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PATHOLOGICAL AND HISTOLOGICAL STUDY ON RETINOBLASTOMA

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INDEX WORDS

retinoblastoma(Rb); pathological study; MIB-1

SYNOPSIS

We studied 30 cases of retinoblastoma (Rb) pathologically and histochemically. Rb cases were classified into two types, 18 cases of differentiated (D-type) and 12 undifferentiated type (U-type) according to International Classification of Diseases for Oncology(1990,WHO). D-type of Rb is found more commonly in younger infants less than 1 year, but U-type found more frequently in aged infants from 2 to 4 years. D-type Rb showed more marked necrotic areas, degeneration and calcification. Immunohistochemical stains were carried out in 21 cases of Rb by use of MIB-1, PCNA, bcl-2, Fas, BAX, NF, SP and Tunel stains, respectively. MIB-1 is a good marker of proliferation of Rb cells and its high positivity correlates closely to more extensive necrotic areas of Rb cases. There are only a few positive cells by Tunel stain suggesting apoptotic cells, but diffuse positive cells by bcl-2 and Fas and completely negative by BAX gene. From these results, it can be seen that differentiation of Rb cells may be affected by bcl-2 and Fas but not by BAX gene.

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INTRODUCTION

Retinoblastoma(Rb) is a malignant tumor arising in the retina and occupying 2 ~ 4% of pediatric neoplasmas[1,5], whereas its pathological background has not yet been understood well enough to date[12,14,16]. Pathological features of Rb are various and are characterised as involveing two opposite biological phenomena, that is, cellular proliferation and differentiation, in addition to necrosis, degeneration and calcification. We then tried to clarify these complicated phenomena by use of immunohistochemical stains. MIB-1 and PCNA were chosen for markers of proliferation of Rb cells, and Tunel stain as a marker of apoptosis, bcl-2 as its suppressor marker[6]. Fas antigen as a marker of mediating apoptotic signal[13] and BAX as an enhancing apoptotic work, and NF and SP as markers of cytoplasmic or membranous differentiation of Rb cells.

MATERIALS AND METHODS

Thirty cases of Rb were studied pathologically, which were diagnosed as Rb and resected at departments of ophthalmology of Kobe University Hospital and Hyogo Prefectural Children's Hospital. Those are composed of 16 male and 14 female eyes, from 1 month to 4 years old (average 1 year and 6 months). Those resected eyes were fixed in 10% buffered formalin solution and cut at the maximum diameter of the Rb mass, and were parafinized, and cut at 4 μ in thickness. Histological specimens were stained by HE stain,routinely.

Histochemical study was done in 23 Rb cases. Histological sections were autoclaved in the 0.01M citrated buffered solution, and stained by MIB-1 antibody by use of LSAB method. PCNA, bcl-2, BAX, Fas, NF, SP, Tunel stains were also done by ABC-procedure, and were compared with each other histochemically.

A. Observation of Rb from the age at time of occurrence

Thirty Rb cases were classified into two categories according to ICD-O(WHO, 1990), 18 differentiated type (DT) and 12 undifferentiated type(UT)[11]. Furthermore, Rb cases were subdivided to three groups: group-a (11 cases) from 1 to 6 months, group-b (9 cases) from 7 months to 1 year and 9 months, group-c (10 cases) from 2 to 4 years, and examined with each other pathologically.

B. Observation of Rb from HE specimens

In the histological examination Rb cases showing rosette formation more than 60% of whole area of Rb specimen were decided as ++, 30± 29% were to be +, none to be U type. In the estimation of necrosis areas Rb cases showing more than 50% of whole area to be ++, 30±19% to be +, less than 10% to be −. The estimation of calcification degree was done as follows: more than 20% of whole areas to be ++, 10±9% to be +, and none to be −.

C. Observation of Rb from histochemical stains

Histochemical stains were estimated as follows: strongly positive to be ++, positive to be +, and negative to be −, respectively. Positive sites of stain of Rb cells were diagnosed intra-nuclear, cytoplasmic, membranous positive, respectively. Histochemical results were compared each other between D- and U-type, or a-~c- groups, and cases to cases. The staining pattern of intact areas of the retina of resected eyes was studied as control of stain.

RESULTS

A. Rb features observed from occurrence age (Table I)

Eleven cases of a-group are composed of 8 D-type Rb(male 5, female 3), and of 3 U-type(male 3, female 0), and 9 cases of b-group are composed of 4 D-type(male 1, female 3), and 6 U-type(male 2, female 4), 6 cases of c-group comprise of 2 males and 4 females. Male to female ratio of D-type is 8:10, and of U-type 8:4, showing male preponderance. In our study, it is suggested that as the age in Rb cases becomes lower, more incidence of cases of D-type appears, but in higher age more U-type of Rb. Rosette formation in D-type Rb showed a tendency to increase in its number as aged from + to ++.

B. Rb features observed by HE stain (Table I)

In cases of D-type Rb there are extensive necrotic and degenerative areas(Figure 1), and calcification, but rather small areas in U-type Rb(Figure 2). However some cases of U-type Rb showed massive hemorrhage without necrosis or calcification. The areas of calcification were only a few , which were seen in the center of necrotic areas(Figure 3). There are no calcification areas in vascular walls in Rb areas.

C. Rb features observed by immunohistochemical stains (Table II)

Most of Rb cases showed more than 50% positivity by MIB-1 stain, showing black coloured nuclei. MIB-1 staining pattern is very clear

Table I . Cases of Rb and pathological findings.

			Histological Features				
			Rb. Histological Features				
Gr	Nr	AGE/SEX	dif	ros	nec	cal	o.n.i
a group	1	1M/M	D	+	++	-	+
	2	1M/F	D	+	+	+	-
	3	2M/M	D	++	++	+	-
	4	2M/M	D	+	+		-
	5	2M/M	U	-	-	-	+
	6	3M/F	D	+	+	+	-
	7	4M/M	U	-	++	-	-
	8	4M/M	U	-	+	+	-
	9	5M/M	D	+	+	-	-
	10	5M/M	D	+	++		-
	11	5M/F	D	++	++	++	-
b group	12	7M/M	U	-	++	-	-
	13	7M/F	D	+	++	+	+
	14	9M/M	U	-	+	-	-
	15	11M/M	U	-	+	-	-
	16	1Y/F	D	+	++		-
	17	1Y/F	D	++	++	++	-
	18	1Y1M/M	D	++	-	-	-
	19	1Y5M/F	D	+	++	-	-
	20	1Y9M/M	D	+	++	+	-
c group	21	2Y/F	D	+	++	-	-
	22	2Y3M/M	D	+	++	-	-
	23	2Y5M/F	U	-	++	+	+
	24	2Y7M/F	U	-	++	+	-
	25	3Y/F	D	+	++	++	-
	26	3Y/F	U	-	+	+	-
	27	3Y6M/F	D	++	++	-	-
	28	3Y8M/M	U	++			-
	29	4Y/M	U	-	++	-	-
	30	4Y/F	U	-	+	+	-

D : differentiated type of Rb

U : Undifferentiated type of Rb

ros:rosette formation

o.n.i : Optic nerve invasion

dif : differentiation

nec : necrosis

Cal : Calcification

Table II . Immunohistochemical study of Rb.

Nr	MIB-1	PCNA	Tunel	Fas	bcl-2	BAX	NF	SP
1	++	++	-	-	-	-	+	++
3	-	+	-	+	+	-	+	++
5	++	+	-	-	++	-	+	++
7	++			-	++	-		++
8	++	++	-	++	+	-	-	++
9	+	+	+	-	+	-	++	++
11	++	++	-	+	-	-	++	++
12	++			++	+	-		++
13	++			+	++	-	++	+
14	++			++	+	-	+	
17	++	+	-	++	+	-	+	+
18	+	+		++		-	+	++
20	++	-	-	-	++	-	+	+
21				++		-	-	
22	++	+	-	++	++	-	+	++
23	++			++	+	-	+	++
24	++			++		-	+	++
25	-			++		-	+	+
26	-			++		-	+	+
27	++	+		++	+	-	+	++
28	++	++	-	-	-	-		++
29	-			++	-	-	+	-
30	++	++	-	++	+	-	+	++

PCNA: Proliferative Cell Nuclear Antigen
Tunel : Terminal deoxy-nucleotidyl transferase-mediated d-UTP-biotin nick end labeling methods
NF:Neurofilament SP:Synaptophysin

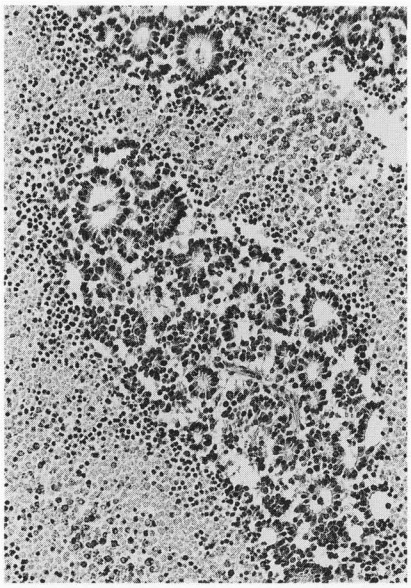


Fig. 1.

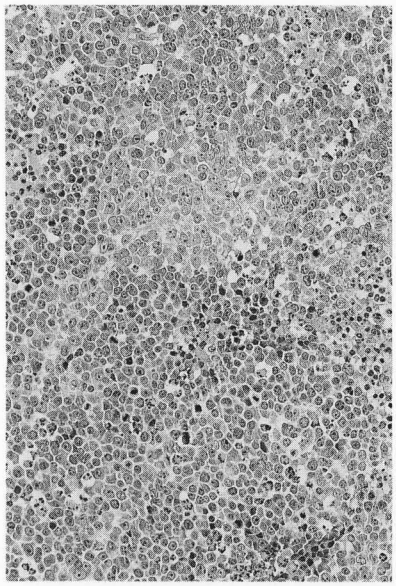


Fig. 2.

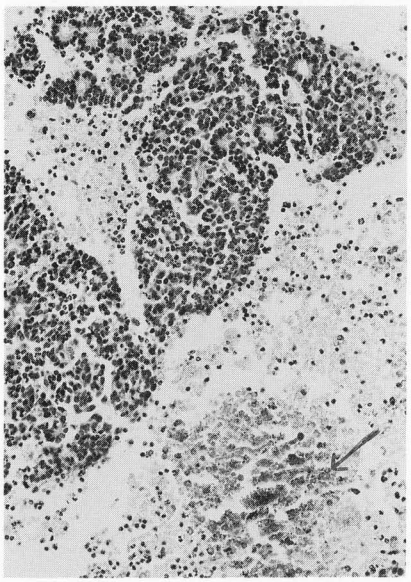
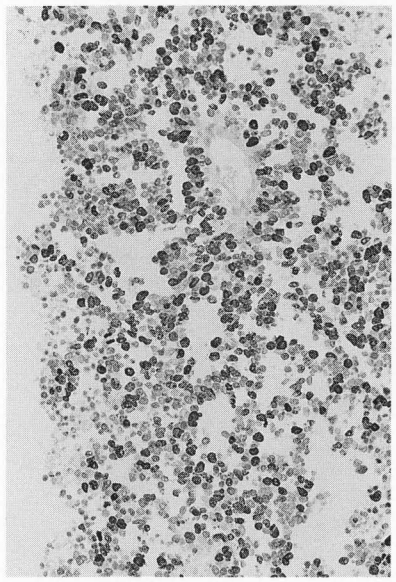


Fig. 3.



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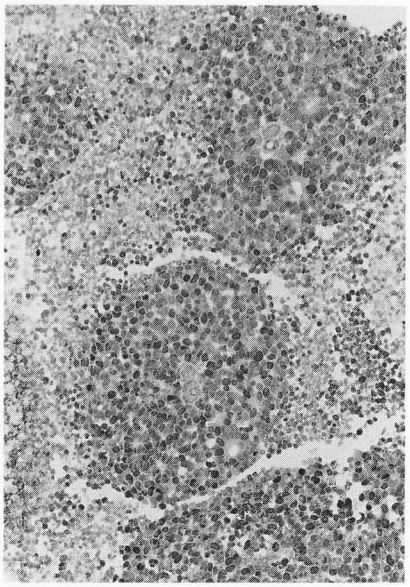

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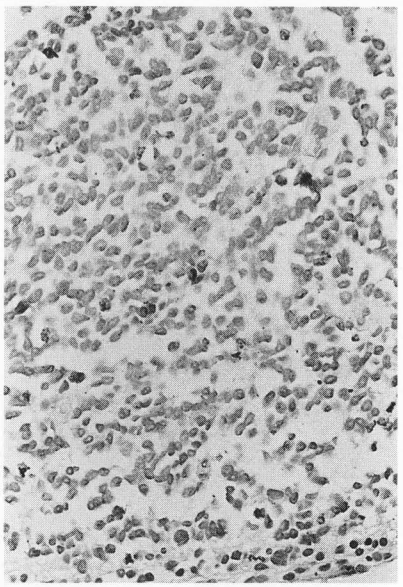

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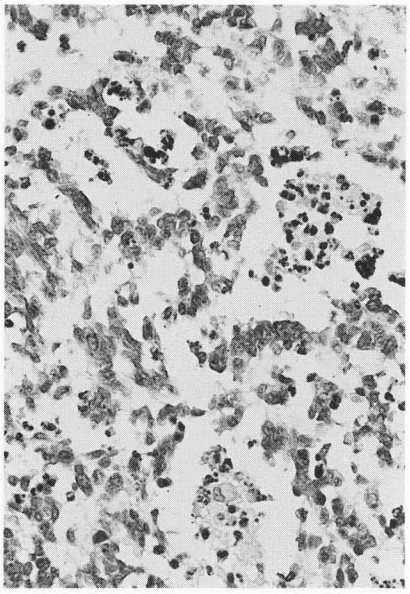

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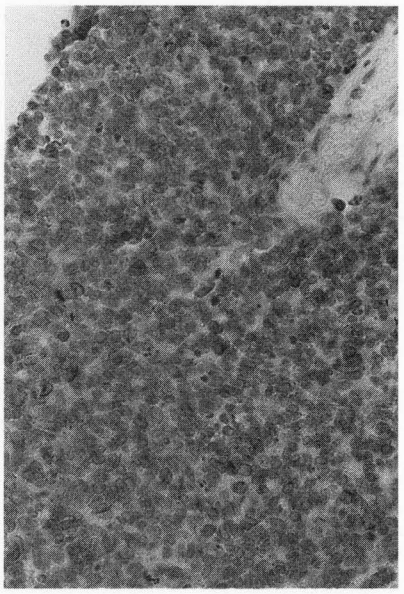

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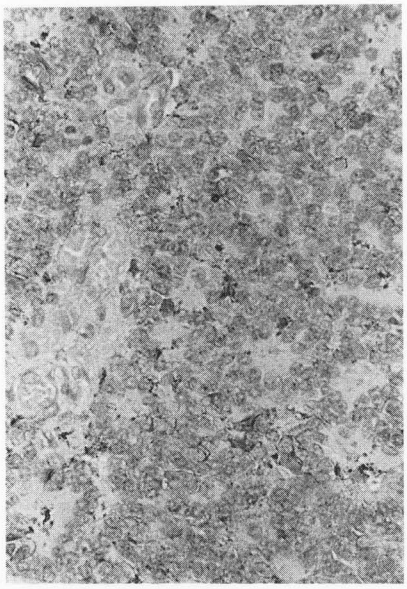

Fig. 9.

Fig.1. D-type Rb, Case 3; 2 months, male; There are many rosette formations and larger of necrosis. HE, ×200.

Fig.2. U-type Rb, Case 8; 4 months, male; There are no rosette formation and a few necrotic foci. HE, ×200.

Fig.3. D-type Rb, Case 11; 5 months, female; There are larger necrotic areas and calcification(arrow). HE, ×200.

Fig.4. D-type Rb, Case 27; 3 year and 6 months, male; Nuclei of Rb cells are positively stained showing black colour. MIB-1 stain, ×200.

Fig.5. D-type Rb, Case 1; 1 month, male, Nuclei of Rb cells are positively stained by black colour. PCNA stain, ×200.

Fig.6. D-type Rb, Case 9; 5 months, male, Positive cells are a few in number and their nuclei are black colour. Tunel stain, ×400.

Fig.7. U-type Rb, Case 30; 4 years, female, Rb cells are stained positively in cytoplasms and membranes. Fas stain, ×400.

Fig.8. D-type Rb, Case 22; 2 years and 3 months, male,Rb cells are positively stained in their membranes,bcl-2 stain ×400.

Fig.9. D-type Rb, Case 18; 1 years and 1 month, male, Rb cells are positive especially in their membrane and faintly positive in their cytoplasms. SP stain, ×400.

(Figure 4), in many cases, the difference of staining pattern by MIB-1 is not found between D-type and U-type Rb, and between male and female , and between a- ~ c-groups. MIB-1 positive cells are highly seen in accordance with an increase of necrotic areas of Rb. However, there are any correlations to calcification degree. On the other hand, PCNA stain is also good staining for the marker of proliferation of Rb cells in some case (Figure 5), but most of cases of Rb are +, and rather difficult to diagnose its staining degree clearly. Positivity ratio by PCNA is different in male and female, suggesting male preponderance. In the Tunel stain there are only a few positive cells (Figure 6), which are distributed only 5% of Rb cells in one case. Fas stain showed positivity in the cytoplasm and the membrane, slightly but diffusely in Rb cells(Figure 7). The bcl-2 stain was positively and diffusely(Figure 8) in the membrane of Rb cells, but all negative by BAX stain. SP and NF stain (Figure 9) and are positive in the membrane and the cytoplasm of Rb cells diffusely, but faintly positive by NF stain.

DISCUSSION

Pathogenesis and pathological background of Rb has not yet been clarified until now. Concerning its pathogenesis there are many disputable subjects such as a theory by glioma of the retinae by Virchow(1864), Rb origin considering to be glial-supporting cells of the retina by Bennedict et. al.(1941), and another Rb theory arising from photoreceptor cells of the retina by Tso, et. al.[18].

On the other hand, the pathological characteristics of Rb are various and have two opposite phenomena such as proliferative or differentiative tendencies, necrosis and degeneration, calcification and others. These Rb's histological variance in case and case may connect to each patient's prognosis of Rb,whereas there are few reports clarifying the backgrounds of various pathological findings[10]. In this study differentiative tendency was studied in D-type Rb by the estimation how many rosette formation is found in histological specimen. Tso et. al, reported the comparison of two types Rb, U-type Rb and D-type with photoreceptor differentiation[17], and the former showed hyper-proliferation of Rb cells and much necrosis and calcification with high formation of rosette of Flexner-Wintersteiner type,but the latter represented neither necrosis nor calcification,but showed the formation of fleurettes. They concluded U-type Rb to be malignant, and Rb with

photoreceptor differentiation to be relatively benign. In our study four cases of Rb invaded into the optic nerve,which were composed of 2 cases of D- and 2 ones of U-type. Our examined U-type of Rb exhibited few fleurette formation, though we have not tried to observe electron-microscopic observation these Rb cases.

On the other hand, proliferative tendencies of Rb is studied by markers of MIB-1 and PCNA[15]. MIB-1 antibody against Ki-67 antigen is known to depend on the binding of a nuclear protein to the DNA, and Ki-67 antigen is a marker for proliferative cells[2,3,8]. In our results MIB-1[9] activity correlated closely to the high degree of necrotic tendencies, and its positivity is seen similarly in both Rb of D-type and U-type. An another marker of PCNA(Proliferative Cell Nuclear Antigen) is also identified a protein (Ki-67) which exsist free or associated with DNA as evidenced by DNA digestion of cells before or after immunolabeling with Ki-67[19]. PCNA positive cells is thought to exhibit S-stage cells, but the decision of positive degree is rather difficult than that by MIB-1. Although there are many necrotic and degenerative areas especially in D-type Rb, there are only a few apototic cells among Rb cells by Tunel stain. It may be correlated to diffuse positivity in the membrane and cytoplasm of Rb by Fas antigen is also related to bcl-2 and to interact apotosis signaling through CD 95(Fas/ARO-1)[6,13]. Negativity in all cases examined by BAX may be also correlated to the appearance of a few apototic cells in Rb cells, which is thought to enhance apotic movement.

The markers of Rb cells' membrane or cytoplasm were chosen NF and SP stains. In conclusion, the better marker is SP but not NF.

Finally, pathogenesis of Rb concerning to Rb-1 gene[4,7] are increasing now, which is a tumor suppressor gene and located at 13q14. Loss of function of mutation of Rb-1 gene may cause Rb occurrence same as osteosarcoma, lung cancer, hepatoma, breast cancer and urinary bladder cancer etc. Almost all cases of Rb show abnormality of Rb-1 gene, especially E2F protein distortior regulating transcriptional function. PCR-SSCP methods of Rb-1 is useful for screening of Rb patients in the near future.

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