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Original contribution

Expression patterns of Trk-A, Trk-B, GRP78, and p75NRT in olfactory neuroblastoma

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Summary Olfactory neuroblastoma is an uncommon neuroectodermal tumor of the sinonasal tract. It represents 2% to 3% of sinonasal neoplasms. Most olfactory neuroblastoma behave locally aggressive with 30% recurrence rates. A subset metastasizes to lymph nodes and/or distant sites. Grading of olfactory neuroblastoma involves a combination of factors with low-grade tumors having better survival than high-grade tumors. The grade does not always predict prognosis, however, as metastases can be seen in all grades of olfactory neuroblastoma. Trk-A, Trk-B, and p75NRT are neurotrophin receptors associated with numerous solid malignancies, particularly pediatric neuroblastoma. GRP78 is an endoplasmic reticulum protein, associated with differentiation of neuroblastic cells. Trk-A, p75NRT, and GRP78 overexpression are favorable prognostic factors in pediatric neuroblastoma, whereas Trk-B is associated with a poorer prognosis in these tumors. Olfactory neuroblastoma is clinically distinct from pediatric neuroblastoma but shares some histological features. Trk-A and p75NRT have been demonstrated in olfactory neuroblastoma previously. Trk-B and GRP78 have not been investigated in olfactory neuroblastoma. None of these markers have been correlated with grade or outcome in olfactory neuroblastoma. To investigate the role of Trk-A, Trk-B, p75NRT, and GRP78, a series of 20 olfactory neuroblastomas was stained with these antibodies. Trk-A and Trk-B stained most cases of olfactory neuroblastoma (90% and 85%). GRP78 stained most cases (90%), although weakly. P75NRT demonstrated focal membranous staining in a sustentacular pattern (60%). None of these markers correlated with Hyams grade. None of these markers definitively correlated with patient outcome. Neurotrophin receptors do not appear to have a prognostic role; however, Trk's may play an oncogenic role in olfactory neuroblastoma.

1. Introduction

Olfactory neuroblastoma (ONB) is an uncommon malignant neuroectodermal tumor thought to arise in the olfactory membrane in the sinonasal tract [1]. It represents approximately 2% to 3% of all sinonasal neoplasms [1]. Most ONB

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behave in a locally aggressive manner with a 30% recurrence rate and a small subset may metastasize to lymph nodes and/or distant sites, including lung and bone [1]. These recurrences and metastases may occur years after the primary treatment. Grading of ONB involves a combination of factors (architecture, rosettes, neurofibrillary stroma, nuclear pleomorphism, mitotic activity, and necrosis) [2]. Low-Hyams-grade tumors have a better overall survival than high-grade tumors; however, the grade of the tumor does not always adequately predict prognosis and metastases can be seen in all grades of ONB [1].

Trk-A, Trk-B, and p75NRT are neurotrophin receptors associated with numerous solid malignancies [3], most notably pediatric neuroblastoma (PNB). GRP78 is an endoplasmic reticulum protein, known to be associated with differentiation of neuroblastic cells [4]. Trk-A, p75NRT, and GRP78 overexpression are known to be favorable prognostic factors in PNB, whereas Trk-B is associated with a poorer prognosis in these tumors making it mutually exclusive from Trk-A [3-5]. ONB is clinically distinct from PNB but shares some histological features. Trk-A and p75NRT have been demonstrated in ONB previously [6]. Trk-B and GRP78 have not been investigated in ONB, and none of these markers have been correlated with either grade or outcome in ONB.

To investigate the role of Trk-A, Trk-B, p75NRT, and GRP78, a series of 20 typical cases of ONB were retrieved and immunostained with these antibodies. The specific staining patterns and potential roles in diagnosis, oncogenesis, and prognostication of these tumors were investigated.

2. Methods

An archival search of the University Health Network (Toronto, Ontario, Canada) for olfactory neuroblastoma was performed with institutional ethics board approval. Cases with both tissue availability and clinical outcomes were retrieved and reviewed for diagnosis and regraded using the Hyams grading scheme [2]. Twenty cases with tissue were retrieved. All surgical specimens were fixed in 10% neutral buffered formalin and processed routinely. Hematoxylin and eosin stains were available on all cases and were performed on 3- to 4-μm-thick sections of formalin-fixed, paraffin-embedded tissue. Immunohistochemical stains were performed in sections from a paraffin block or from unstained slides, using the ultrastreptavidin-horse radish peroxidase (HRP) detection system (ID Labs Biotechnology, London, Ontario, Canada). Color development was performed using the NovaRed substrate kit (Vector Labs, Burlingame, CA). All the immunohistochemical studies were performed manually. Antibodies used and their sources and dilutions are listed in Table 1. Positive controls were used for all antibodies, which consisted of a Trk-A– and Trk-B–positive pediatric neuroblastoma and GRP78 and p75NRT positive schwannoma and ganglioneuroma (1 each). Trk-A and Trk-B were scored as positive with nuclear staining, GRP78 was scored as positive with cytoplasmic staining, and p75NRT was scored as positive with cytoplasmic membrane staining. Staining was scored as follows: F+, (isolated cells staining); +, (10%-25% of cells staining); ++, (25%-50% of cells staining); and +++, (> 50% of cells staining).

Table 1 Immunohistochemical antibodies employed

Antibody	Source	Dilution	Pretreatment
Trk-A	Abcam Cat# ab10838 rabbit polyclonal	1/60	Microwave
Trk-B	R&D Cat# MaB397 mouse monoclonal clone 75133	1/60	Microwave
p75NRT	Abcam Cat# ab10495 mouse monoclonal clone ME20.4	1/2000	Pepsin
GRP78	Santa Cruz Cat#SC1050 goat polyclonal	1/800	Microwave

Statistical analysis was performed using SPSS v 16.0 (SPSS, Chicago, IL). χ^2 or Fisher exact test was used to assess the relationship between Hyams grade and immunohistochemical markers. Hyams was grouped into low-grade (grades 1and 2) and high-grade (grades 3 and 4). Immunohistochemical predictors of survival (overall and recurrence free) were analyzed with the Kaplan-Meir method using the log-rank test. All statistical tests were 2-sided, and a P value of .05 was considered significant.

3. Results

3.1. Clinical findings

There were a total of 20 patients (13 men and 7 women) with an age range of 20 to 79 years and an average age of 49.3 years. They presented with left [9], right [6] or bilateral [5] nasal masses. The symptom duration ranged from 1 to 24 months with an average of 10.3 months before presentation. The symptoms included one or more of anosmia, visual defects, nasal obstruction, epistaxis, or headache. Three patients (15%) presented with neck masses. The patients were treated with surgery [4], surgery with preoperative radiation [10], or postoperative radiation [5] or combined chemoradiation without surgery [1]. Follow-up ranged from 3 to 152 months, with an average of 58.6 months. At last follow-up, 13 patients are alive with no evidence of disease at an average of 59.4 months. Five patients are alive with persistent local disease at an average of 72.6 months, 2 of which have distant metastases (1 each in the lung and brain). Two patients have died of disease, the first at 3 months due to progressive local disease and the second at 33 months due to extensive meningeal spread.