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Clinical Study

Clinicopathological features of myxopapillary ependymoma

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ABSTRACT

Myxopapillary ependymoma (MPE) is a rare and distinct variant of ependymoma with a tendency for local recurrence and metastasis. Its clinicopathological spectrum is heterogenous, underscoring the need to understand and characterize MPE for better diagnosis and treatment. The purpose of this study was to explore the tumor biology and assess the management of patients with MPE. Tumors from a cohort of 19 patients were analyzed by light microscopy, electron microscopy, immunohistochemistry and fluorescence *in situ* hybridization (FISH). Clinical characteristics, therapeutic options and clinical follow-up data were also analyzed. Back pain was the most common presenting symptom. The main pathological morphology observed was papillae embedded in a myxoid background, but other rare morphologies were also present. Immunostaining revealed epidermal growth factor receptor (EGFR) expression in four MPE, while FISH for EGFR was negative. No correlation between tumor recurrence and EGFR overexpression was found. Ultrastructural examination revealed adherens junctions and intracytoplasmic lumina with microvilli. Patients with gross-total resection (GTR) had no tumor recurrence ($p = 0.021$). Also, patients with subtotal resection (STR) followed by radiotherapy showed a higher local control rate than patients with STR alone ($p = 0.043$). The diagnosis of MPE should be made considering the histology, immunohistochemistry, imaging studies and anatomical site. GTR of the tumor or STR followed by radiotherapy are more likely to avoid tumor recurrence than STR alone. Based on our findings, there is no correlation between tumor recurrence and EGFR expression.

1. Introduction

Myxopapillary ependymoma (MPE) is a rare distinct variant of ependymoma that affects middle-aged individuals and occurs most commonly in the cauda equina and filum terminale of the spinal cord [1,2]. It is considered a grade I tumor by the World Health Organization (WHO), but it can sometimes be locally aggressive and metastasize [3,4]. Microscopically, the tumor reveals papillary formation surrounding areas containing both hyalinized blood vessels and myxoid degeneration. Since the clinicopathological spectrum is heterogenous [5], it is important for both pathologists and clinicians to recognize these rare tumors. Little is known about the genetic mechanisms underlying MPE and few molecular markers of clinical relevance have been identified until now. Epidermal growth factor receptor (EGFR) inhibitors may be promising because of the presence of EGFR in ependymoma [6]. Mendrzyk et al. reported that gain of 1q25 and EGFR overexpression correlated to poor prognosis of ependymoma [7]. The optimal management of MPE remains somewhat controversial. Excellent outcomes may be obtained with the use of aggressive surgical techniques. Here, we present 19 patients with MPE and discuss the clinicopathological features, EGFR protein expression level and current management options for this tumor.

2. Materials and methods

2.1. Patients

Nineteen patients diagnosed with MPE at Jinling Hospital and Affiliated Hospital of Nantong University were included in this study, which was approved by the Institutional Review Board. All patients consented to the study. Specimens were obtained from gross-total resection (GTR) or subtotal resection (STR) surgery from 1990 to 2011. At the time of collection, samples were fixed in 10% buffered formalin and embedded in paraffin. Hematoxylin and eosin stained slides for each patient were histologically examined

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according to WHO criteria. Data regarding age at diagnosis, sex, location of disease, symptoms, type of surgery, tumor size, imaging and follow-up was obtained from paper charts and electronic medical records.

2.2. Immunohistochemistry

Immunostaining was performed on archived paraffin embedded specimens. Serial sections of 5 μm were cut from the tissue blocks, deparaffinized in xylene, rehydrated through a graded series of alcohol and placed in running water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase. Antigen retrieval was performed by microwave heating at high power (750 W) in 10 mM sodium citrate buffer (pH 6.0) for 10 minutes. After blocking, slides were incubated with primary antibody for glial fibrillary acidic protein (GFAP), vimentin, S-100, epithelial membrane antigen (EMA), cytokeratin (CK), EGFR and Ki-67. Diaminobenzidine was used as chromogen. Slides were counterstained with hematoxylin. Samples incubated with phosphate buffered saline instead of primary antibodies were used as negative controls.

2.3. Ultrastructural observation

Fresh samples were fixed in 3% cold glutaraldehyde at 4 °C overnight. This was followed by rinsing in cold phosphate buffer solution for 1 hour. The samples were then fixed in 1.3% osmium tetroxide for 1 hour, followed by acetone gradient dehydration and embedding in Epon 812 resin. Ultra-thin sections were counterstained with uranyl acetate and lead citrate, and examined using a JEM-1011 transmission electron microscope (JEOL, Tokyo, Japan).

2.4. EGFR fluorescence in situ hybridization assay

Fluorescence *in situ* hybridization (FISH) was performed on tumors with EGFR positive immunostaining. The DNA probe used was Vysis LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen Probes (Abbott Laboratories, Abbott Park, IL, USA). Section preparation and hybridization were performed as described [8]. The EGFR gene was visualized as an orange signal, the CEP7 probe as a green signal, and the nucleus as a blue signal with a DAPI filter. At least 100 nuclei were scored for both EGFR gene signals and chromosome 7 signals for FISH evaluation. EGFR gene amplification was defined according to one of the following criteria: [9] (1) EGFR to CEP7 ratio >2 over all scored nuclei; (2) the presence of gene cluster ($\geqslant$4 spots) in $\geqslant$10% of tumor cells; or (3) at least 15 copies of the EGFR signals in $\geqslant$10% of tumor cells.

2.5. Statistical analysis

Prism (GraphPad Software, La Jolla, CA, USA) software was used to calculate survival and local control (LC) rate based on the Kaplan-Meier method [10]. Statistical difference was calculated using the log-rank test. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

We included a total of 19 patients in this study. Patient characteristics are described in Table 1. Out of 19 patients, eight were females. The age of these patients varied from 14 to 72 years with an average age of 33 years. Back pain was the most common presenting symptom. Motor or sensory abnormalities and bladder dysfunction were also seen. Surgical resection was performed for all patients. MRI revealed the majority of tumors were isointense or hypointense on T1-weighted images, hyperintense on T2-weighted images and enhanced on post-contrast T1-weighted images (Fig. 1).

Table 1
Characteristics and treatment of patients with myxopapillary ependymoma

Variable	n
Patients	19
Age, years	
Median	33
Range	14–72
Sex	
Female/Male	8/11
Symptoms	
Back pain	17 (89.5%)
Weakness	7 (36.8%)
Numbness	9 (47.4%)
Abnormal gait	6 (31.6%)
Urinary dysfunction	4 (21.1%)
Symptom duration, months	
Median	10
Range	1–68
Tumour location	
Thoracolumbar	8 (42.1%)
Lumbosacral/cauda equina	11 (57.9%)
Treatment	
GTR	9 (47.4%)
STR alone	5 (26.3%)
STR + RT	5 (26.3%)
Recurrence after resection	
No	14 (73.7%)
Yes	5 (26.3%)

GTR = gross-total resection, RT = radiotherapy, STR = subtotal resection.

3.2. Pathologic findings

Macroscopically, the tumors were soft to rubbery and greyish to reddish-brown. Complete to partial encapsulation was identified. By light microscopic study, the tumors showed a variegated appearance and the majority of the tumors displayed classical pseudopapillary arrangements of cuboidal cells with hyalinized fibrovascular cores, which often revealed prominent myxoid change (Suppl. Fig. 1A). Six tumors predominantly had a multicystic or reticular appearance. The cyst-like spaces had deposition of eosinophilic hyaline material in the lumen surrounded by neoplastic cells resembling an adenoid cystic pattern of growth (Suppl. Fig. 1B). Two tumors consisted mostly of areas with low cellularity. In these less cellular areas, conspicuous hyaline change was observed in both blood vessel walls and stroma (Suppl. Fig. 1C). Overall, cellular atypia was not conspicuous and necrosis could not be identified. Immunohistochemically, the tumor cells showed strong positivity for GFAP (Suppl. Fig. 1D), vimentin (Suppl. Fig. 1E) and S-100 protein, whereas they were negative for EMA and CK. The Ki-67 labeling index was about 1–6%. In addition, immunostaining for EGFR was strongly positive (Suppl. Fig. 1F) in the tumor of one of the five patients who relapsed, and intermediately positive in three of the 14 tumors in patients without recurrence. Electron micrographs of the tumors showed intracytoplasmic lumina with microvilli, desmosomal junctions and abundant glycogen (Fig. 2). These results confirmed the origin of these neoplastic cells as ependymal and were consistent with the diagnosis of MPE.