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Gonadotropin-releasing hormone (GnRH) and its receptor in human meningiomas

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ABSTRACT

Objective: Meningiomas are the most common neoplasms of the central nervous system and are more frequent in women than in men. Many studies have been conducted to determine whether the progesterone receptor (PR) and estrogen receptor (ER) are present or absent in meningiomas. No previous studies, however, have investigated the status (presence or absence) of gonadotropin-releasing hormone (GnRH) and its receptor (GnRH-R), two major factors related to PR and ER, in meningiomas. This study aims to determine the status of GnRH and GnRH-R and to elucidate the correlations of GnRH and GnRH-R with PR, ER, and clinical features in meningiomas.
Methods: Eighty-two specimens of human meningiomas were obtained for immunohistochemical analysis with anti-GnRH, anti-GnRH-R, anti-PR, anti-ER, and anti-Ki-67 (MIB-1) antibodies, and for RT-PCR analysis of the mRNA expressions of GnRH and GnRH-R. Correlations of GnRH and GnRH-R with PR, ER, Ki-67, and clinical features such as age, sex, tumor grade, and tumor histology were assessed.
Results: Seventy-eight (95.1%) of the 82 meningiomas reacted positively in the cytoplasm for the GnRH-R. Forty-nine (59.8%) of the 82 cases reacted positively in the cytoplasm for the GnRH. The positive immunoreactivity for GnRH-R and GnRH was confirmed by the RT-PCR analyses of mRNA. Forty-seven (96%) of the 49 cases with positive immunoreactivity for GnRH-R also had positive immunoreactivity for GnRH. PR expression was higher in the tumors positive for GnRH-R ($p = 0.002$), and a significantly higher proportion of tumors from male patients exhibited positive immunoreactivity for GnRH ($p = 0.02$). No significant correlations were found between the status of GnRH-R or GnRH with other clinicopathological features.
Conclusion: Over half of meningiomas may be regulated by GnRH–GnRH-R expression in an autocrine fashion. This unique expression profile of GnRH and GnRH-R may open the way to the development of GnRH analogs as a treatment tool in the future.

1. Introduction

Meningiomas are relatively common neoplasms, accounting for 20–30% of all intracranial tumors reported [1]. Most meningiomas are benign, though some are recurrent or unresectable. Most of the unresectable tumors are located at the base of the skull, adjacent to vital structures such as the cranial nerves and brainstem. Common chemotherapeutic agents are generally ineffective. Even in cases administered radiation therapy postoperatively, the recurrence rate of incompletely resected cases is 12–23% [2,3].

A higher incidence of meningiomas in women than men [4], the relapse and remission of symptoms during and at the termination of pregnancy [5], and a reported epidemiological link between meningiomas and breast carcinomas [6,7] have led to a general consensus that sex steroid hormones influence the growth of tumor cells [8]. Many investigations have been driven by the hope that hormone manipulation might eventually serve as a valid medical intervention for patients with recurrent or unresectable meningiomas. Studies on the presence and absence of the progesterone receptor (PR) and estrogen receptor (ER) in meningiomas seem to establish that most meningiomas contain the PR and are devoid of the ER [9–16]. Closer examinations of PR and ER have failed to elucidate the significance of receptor status in progression, and reports on the correlation between receptor status and tumor proliferative indices are limited [17–19]. And to our knowledge, there have been

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no studies on the status of other major hormones and receptors related to PR, ER, and other sex steroid hormones.

Gonadotropin-releasing hormone (GnRH) and its receptor (GnRH-R) are major factors related to progesterone and estrogen. GnRH is normally present in pre-optic nucleus and hypothalamus. GnRH binds with its receptor in the pituitary gland, and releases the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Peripherally produced hormones include the sex steroids and inhibins that are primarily of gonadal origin, while activins and follistatin are produced in all tissues including the gonads. Above cascade of hormones is called the hypothalamic–pituitary–gonadal (HPG) axis. The concentration of each of the HPG axis hormones is regulated by complex feedback loops [20]. Because GnRH and GnRH-R, members of the HPG axis, exist within the brain, meningiomas located intracranial region may have a chance to be under control of these hormones rather than the sex steroid hormones. In the current study we attempted to determine the status of intracranial meningiomas excised from 82 patients, through analyses using monoclonal antibodies specific for GnRH and GnRH-R, and an RT-PCR method for both factors. We also analyzed the significance of our results on GnRH and GnRH-R, together with our findings on PR, ER, the mitotic index, and other clinicopathological features.

2. Materials and methods

2.1. Materials

Eighty-two tumor specimens of human memingioma were obtained at surgery in the Department of Neurosurgery, Kanazawa University Hospital (and associated institutions), Japan, between 1999 and 2002. Tumors were classifies into meningothelial (WHO grade I, 33 cases), fibrous (WHO grade I, 7 cases), transitional (WHO grade I, 33 cases), psammomatous (WHO grade I, 4 cases), angiomatous (WHO grade I, 1 case), atypical (WHO grade II, 3 cases) and anaplastic (WHO grade III, 1 case). The specimens of human placenta from patients of whom informed consent was obtained were distributed by our department of pathology. Control slides of human mammary cancer were purchased from DAKO Japan (Kyoto). All tumor specimens were fixed in 4.0% paraformaldehyde and embedded in paraffin for immunohistochemistry. Samples of 21 meningiomas were snap-frozen in liquid nitrogen immediately after surgical removal and stored at −130 °C until RNA extraction.

2.2. RNA extraction and RT-PCR

Total RNA was extracted from frozen tissue samples using RNeasy Kit (QIAGEN, Hilden, Germany). Random hexamer-primed single-strand cDNA was synthesized using the First-strand cDNA Synthesis Kit (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions. Ten microliters of cDNA was employed for PCR. The GnRH-R specific primer sequences were 5′-CGGATCTTTCCAGACAAGGTCA-3′ and 5′-GCCTCTCCTGAACAGAATCAAA-3′. The GnRH specific primer sequences were 5′-TCCACGCACCAAGTCAGTAGAAT-3′ and 5′-TTGGCTCTCTGCCTCTAAACAG-3′.

2.3. Antibodies

Anti-GnRH-R antibody (A9E4) was purchased from Quartett (Berlin, Germany). Anti-GnRH antibody (NT109) was purchased from Affiniti Research Products (Exeter, UK). Anti-PR antibody (PR636), anti-ER antibody (1D5), and anti-Ki-67 antibody (MIB-1) were purchased from Dako Japan (Kyoto, Japan).

2.4. Immunohistochemistry

The sections were deparaffinized in xylene and dehydrated in graded ethanol.

The endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 solution in methanol. For GnRH-R, GnRH, PR and ER immunostaining, the sections were boiled in 10 mM sodium citrate for 25 min in a pressure cooker. For Ki-67 immunostaining, the sections were boiled in sodium citrate for 15 min (five times 3 min) by a microwave. Anti-GnRH-R antibody, anti-GnRH antibody, anti-PR antibody, anti-ER antibody, and anti-Ki-67 antibody were used at 1:100, 1:200, 1:50, 1:50, and 1:50 dilution, respectively. All sections were incubated with each antibody at 4 °C overnight. The reaction was visualized using 3,3′-diaminobenzidine (DAB) substrate (Sigma, St. Louis, USA). The sections were counterstained with hematoxylin. As positive controls, tissue sections of human placenta were used in GnRH-R and GnRH immunostaining. Control slides of human mammary cancer were used in PR and ER immunostaining. Tissue sections of metastatic brain tumor of human lung cancer were used in Ki-67 immunostaining. Negative controls were made by omitting the primary antibody during the immunostaining. The expression of GnRH-R and GnRH was examined in the arachnoid villi which was origin of memingioma.

2.5. Scoring of immunoreactivity

Scoring was performed on high-power fields (400×) using light microscope. The immunoreactivity for GnRH-R and GnRH were scored as positive in more than 10% of the tumor cells. The immunoreactivity for PR, ER and Ki-67 were detected in the nuclei, and the percentage of immunoreactivity (labeling index, LI) was determined. The immunoreactivity for PR and ER were scored as negative (−) in less than 10%, weak positive immunostaining (+) from 10% to 40%, positive immunostaining (++) from 40% to 70% and strong positive immunostaining (+++) in more than 70%. The immunoreactivity for Ki-67 were scored as negative (−) in less than 1%, weak positive immunostaining (+) from 1% to 3% and positive immunostaining (++) in more than 3%.

2.6. Statistical analysis

Statistical differences between immunoreactivity for GnRH-R or GnRH and parameters; tumor grade, histological subtype, presence of recurrence, presence of multiple lesions, patient age, and sex were evaluated using χ^2-test or Fisher's exact test. Statistical differences between immunoreactivity for GnRH-R or GnRH and parameters; patient age, tumor size, PR LI, ER LI and Ki-67 LI were evaluated using Student's *t*-test or Welch's *t*-test. *p*-Value less than 0.05 was considered significant.

3. Results

3.1. Patient profile

The patient group consisted of 27 males and 55 females, with a mean age of 58.0 ± 13.1 (20–82) years at their presentation. The 82 cases of meningiomas were classified as 78 benign, 3 atypical, and 1 anaplastic (Table 1).

3.2. GnRH-R and GnRH mRNA expression in meningiomas

Based on the storage condition of tissues, only 21 (25.6%) of the 82 cases of meningiomas could be analyzed by RT-PCR. All (100%)