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RETINOBLASTOMA: CLINICAL AND IMMUNOCYTOCHEMICAL OBSERVATIONS

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INDEXING WORDS

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SYNOPSIS

The clinical features of three cases of retinoblastoma and the histopathgological and immunocytochemical findings of the retinoblastoma specimens of these patients are described. The other eye of each patient was normal. There was no family history of retinoblastoma. Fundal examination disclosed white masses in the posterior segments of the eyes. B-scan ultrasonography showed echo dense areas in the masses. Orbital tomograms showed no evidence of tissue densities. There was neither extraocular extension nor optic nerve involvement. Immunocytochemistry of paraffin-embedded tissues of the neoplasms revealed immunoreactivity with antibodies against S-100 protein, glial fibrillary acidic protein(GFAP), vimentin, neuron-specific enolase(NSE) and *bcl*-2. S-100, GFAP and vimentin were positive only

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for glial elements(reactive astrocytes) and, NSE and *bcl*-2 for almost all tumor cells.

These findings support the previous reports that the majority of retinoblastomas are sporadic. The results of this study also support the views that retinoblastomas are composed of neuron-committed cells.

INTRODUCTION

Retinoblastoma is the most common tumor of the retina and arises from the sensory part of the retina. It may be unilateral or bilateral. In Japan, 34% of the cases are bilateral.[2] In the literature it has been described that 40% of the cases are hereditary and always bilateral and, 60% of the cases are non-hereditary affecting only one eye.[3] The clinical features of retinoblastoma have been well studied and these studies have shown a number of cellular changes consisting of well-differentiated, poorly differentiated or undifferentiated tumors.[12,14,16] In recent years the origin of this tumor has provoked profound interest. Numerous culture and immunocytochemical studies have shown that the tumor cells may originate from multipotential precursor glial and neuronal cells of the retina or from neuronal cells alone.[4-10,13] Here we describe some informations on clinical and immunocytochemical studies of retinoblastoma. In immunocytochemistry S-100 protein, GFAP, and vimentin were used as glial markers and, NSE and *bcl*-2 as neuronal markers.

CLINICAL SUMMARY

Three cases of retinoblastoma from the Registry of Department of Ophthalmology, Kobe University School of Medicine constitute the basis of this study.

Case-1

The first case was a male child of 1-year, 6-month-old and was consulted for the history of leukocoria in the left eye for approximately 3 months. The patient did not give any history of systemic or eye diseases. His family members were free from any major eye diseases or neoplasms.

Visual acuity was 0.1 in the right eye(measured by Preferential Looking Method) and finger movement perception in the left eye without glasses. No abnormality was found in the eyelids, extraocular muscles, conjunctiva, cornea and sclera of both eyes. The anterior chamber was normal in depth without any cell and there was no lental opacity in each eye. A white mass was seen to extend from the supero-posterior part of the left fundus to the posterior surface of the lens. Pupils were circular and reactive to light and accommodation.

On laboratory examination, no abnormality was detected in blood, urine or in the liver. Carcinoembryonic antigen and α-feto-protein tests were negative. A B-scan ultrasound examination showed an echodense area of the tumor mass. Orbital CT showed no abnormality in both eyes. An enucleation was done in treating the lesion.

Case-2

The second case was a 3-year-old girl with a history of leukocoria in the right eye for about 2 months. There was no history of systemic or other illnesses. Family history was positive only for diabetes mellitus.

Visual acuity without aid was 0.1 in the right eye and 1.0 in the left eye. The findings of the anterior segment examinations were same as the first case. Upon funduscopy a whitish mass was seen to cover the lower quadrant of the retina of right eye.

The results of laboratory examinations showed the same findings as the first case with an echo-dense area of the mass on B-scan. The patient underwent an uncomplicated enucleation of the right eye.

Case-3

The third case was a boy of 2-year-old and was consulted for leukocoria in the right eye for approximately 4 months duration. The personal and family histories continued to yield the same findings as the first case.

The acuity of vision was perception of finger movement in the right eye and 0.075 in the left eye(by Preferential Looking Method) without correction. The anterior segments were normal. On ophthalmoscopy a large mass was detected behind the lens of right eye and the fundus was not clearly visible.

The findings of laboratory tests were same like the previous two cases. B-scan ultrasonography also showed an echo-dense area of the mass. An enucleation was performed in the right eye to treat the neoplasm.

METHODS

(FOR HISTOPATHOLOGY AND IMMUNOCYTOCHEMISTRY)

Sections of 5μm thick formalin-fixed and paraffin-embedded retinoblastoma specimens were used for routine hematoxylin-eosin(Fig.1) and immunocytochemical stainings(Figs.2-5). For immunocytochemical study sections were mounted on slides treated with poly-*L*-lysine. They were processed by the labelled streptoavidin biotin(LSAB) method. Primary antibodies against the following antigens were used: S-100 protein(polyclonal, 1:400), GFAP(monoclonal, 1:50),

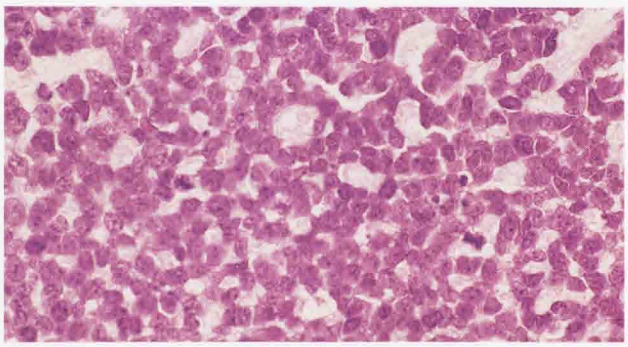

Fig.1. Moderately differentiated area of retinoblastoma containing Flexner-Wintersteiner rosettes(HE, x400).

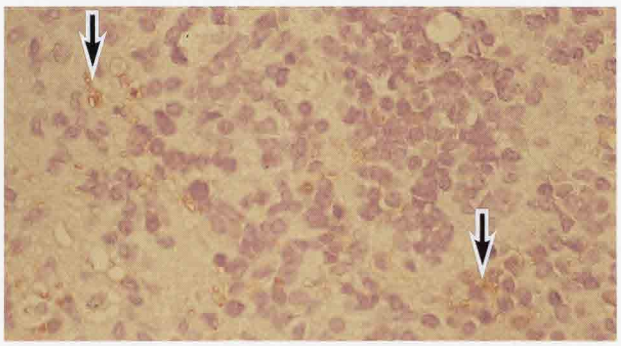

Fig.2. Scattered immunoreactivity of S-100 protein is seen in the reactive astrocytes of retinoblastoma(arrows) (LSAB, x400).

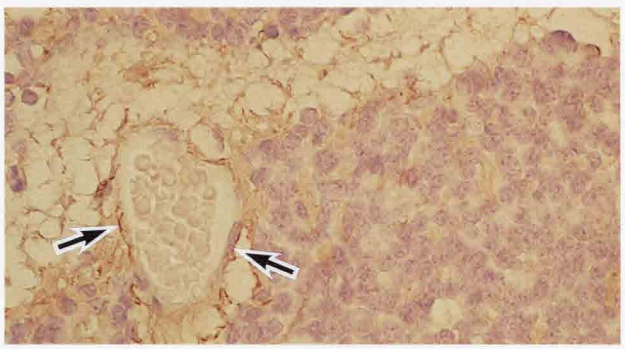

Fig.3. GFAP positive perivascular glial cells are evident in the retinoblastoma(arrows) (LSAB, x400).

vimentin(monoclonal, 1:250), NSE(polyclonal, 1:100) and *bcl*-2(monoclonal, 1:40). These were procured from Dako, Glostrup, Denmark. Negative controls were provided by omission of the primary antibodies and positive controls by the morphologically normal appearing retina in retinoblastoma specimens.

RESULTS

Macroscopic findings

Case-I: The eye measured 20x20x17mm. The eye was firm in consistancy and was sectioned in the horizontal plane. The anterior chamber was normal. The lens was in proper place. The ciliary body was normal and the vitreous was liquid. The tumor mass measured 15x8x8mm and was seen to occupy the upper half of the eyeball. The entire mass was examined and it was solid in nature. No infiltration was observed in optic nerve or in sclera.

Case-II: The eye measured 21x21x18mm and it was opened in the horizontal plane. The anterior chamber was normal and its angle was open. The lens was normal in place. The ciliary body was found to be normal. The retina was infiltrated by a large whitish tumor mass extending from the posterior pole of the globe to the ora serrata. The tumor measured 18x18x10mm. There was vitreous seeding. The optic nerve and the choroid were unremarkable.

Case-III: The eyeball measured 21x21x19mm. The globe partially transmitted light. The eye was sectioned in the horizontal plane. The retina was infiltrated by a moderate size of tumor measuring approximately 10x6x6mm. The vitreous was liquid. Infiltration was not observed in sclera, ciliary body or optic nerve.

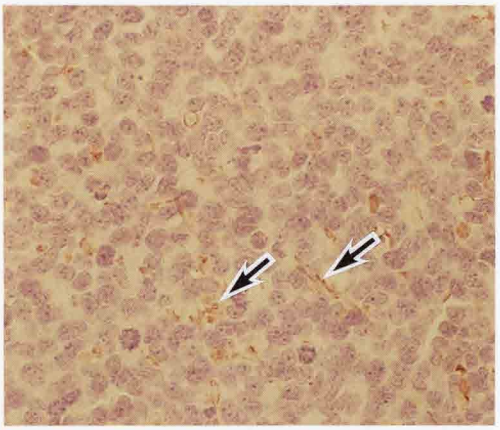

Fig.4. Vimentin positive astrocytes are dispersed among the negatively reactive tumor cells(arrows) (LSAB, x400).

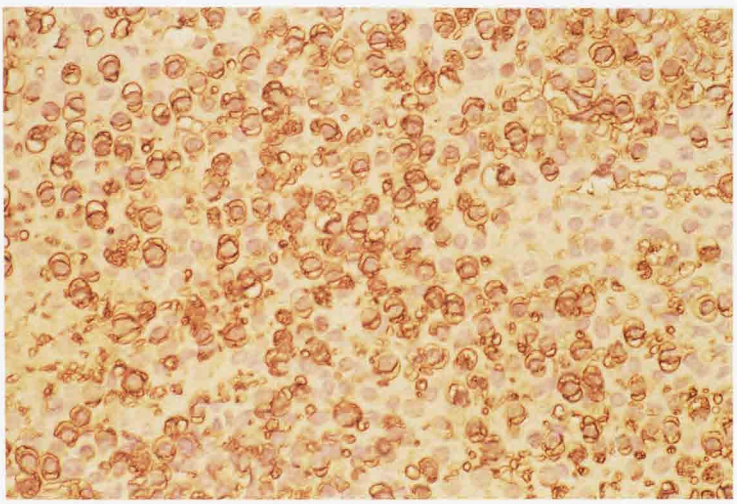

Fig.5. Extensive neuronal differentiation in the tumor with anti-NSE immunoreactivity(LSAB, x400).

Microscopic findings

On microscopic examination the cornea, sclera, trabecular meshwork, iris, lens and ciliary body were found to be normal in all three cases. The tumors displayed endophytic appearences. There were areas of tumor necrosis in case-II and case-III. The neoplastic cells were mostly poorly differentiated in all cases except some areas of Flexner-Wintersteiner rosettes in cases I and II(Fig.1). The rosettes were composed of one or more layers of cuboidal cells surrounding a central lumen. No rosette was found in case-III. Cases I and II were diagnosed as moderately differentiated retinoblastoma and case III as poorly differentiated retinoblastoma.

Immunocytochemical stainings showed positive reactions of S-100 protein(Fig.2), GFAP(Fig.3) and vimentin(Fig.4) for reactive astrocytes. These reactivities were found predominantly around vessels. The neoplastic cells showed strong reactions with anti-NSE(Fig.5) and weak reactions with anti-*bcl*-2.

DISCUSSION

Retinoblastoma is the most common ocular malignant neoplasm of infancy and childhood. It is the first malignant neoplasm to be identified with a tumor suppressor gene, *Rb1* and its protein product $p110^{Rb1}$ has been isolated.[1] This neoplasm is most probably derived from embryonal retinal cells and can be differentiated in the form of Flexner-Wintersteiner rosettes and fleurettes.[16] The histologic features of retinoblastoma are variable. Some tumors may show marked necrosis and prominent foci of calcification. A few neoplasms display areas of glial differentiations.[16] Cell cultures of retinoblastoma have proved that it can exibit glial and neuronal differentiation.[11] In general more

anaplastic tumors are more sensitive to radiation and the more benign tumors are resistant to radiation.[15] Clinically our cases presented with leukocoria with relatively slow progression. Since there was no family or hereditary history, we classify our cases as sporadic retinoblastomas.

The histogenesis of retinoblastoma is still disputed. In our study S-100 protein, GFAP and vimentin showed strong reactions in the glial elements and we interpreted these immunoreactivities as reactive astrocytes. These astrocytes were distributed 1) predominantly around vessels and 2) sparsely among the tumor cells. Tumor cells themselves were devoid of the reaction. We have shown that the retinoblastoma tumor cells exhibit neuronal differentiation with staining of the tumor cells with NSE and *bcl*-2. NSE reacted intensely while *bcl*-2 weakly.

The data of this study supports the views that most of the cases of retinoblastoma are sporadic. Based on the staining with glial and neuronal markers, the data also indicates that these tumors are predominantly neuronal tumors.

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