High FABP5 Versus CRABPII Expression Ratio in Recurrent Craniopharyngiomas: Implications for Future Treatment

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■ BACKGROUND AND OBJECTIVE: **Recurrence is a major problem in craniopharyngioma (CP) management. Recent study shows that high FABP5/CRABPII may be related to tumor growth and that all-*trans* retinoic acid (ATRA) may suppress primary CP growth. We studied the expression profile of FABP5 and CRABPII in recurrent CP tissue and the effect of ATRA on recurrent CP cells.**

■ METHODS: **Fifty cases of patients with CP were enrolled in the retrospective study. Among them, 15 were recurrent. Fresh specimens were collected for immunohistochemistry, reverse transcription polymerase chain reaction, and western blotting analysis of FABP5 and CRABPII. Fresh specimens from 6 primary and recurrent CPs were collected and subjected to cell culture using an explants method. ATRA at various concentrations was applied to recurrent CP cell culture, and cell growth was recorded and analyzed.**

■ RESULTS: **Immunohistochemistry, reverse transcription polymerase chain reaction, and western blot study showed that FABP5 was expressed significantly higher in recurrent tumors, whereas CRABPII was expressed significantly higher in primary tumors. The FABP5/CRABPII ratio was significantly higher in recurrent rather than primary tumors. Recurrent CP cells grew faster than primary cells, and ATRA induced cellular apoptosis and inhibited CP cell growth in a dose-dependent manner.**

■ CONCLUSIONS: **A high expression ratio between FABP5 and CRABPII may be related to CP tumor recurrence and ATRA could be a potential therapeutic agent for CP chemotherapy.**

INTRODUCTION

Craniopharyngioma (CP) is associated with frequent relapse, and as much as 60% of CPs could recur at 5 years after surgery.[1] Several factors, such as degree of resection and expression of certain molecules may be used to predict CP recurrence.[1] Recent studies have shown that all-*trans* retinoic acid (ATRA), an intrinsic derivative from vitamin A, can facilitate tumor growth or induce apoptosis at the physiologic level (10^{-9}M).[2-6] The paradoxic effect of ATRA is determined by the relative level between cellular retinoic acid binding protein II (CRABPII) and fatty acid binding protein 5 (FABP5).[5] With higher CRABPII expression, ATRA is directed to retinoic acid receptor α, activation of which leads to the classic anticancer signaling pathway; but with higher FABP5 expression, ATRA is directed to peroxisome proliferators activated receptor β/δ and the proproliferative pathway is activated.

Clinically, a high FABP5/CRABPII ratio has been related to gliomas and breast cancers with worse pathologic grading, higher recurrence, and shorter patient survival time.[7-9] In addition, retinoic acid receptors have been found in adamantinomatous CPs, which suggests that retinoic acid signaling pathways, possibly both of the opposing pathways, may exist in CPs.[10] Therefore, in the presence of physiologic level ATRA in human serum,[11] it is reasonable to assume that the FABP5/CRABPII expression

Key words
- Apoptosis
- CRABPII
- Craniopharyngioma
- FABP5
- Retinoic acid

Abbreviations and Acronyms
AC: Adamantinomatous craniopharyngioma
ATRA: All-*trans* retinoic acid
cDNA: Complementary DNA
CP: Craniopharyngioma
CRABPII: Cellular retinoic acid binding protein II
FABP5: Fatty acid binding protein 5
IHC: Immunohistochemistry
MTT: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide
PC: Papillary craniopharyngioma
RT-PCR: Reverse transcription polymerase chain reaction
SD: Standard deviation

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pattern may be an important determinant for CP recurrence and growth potential. In the present study, we examined the expression pattern of FABP5-CRABPII in both primary and recurrent CPs. Also, in our previous study, we showed that pharmacologic level ATRA inhibited primary CP cell growth. In the present study, we studied the effect of ATRA on recurrent CP cells.

METHODS

Patients and Study Design

Fifty patients with CP were enrolled in this retrospective study from September 2009 to March 2012. Their age ranged from 3 to 62 years, with mean age 32.9 years. There were 30 cases of adamantinomatous CPs (AC), 10 of which were recurrent. There were 20 cases of papillary CPs (PC), 5 of which were recurrent. Patients who underwent radiation or chemical therapy before surgery were excluded. The degree of resection was assessed using enhanced magnetic resonance imaging within 72 hours after surgery. Follow-up ranged from 8 to 12 months. This study was reviewed and approved by the ethics committee of West China Hospital, and all patients or their guardians gave informed consent.

Immunohistochemistry Analysis

Fresh tumor specimens were divided and preserved differently for immunohistochemistry (IHC), messenger RNA (mRNA), or protein extraction, and cell culture after confirmation of CP by frozen sectioning. For IHC, tumor specimens were preserved in 10% paraformaldehyde and then embedded in paraffin. Mouse anti-human FABP5 monoclonal antibodies were obtained from R&D Systems (Minneapolis, Minnesota, USA) and goat anti-human CRABPII polyclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, California, USA). Staining of 4-μm-thick sections for FABP5 and CRABPII (1:200 at 4°C overnight for primary antibodies and 1:1000 at 25°C for 2 hours for secondary antibodies) was performed using a standard streptavidin and peroxidase kit (Boster, Wuhan, Hubei, China) according to the manufacturer's instructions. Sections incubated without primary antibody were used as control. Ten randomly selected microscope fields for each slide (at 400×, Olympus BX51, Tokyo, Japan) were obtained with the same conditions (eg, white balance, exposure time, light brightness). Ten random high fields (400×) of each specimen were recorded and 100 cells were counted under each field and added. The percentage of immunopositive cells was calculated as the number of immunopositive cells/1000 × 100%, and mark A was assigned to each specimen as follows: <10%, 0; 10%–25%, 1; 25%–50%, 2; >50%, 3.

Brown staining was also classified in each high field as follows: no stain, 0; weak stain, 1; medium, 2; strong, 3. The staining points from 10 fields of the same specimen were added to generate an average score for each specimen, mark B. The 2 points for each specimen (mark A and B) were added to generate an immunoreactive point (mark C) for each specimen as follows: 0–1, negative, −; 2–4, weak positive, +; >5, strong positive, ++. The number of specimens with a different mark C in each group was counted and recorded.

Reverse Transcription Polymerase Chain Reaction Analysis

For reverse transcription polymerase chain reaction (RT-PCR) and western blot, fresh tumor specimens were preserved in liquid nitrogen immediately after resection. Total RNA was extracted using TRIzol reagent from 50 μg of tumor tissue (Invitrogen, Grand Island, New York, USA) according to the manufacturer's instructions. For reverse transcription, complementary DNA (cDNA) was generated using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada) in a 20-μL volume system. The cDNA was amplified with specific primers for human FABP5 and CRABPII (FABP5 sense 5′ ATG GCC ACA GTT CAG CAG CTG 3′, antisense 5′ CAG GTG ACA TTG TTC ATG AC3′; CRABPII sense 5′ GCC CAA CTT CTC TGG CAA CTG3′, antisense 5′ GTC TGC TCC TCA AAC TCC TCC C 3′). Human glyceraldehyde-3-phosphate dehydrogenase primers were used to normalize the quantity of PCR product in each condition (GADPH sense 5′ ACC ACA GTC CAT GCC ATC AC 3′, antisense 5′ TCC ACC ACC CTG TTG CTG TA 3′). The level of mRNA expression was quantified using integrated optical density in Quantity One 4.5 software for Windows (Bio-Rad, Hercules, California, USA). The ratio between FABP5 and CRABPII was calculated using the mean value of each specimen and they were added together to yield a mean value of the ratio in each group, which was recorded as mean ± standard deviation (SD).

Western Blotting Analysis

Fresh tumor tissue (100 μg) was lysed in radioimmunoprecipitation assay buffer (50 mM Tris, pH 7.6; 150 mM NaCl; 1% Nonidet P-40) containing protease inhibitor cocktails (Roche, Basel, Switzerland) for 10 minutes on ice. The total lysate was loaded for gel electrophoresis and transferred onto a 0.22-μm nitrocellulose membrane. After blocking with TBS buffer with nonfat powdered milk (5%) at 4°C overnight, the membrane was incubated using 1:500 diluted mouse anti-human FABP5 monoclonal antibodies (R&D) at 4°C overnight. Blots were developed using secondary antibodies at a dilution of 1:5000 and visualized using an enhanced chemiluminescent method according to the manufacturer's instructions (Boster). Immunoblots against CRABPII and β-actin were performed in a similar manner. Goat anti-human CRABPII polyclonal antibodies were purchased from Santa Cruz Biotechnology. Mouse anti-human β-actin monoclonal antibodies were purchased from Boster. Proteins were quantified using integrated optical density in Quantity One 4.5 software for Windows (Bio-Rad). The ratio between FABP5 and CRABPII was calculated using the mean value of each specimen and they were added together to yield a mean value of the ratio in each group, which was recorded as mean ± SD.

Recurrent CP Cell Growth and ATRA Treatment

Fresh tumor tissue from 6 cases each of recurrent and primary specimens was obtained and quickly preserved in defined keratinocyte serum-free medium (Invitrogen, Grand Island, New York, USA) at 4°C. Primary cell cultures were established using an explant method as previously described.[12] Tumor was dissected into pieces of 3 mm and placed on the bottom of a standard 25-cm² flask, which was placed vertically in a standard incubator. Six hours later, the flask was turned to the normal position and cultured. When cells reached 50%–60% confluency, the