplasmic staining was evaluated as weak (+1) or intense (+2). Because there was no variation in the intensity of p75NTR immunohistochemical staining, p75NTR expression was scored semi-quantitatively as follows: 0, no staining; +1, staining <1/3 of tumor cells; +2, staining 1/3-2/3 of tumor cells; +3, staining >2/3 of tumor cells.

2.4. Statistical analysis

Statistical differences in the average values were examined with the Student’s t-test. To compare the categorical variables, the chi-square test was performed. The Mantel-Haenszel chi-square test was used to evaluate
trends in scores. The survival rate was estimated by the Kaplan-Meier method, and differences in survival were determined with the log-rank test. Deaths that were related to a thymoma were considered as events, and all deaths that were not related to the tumor were considered as censored observations. Deaths resulting from the deterioration of myasthenia gravis (MG) were considered as thymoma-related. A \( P \)-value of less than 0.05 was considered to demonstrate statistical significance.

3. Results

3.1. Clinical features

Patients included 59 men and 40 women, with a median age of 46 years (range 20–83 years). MG was associated in 40 patients (40.4%). Forty-six patients were in stage I, 33 patients were in stage II, 12 patients were in stage III, and 8 patients were in stage IV. Extended thymectomy was performed on 69 patients (69.7%), thymomectomy was performed on 27 patients (27.3%) and debulking was performed on 3 patients (3.0%). Complete (R0) resection was achieved in 80 patients (80.8%).

Thymomas were histologically classified at type A \((n=6)\), type AB \((n=21)\), type B1 \((n=15)\), type B2 \((n=24)\), type B3 \((n=22)\), or type C \((n=11)\). Seven cases of type B2 thymomas showed minor components of B3 histology (less than 10% of tumor cells). Eleven cases of type B3 thymomas showed components of B2 histology, with the area of B2 histology making up 5-50% of the total area. The clinical features of each histologic subtype are summarized in Table 1. There was no significant difference in tumor size among the subtypes of thymoma. MG was most frequent in type B thymomas and tended to be more frequent in type B2 or B3 thymomas. A significant correlation between the histologic subtype and the Masaoka stage was noted \((P<0.05)\), and the proportion of invasive tumors gradually increased from type A to type C.

3.2. Expression of neurotrophin receptors in non-tumorous thymus

In the attached non-tumorous thymus from surgical samples, the cortical and medullary epithelial cells were stained by Trk-A, while thymocytes demonstrated negative immunostaining (Fig. 1). No cells in the thymus were stained by Trk-B or Trk-C. Medullary spindle cells, some septal cells and perivascular nerve fibers were immunostained by p75NTR. In thymic tissue with follicular hyperplasia, follicular dendritic cells in the germinal centers were strongly immunostained by p75NTR.

![Fig. 1. Expression of Trk-A and p75NTR according to the WHO histologic subtypes. (A) Trk-A expression in non-tumorous thymus \((\times 200)\). (B) p75NTR expression in non-tumorous thymus \((\times 200)\). (C) Trk-A expression in type B3 tumor \((\times 200)\). Weak positive staining is observed. (D) Trk-A expression in type C tumor \((\times 100)\). Diffuse, intense immunoreactivity is noted. (E) p75NTR expression in type A tumor \((\times 100)\). Diffuse positive membranous staining of the spindle shaped tumor cells. (F) p75NTR expression in type AB tumor \((\times 200)\) . Tumor cells show the collision tumor-like pattern composed of sheet-like proliferation of plump spindle cells abutting lymphocyte-rich area. (G) p75NTR expression in type B1 tumor \((\times 100)\). Several immunoreactive cells are noted in the medullary area. (H) p75NTR expression in type C tumor \((\times 100)\). Tumor cells are uniformly negative.](image-url)
3.3. Expression of neurotrophin receptors in thymic epithelial tumors

All thymic tumors, except one type C squamous cell carcinoma, demonstrated cytoplasmic immunoreactivity for Trk-A (Fig. 1). The immunostaining pattern was diffuse and some variation of intensity was observed. The proportion of tumors which demonstrated intense (C2) immunoreactivity for Trk-A gradually increased from type A to type C (Mantel–Haenszel \( \chi^2 = 7.10, P = 0.008 \)), as shown in Table 2. As was found in non-tumorous thymic tissues, immunoreactivity for Trk-B or Trk-C was not observed in any type of thymoma. Thymocytes in the tumors were not immunostained for any of the family of Trk receptors.

The membranous immunoreactivity for p75NTR demonstrated characteristic patterns according to the WHO histologic classification of thymomas, as shown in Fig. 1. All type A thymomas, with the exception of one case, showed scattered patch or diffuse positive membranous immunostaining of the spindle tumor cells. Ten cases of type AB thymomas demonstrated scattered positive staining in less than 1/3 of the tumor cells, and 11 cases of type AB thymomas demonstrated p75NTR immunoreactivity in over 1/3 of the tumor cells. The cases with over 1/3 immunoreactivity for p75NTR showed a collision tumor-like histologic pattern composed of a sheet-like proliferation of plump spindle cells abutting lymphocyte-rich areas, while cases with less than 1/3 immunoreactivity for p75NTR showed a histologic pattern of lymphocyte-rich tumor islands separated by relatively thin bundles of slender spindle cells. In type B1 thymomas, six cases (40.0%) showed on p75NTR immunoreactive tumor cells, while nine cases demonstrated several p75NTR immunoreactive cells in the medullary area. Nineteen cases (79.2%) of type B2 thymomas and 17 cases (77.3%) of type B3 thymomas showed no p75NTR immunoreactive tumor cells. In one type B2 thymoma and three type B3 thymomas, the tumor cells demonstrated diffuse, intense positive reactions to p75NTR. Ten cases (90.9%) of type C thymomas showed no p75NTR immunoreactivity, while one case demonstrated diffuse p75NTR immunoreactivity. A significant correlation between the proportion of p75NTR-negative tumors and the histologic subtype was noted, as shown in Table 2. The proportion of p75NTR-negative tumors tended to increase from type A to type C (Mantel-Haenszel \( \chi^2 = 31.8, P < 0.0001 \)).

3.4. Survival outcomes

A follow-up survey was successfully completed for all patients, with a median follow-up time of 42 months (range 2.0–122.0 months). During the follow-up period, 17 deaths occurred. Eleven patients (64.7%) died of tumor-related causes, and the remaining 6 patients (35.3%) died of other causes (cardiovascular disease \( n = 3 \), traffic accidents \( n = 1 \), delayed mediastinitis \( n = 1 \), and primary lung cancer \( n = 1 \)). Tumor-related survival rates at 5 and 10 years were 88.6 and 83.0%, respectively.

Survival curves according to the WHO histologic subtypes are shown in Fig. 2. There was no tumor-related death among type A, AB and B1 thymomas, and the survival rate gradually decreased from type A/AB/B1 to type C. The tumor-related survival rates among type B3 and type C thymomas were significantly lower than that of type A/AB/B1 thymomas \((P < 0.05)\). Type C thymomas showed the worst prognosis, and majority of the patients died before within 5 years of their surgery. Type B3 thymomas had an intermediate prognostic ranking relative to the other tumor types. Survival outcomes were further analyzed according to

<table>
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<th>Type</th>
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<th>P75NTR(^b), no. (%)</th>
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<tr>
<td></td>
<td>0</td>
<td>+1</td>
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<tr>
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<td>16 (76.2)</td>
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<td>B2</td>
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<td>12 (50.0)</td>
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<td>B3</td>
<td>0 (0.0)</td>
<td>11 (50.0)</td>
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<td>C</td>
<td>0 (0.0)</td>
<td>3 (27.3)</td>
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\(^a\) (+1), weak cytoplasmic staining; (+2), intense cytoplasmic staining.

\(^b\) (+1), staining <1/3 of tumor cells; (+2), staining 1/3–2/3 of tumor cells; (+3), staining >2/3 of tumor cells.

![Fig. 2. Survival curves according to the WHO histologic subtypes. There was no tumor-related death among type A, AB and B1 thymomas, and the survival rate gradually decreased from type A/AB/B1 to type C. The tumor-related survival rates among type B3 and type C thymomas were significantly lower than that of type A/AB/B1 thymomas \((P < 0.05)\). Type C thymomas showed the worst prognosis, and majority of the patients died before within 5 years of their surgery. Type B3 thymomas had an intermediate prognostic ranking relative to the other tumor types. Survival outcomes were further analyzed according to...](image-url)
the expression of neurotrophin receptors, as shown in Fig. 3. Differences in survival were not observed between the Trk-A (+2) tumors and Trk-A (0 or +1) tumors. As for p75NTR, the 5- and 10-year survival rates were 82.8 and 77.2%, respectively, in p75NTR-negative tumors and 95.5 and 89.5%, respectively, in p75NTR-positive tumors. Although the survival rate of p75NTR-negative tumors was lower than that of p75NTR-positive tumors, a statistically significant difference was not observed (P = 0.15).

4. Discussion

In the present study, we evaluated the expression of neurotrophin receptors in thymomas using an immunohistochemical method. We also analyzed the relationship between neurotrophin receptor expression and WHO histologic subtypes, as well as their prognostic relevance. In a variety of human tumors, an altered expression of neurotrophin receptors is known to be associated with tumor development and metastasis [5-8]. For example, activation of Trk-A is a frequent event in malignant mesothelioma and is predominately seen in effusions and peritoneal lesions. In contrast, p75NTR is infrequently expressed in malignant mesothelioma and is further reduced in effusions [14]. The pattern of neurotrophin receptor expression is known to vary according to the organ and the type of malignancy [8], but little had been known about their role in thymic epithelial tumors.

In non-tumorous thymic tissues, we observed that Trk-A was expressed in the cortical and medullary epithelial cells and that p75NTR was expressed in medullary spindle cells, some sepal cells, and pericapsular nerve fibers. The same pattern of expression was observed in a study of normal thymuses, as Labouyrie et al. reported [11]. Among the neurotrophin receptors examined, the pattern of p75NTR expression was closely associated with the WHO histologic classification of the thymoma. All type A/AB tumors, with the exception of one case, showed scattered or diffuse membranous immunostaining of the tumor cells. Tumor cells were spindle- or oval-shaped, and the foci of tumor cells were sharply demarcated with the lymphocyte-rich areas in type AB tumors. In some type B tumors, the polygonal-shaped neoplastic epithelial cells showed membranous immunoreactivities for p75NTR. Although 60% of type B1 tumors showed p75NTR expression, the majority of type B2 and B3 tumors demonstrated negative p75NTR expression, as shown in Table 2. None of the type C tumors demonstrated p75NTR expression, except one squamous cell carcinoma. Regarding the histogenesis of thymoma, our findings support the current hypotheses which attribute each subtype (cortical or medullary) of thymoma to different groups of thymic epithelium [15], since almost all the tumors with a medullary epithelial type (WHO subtype A or AB) demonstrated p75NTR expression, while a significant portion of the tumors with a cortical epithelial type showed negative p75NTR expression. p75NTR has been shown to be capable of mediating cell death [16], and we assume that this may explain why thymomas with a medullary epithelial component show a better prognosis.

With regard to the prognostic relevance of neurotrophin receptors, neither Trk-A nor p75NTR were independent prognostic factors affecting postoperative tumor-related survival. Although the survival rate of p75NTR-positive tumors was higher than that of p75NTR-negative tumors, a statistically significant difference was not observed (P = 0.15). The failure of neurotrophin receptor expression in thymomas to demonstrate prognostic value could be explained as follows. First, given the emerging complexity of the p75NTR-Trk signaling system, it may be difficult to make accurate prediction about the effects of p75NTR mutations or other derangements. p75NTR may function as both death receptor and dependence receptor, depending in part on the expression of Trk and the presentation of NGFs. For example, in a p75NTR-dominant system, withdrawal of NGF leads to apoptosis induction that is rescued by the addition of NGFs binding p75NTR. In a Trk-dominant system, the addition of a mismatched NGF induces apoptosis, whereas addition of a matched NGF blocks apoptosis [17,18]. Thus, we believe that the expression status of p75NTR alone cannot provide a sufficient explanation of prognosis. Second, the expression of neurotrophin receptors did not show a significant correlation with the Masaoka staging system (data not shown), known to be the most important prognostic factor in resected thymic epithelial tumors [19,20].

Our observations may have diagnostic value, especially in the setting of biopsy specimens. Occasionally, it is difficult to make a definitive diagnosis of thymomas due to the small amount of specimen. In these cases, immunostaining with neurotrophin receptors (Trk-A and p75NTR) can aid in the diagnosis by highlighting the neoplastic epithelial cells and the cytoarchitectural pattern. This may prevent the misdiagnosis of a thymoma as lymphoma or other diseases.

A recent study reported that a switch to a Trk-A negative/p75NTR positive phenotype was observed in thymic carcinomas and was associated with cell proliferation-associated MIB1 expression, while epithelial cells of normal thymuses or benign thymomas exhibited a Trk-A positive/p75NTR negative phenotype [21]. This is somewhat in conflict with the present study. Although the authors used various techniques including molecular studies, the number of patients was small (n=10) and only two cases were thymic carcinoma. To the best of our knowledge, our report is the largest study of the expression of neurotrophin receptors in thymomas, and we demonstrated detailed clinical profiles including time-series analysis. Further studies are needed regarding the role of neurotrophin and its receptors in thymic epithelial tumors.
In conclusion, the pattern of \( p75^{NTR} \) expression was closely correlated with WHO subtypes of thymomas and further research of \( p75^{NTR} \) may aid in understanding the biology of thymic epithelial tumors.

Acknowledgements

This work was supported by the Yonsei University Faculty Research Fund (2004).

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