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Expression of p53 and Mdm2 in Human Retinoblastoma

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Abstract

Retinoblastoma (Rb) is a malignant tumor of the developing retina. Retinoblastomas arise within the retina to form soft white tumor masses that expand within the vitreous cavity of the eye, and if untreated, mostly invade through the sclera, or along the optic nerve, or hematogenously via the choroids, in turn posing a risk for metastasis. However, it is an uncommon tumor accounting for 3% of all childhood malignancies in developed countries. Very few studies on p53 expression in retinoblastoma patient's inspite of its crucial role in tumorigenesis are officially recorded. Therefore, we detected the expression of p53 and mdm2 in retinoblastoma by immunohistochemistry, analyzed their relationships with clinicopathological parameters and then investigated the influence on the biological activity of retinoblastoma.

Keywords: Retinoblastoma (Rb); Tumor; p53; mdm2

Introduction

Retinoblastoma (Rb) is a malignant tumor of the developing retina that occurs in children, usually before age of five years [1]. However, it is an uncommon tumor accounting for 3% of all childhood malignancies in developed countries [2]. There is indirect evidence that it may be more frequent in some developing areas, such as Latin America, Africa, and India [3]. The estimated incidence of retinoblastoma in India is about 2000 a year [4]. An epidemiological study of pediatric tumors at a tertiary care centre in India revealed that retinoblastoma affected 14% of the study population. Retinoblastomas arise within the retina to form soft white tumor masses that expand within the vitreous cavity of the eye, and if untreated, mostly invade through the sclera, or along the optic nerve, or hematogenously via the choroids, in turn posing a risk for metastasis [5]. Current management modalities for retinoblastoma include enucleation, external beam radiotherapy, plaque radiotherapy, laser photocoagulation, hyperthermia and cryotherapy [6].

With the development of techniques, molecular biology research has indicated that p53 tumor suppressor gene plays an important role in DNA transcription, cell growth and proliferation, DNA repair and various metabolic processes. p53 abnormalities such as gene mutation and depletion can lead to the altered intracellular signal transduction pathways as well as loss of the regulation of cell growth, apoptosis, and DNA repair, which are responsible for carcinogenesis [7]. Murine double minute gene 2 (Mdm2), is an oncogene (the corresponding human homologous gene is hdm2). Mdm2 protein can be combined with p53 to inhibit p53 function of growth supervision, leading to cell overgrowth into tumor [8]. There are not many studies on p53 expression in retinoblastoma patient's inspite of its crucial role in tumorigenesis. Knowledge on Mdm2 expression in retinoblastoma patients is also not adequate. Therefore, we detected the expression of p53 and mdm2 in retinoblastoma by immunohistochemistry, analyzed their relationships with clinicopathological parameters and then investigated the influence on the biological activity of retinoblastoma. Ours seems to be the first report to demonstrate the p53 and Mdm2 expression and their correlation with histopathological features.

Materials and Methods

The specimens of patients with retinoblastoma treated by enucleation, either as a form of primary treatment or because of conservative treatment failure, were included in the study. All human retinoblastoma tissue samples were obtained in accordance with the tenets of the Declaration of Helsinki. Paraffin embedded blocks from 60 cases derived from enucleation of retinoblastomas was used for immunohistochemistry. The tumors were classified into two groups: group 1 (n=49), tumour samples obtained from enucleated eyes as a part of the treatment and the patients were not subjected to either preoperative or postoperative chemotherapy; group 2 (n=11), tumor samples obtained from enucleated eyes of patients who received preoperative chemotherapy.

Antibodies and chemicals

For the detection of p53 protein, mouse monoclonal anti-p53 antibody (clone DO-7, Santacruz Biotechnology, USA) was used. Primary mouse monoclonal anti-Mdm2 antibody (SMP14, Santacruz Biotechnology, USA) was used for Mdm2 staining. The immunostaining kit (Thermo Scientific, USA) was used for immunohistochemical detection.

Histopathological features

Retinoblastomas were graded microscopically into two groups according to the predominant pattern of differentiation. All tumor slides were reviewed and examined for invasion of choroid, optic nerve, and orbital invasion. Choroidal invasion was classified as either focal invasion or diffuse invasion of the choroid. For optic nerve invasion, postlaminar involvement and invasion of the surgical end of the optic nerve were considered. Invasions were expressed in progressive grades as moderate and strong.

Immunohistochemistry

The immunohistochemical expression of p53 and Mdm2 consisted

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of several steps. Four micrometer thick specimen sections were deparaffinized with xylene and rehydrated through graded ethanol washes. Endogenous peroxidase activity was blocked by a 10 min wash of 3% hydrogen peroxide in methanol. To optimize immunostaining, antigen exposure was performed by microwave heating of the sections in citrate buffer (pH 6.0) for 15 minutes. Nonspecific protein binding was blocked using a 1% bovine serum albumin (BSA)/ Tris-buffered saline (TBS, pH 7.6) solution during a 30 min wash. Sections were incubated with primary antibody. After 1 hour incubation with the primary antibody at room temperature, sections were washed and incubated with biotinylated secondary antibody (Labvision, USA) for 30 min. Immunostaining visualization was enabled by the 3, 3-Diaminobenzidine (DAB) chromogen. Sections were lightly counterstained with hematoxylin. Breast carcinoma sections were used as positive control for p53 and Mdm2 expression. Immunoreactivity was scored as the number of positive tumor cells over total tumor cells. Percentage scores were subsequently categorized using the 0% cutoff as negative, cutoff of ≤ 10% as moderate and ≥ 10% as strong expression. .

Statistical analysis

The Mann-Whitney test was used to analyze the relation between the percentage of p53 and Mdm2 postive tumors and their invasion and differentiation. Pearson Chi-square test was used to see the statistical significance between parameters. Statistical analysis was performed on computer (SPSS ver. 11.5; SPSS, Chicago, IL).

Results

We have studied tumor samples of 60 retinoblastoma patients having mean age of 2.8 ± 1.85 years (Table 1). The sample size comprised of 60 eyes enucleated for retinoblastoma from 38 male patients (63.3%) and 22 female patients (36.7%). Twenty two (36.7%) of the 60 cases were bilateral. Histopathological assessment of tumors revealed that 8 (13.3%) tumors were well differentiated while rest was categorized as poorly differentiated. Of the 60 eyes, 11 (18.3%) had a history of prior treatment i.e. chemotherapy while 49 (81.7%) had received no prior treatment. The incidence of high-risk histopathologic features was reported in 38 (64.4%) cases (Table 1).

Although both groups had advanced disease, there was no significant difference between the groups in variables such as age (p=0.67), sex (p=0.82) and tumor laterality (uni- or bilateral) was observed (p=0.74).

There were 22 (36.7%) tumors with no high risk features i.e. invasion of optic nerve, choroid, ciliary body and sclera while 38 (63.3%) tumors having high risk features (Table 2). Among the 38 tumors with invasion, 24 (40%) had optic nerve invasion. There were 21 (35%) tumors with choroidal invasion. There were 12 (20%) tumors with invasion of both choroid and optic nerve. The cases have shown a correlation between degree of differentiation and high risk features. Six out of 8 (75%) well differentiated tumors have not shown any high risk feature (p=0.04). Severe necrotic area was reported in 22 out of 38 (57.9%) patients having high risk features.

Considering all the patients, p53 expression was observed in 21 (35%) of the tumors (Figures 1A and 1B). Six out of these 21 (28.6%) cases have shown strong positivity for p53. Three group 2 patients have shown mild immunoreactivity for p53 while no patient had strong p53 expression. Sixteen high risk patients expressed p53, out of which 4 (25%) have shown strong positivity. Though there was no correlation in p53 and high risk features statistically. Positive Mdm2 expression was reported in 21 (35%) of the tumors (Figures 1C and 1D). Strong immunoreactivity against Mdm2 was reported in 3 (14.3%) of 21 cases. Five patients from group 2 have shown Mdm2 immunopositivity out of which only one was strong expression. Positive expression of Mdm2 was shown by 16 patients showing high risk features though only 3 (18.75%) cases were strongly positive. There were 6 (10%) patients who were positive for both p53 and mdm2. Strong expression of both p53 and Mdm2 was not reported in any of cases. Out of these 6 patients, four (75%) have also shown high risk features (r=0.78, p=0.03).

Clinical outcome

Patients were followed up by minimum 16 months. There was recurrence in two high risk cases in which one patient had undergone preoperative chemotherapy while one patient had not received any prior treatment before enucleation. Three patients had died due to non ocular reasons during follow-up.

Discussion

p53 tumor suppressor gene located at 17q13.1, which can induce cell apoptosis [10]. Wild-type p53 protein inhibits cell proliferation, halts cell division at the G_1 checkpoint, and facilitates the injured DNA repair [10]. p53 protein can induce cell apoptosis to prevent the mutated DNA passage to the next generation in case of the failed DNA repair [11]. Due to the loss of cell supervision of p53 protein after mutation, cell is susceptible to entry of S phase with injured DNA and the genetic instability is the source of gene mutation and chromosomal aberration, leading to cell malignant change and tumor formation. Half life of wild type p53 is very short, hence its immunohistochemical detection is not possible but mutated p53 or wild type p53 stabilized by some other mechanism can be detected by immunohistochemistry [12]. p53 mutations are rarely reported in Rb. Mdm2, also an oncogene, is located at 12q13.14 [13]. The major function of Mdm2 is to inhibit the transcription activation by p53 as well as to prevent carcinogenesis [14]. As the target gene of p53 transcription, Mdm2 can combine with p53 to form a refined feedback regulation loop [15]. Wild type p53 gene induces the high expression of Mdm2 protein, which, in turn, inhibits p53 transcription activity and strictly controls p53 protein level [16]. Mdm2 overexpression can block the p53-mediated transactivation,

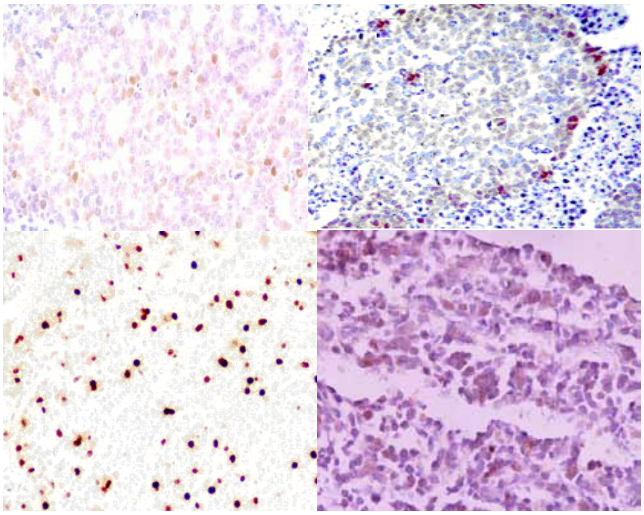

Figure 1: Immunohistochemical detection of p53 and Mdm2 protein in retinoblastoma tissue. A) and B) Retinoblastoma sections showing nuclear reactivity for p53, C) and D) Retinoblastoma sections showing nuclear reactivity for Mdm2.

S.No.	Sex	Age (Years)	Therapy	Laterality	Differentiation	High risk	p53	Mdm2
1	Female	3	No	Unilateral	Poorly	High risk	2	-
2	Male	0.3	No	Unilateral	Poorly	-	-	-
3	Female	5	No	Bilateral	Poorly	High risk	-	-
4	Female	5	No	Unilateral	Poorly	-	2	1
5	Female	1.5	No	Unilateral	Poorly	-	-	1
6	Female	3	No	Unilateral	Poorly	High risk	-	1
7	Female	4	chemo	Bilateral	Poorly	-	-	-
8	Male	4.5	No	Bilateral	Poorly	-	1	-
9	Female	1	No	Unilateral	Well	-	-	-
10	Female	1	No	Unilateral	Well	-	2	-
11	Female	3	No	Unilateral	Poorly	-	-	1
12	Male	7	No	Bilateral	Poorly	High risk	-	1
13	Female	5	No	Unilateral	Poorly	High risk	-	-
14	Male	4	chemo	Unilateral	Poorly	High risk	-	1
15	Female	3	No	Unilateral	Poorly	High risk	2	-
16	Male	5	No	Bilateral	Poorly	High risk	-	1
17	Male	0.1	No	Unilateral	Poorly	High risk	1	-
18	Male	0.3	No	Bilateral	Poorly	High risk	-	1
19	Female	3	No	Unilateral	Poorly	High risk	-	1
20	Male	4	chemo	Unilateral	Poorly	-	-	-
21	Male	0.75	chemo	Bilateral	Well	-	-	-
22	Male	2	No	Unilateral	Poorly	-	1	1
23	Male	0.3	No	Unilateral	Well	-	-	-
24	Male	2	No	Bilateral	Well	-	-	-
25	Female	1.5	No	Unilateral	Poorly	High risk	-	1
26	Male	3	No	Unilateral	Poorly	High risk	-	1
27	Male	6	No	Unilateral	Poorly	-	-	1
28	Male	2.5	No	Unilateral	Well	-	-	-
29	Male	6	No	Bilateral	Poorly	High risk	1	-
30	Female	5	No	Unilateral	Poorly	High risk	-	-
31	Male	5	chemo	Unilateral	Poorly	High risk	-	-
32	Male	0.75	chemo	Unilateral	Poorly	High risk	1	-
33	Male	4	No	Bilateral	Poorly	High Risk	-	2
34	Male	1.5	No	Bilateral	Poorly	High risk	-	-
35	Male	6	No	Unilateral	Poorly	High risk	-	-
36	Female	2.5	No	Unilateral	Poorly	High risk	1	-
37	Male	1	No	Unilateral	Poorly	-	-	-
38	Female	3.5	chemo	Unilateral	Poorly	-	-	-
39	Male	0.5	No	Bilateral	Poorly	High risk	-	2
40	Female	1.5	No	Unilateral	Poorly	-	-	-
41	Male	1.3	No	Unilateral	Poorly	High risk	-	-
42	Male	1	No	Bilateral	Poorly	High risk	1	-
43	Male	4	No	Bilateral	Well	High risk	2	1
44	Female	2.5	No	Bilateral	Poorly	High risk	1	-
45	Female	3	No	Bilateral	Poorly	-	-	-
46	Male	5	No	Unilateral	Poorly	High risk	1	-
47	Female	1.5	No	Unilateral	Well	High risk	-	1
48	Male	0.5	No	Unilateral	Poorly	High risk	1	-
49	Male	6	chemo	Unilateral	Poorly	-	-	1
50	Male	3	chemo	Bilateral	Poorly	High risk	-	-
51	Male	1.5	No	Bilateral	Poorly	High risk	-	-
52	Male	2.5	No	Bilateral	Poorly	-	1	-
53	Male	2.5	No	Bilateral	Poorly	High risk	-	-
54	Male	0.5	chemo	Unilateral	Poorly	High risk	1	1
55	Male	2.5	chemo	Unilateral	Poorly	High risk	1	1
56	Male	2	No	Unilateral	Poorly	High risk	1	-
57	Male	7	chemo	Unilateral	Poorly	High risk	1	2
58	Male	0.3	chemo	Bilateral	Poorly	-	-	-
59	Female	1.2	No	Unilateral	Poorly	High risk	2	-
60	Female	2	No	Unilateral	Poorly	-	-	-

Table 1: Clinicopathological features as well as p53 and Mdm2 expression in retinoblastoma patients.

Case No	ON	Choroid	Sclera	Ant. Seg.	Iris	CB	Necrosis	Calc.	Postenucleation	Chemoprophylactic treatment/present status
1	++	0	0	0	0	0	0	0	+	
3	++	+	0	0	0	0	+	+	+	
6	++	+	0	0	0	0	0	0	+	
12	0	0	0	0	+	0	++	+	-	
13	++	+	0	0	0	0	0	0	+	
14	0	+	0	+	+	+	++	+	-	
15	0	+	0	0	0	0	++	+	-	
16	0	+	0	+	+	+	0	0	-	
17	+	0	0	0	0	0	++	+	+	
18	++	++	+	0	+	+	++	+	+	
19	++	+	0	0	0	0	0	0	Recurred	
25	+	0	0	0	0	0	++	0	+	
26	0	0	0	+	+	+	+	0	-	
29	++	+	0	0	0	0	0	0	+	
30	++	+	0	0	0	0	0	0	+	
31	0	+	0	0	0	0	0	0	-	
32	0	+	0	0	0	0	0	0	-	
33	++	0	0	0	0	0	0	0	+	
34	++	0	0	0	0	0	++	0	+	
35	++	+	0	0	0	0	++	0	+	
36	+	0	0	0	0	0	0	0	-	
39	0	+	0	+	+	+	0	0	-	
41	0	+	0	0	0	0	+	0	-	
42	0	0	0	0	+	+	+	+	-	
43	0	+	0	0	0	0	++	0	-	
44	+	0	0	0	0	0	++	0	-	
46	++	++	0	0	0	0	++	+	+	
47	++	0	0	0	0	0	+	0	+	
48	+	0	0	0	0	0	0	+	-	
50	0	0	0	0	+	+	+	+	-	
51	+	++	0	0	0	0	++	0	+	
53	+	0	0	0	0	0	0	0	-	
54	+	+	0	0	0	0	+	+	+	
55	0	++	+	0	0	0	+	+	+	
56	+	+	0	0	0	0	+	0	+	
57	++	0	0	0	+	+	+	+	Recurred	
59	+	0	0	0	0	0	0	0	+	

Table 2: Patients with High-risk histopathologic features.

depriving p53 gene of antineoplastic activity [17]. Mdm2 gene amplification has been found in 36% of all types of sarcomas, 10% of well differentiated glioma as well as esophageal cancer, neuroblastoma, anaplastic astrocytoma [18].

It has been shown in our study that p53 protein expression rate was 28.6% (6/21) in Mdm2 positive cases and 38.5% (15/39) in Mdm2 negative cases. Four out of six (67%) samples showing coexpression of p53 and Mdm2 were also having poor prognostic histopathological features hence indicating a possible role of p53 and Mdm2 expression in aggressiveness of tumor. We found that immunoreactivity of both p53 and Mdm2 was within the nucleus in the cases having coexpression of both the protein. A well documented model for Mdm2 regulation of p53 is that Mdm2 binds to p53 transactivation domain and p53 is transported to cytoplasm, ubiquitinated and degraded in cytoplasmic proteasomes [19]. However there are other proteins like p14ARF inhibits Mdm2-directed transport of p53 to the cytoplasm and attenuates Mdm2-mediated degradation of p53 causing stabilization of wild type p53 [20]. Epigenetic silencing of p14ARF is reported in many tumors [21]. It is thus interesting to know the role of p14ARF in interaction with p53/Mdm2 expression in retinoblastoma. In p53 postive/Mdm2 negative cases transactivating loop between p53 and Mdm2 may be disturbed and expression of p53 does not induce the expression of Mdm2, which in turn can not perform the process of ubiquitination. It has been shown in previous studies that overexpression of Mdm2 is not necessary in Rb as well as in many other tumors [22,23].

Seventy five percent of well differentiated tumors have not shown any invasion which was in accordance with most of the solid tumors. Invasive tumors have shown dense nectrotic area as reported in many other tumors. Calcification was also reported in necrotic cases which were consistent with the observation of Reese and Shivde et al. [24]. Most of the patients showing high risk features were on postoperative chemoprophylactic treatment. There was not many recurring events hence we could not do any survival analysis.

The findings in our study suggest that the transactivating negative feedback loop was disturbed in p53 positive/Mdm2 negative cases and hence p53 expression was independent of Mdm2 expression. Though coexpression of p53 and Mdm2 may be caused by some other players of the transactivating loop. The poor prognostic features are correlating positively to each other and though they are correlating with p53

and Mdm2 coexpression, it gives a picture of the correlation among different histopathological features and cell cycle regulatory proteins' expression. These results indicate that p53 may not always regulate expression of its own inhibitor, Mdm2 in human retinoblastoma. All these findings suggest that along with Mdm2, some other regulatory mechanisms as well as molecules may be likely to involve in p53 expression and dysfunction.

References

1. Chung EM, Specht CS, Schroeder JW (2007) Pediatric Orbit Tumors and Tumorlike Lesions: Neuroepithelial Lesions of the Ocular Globe and Optic Nerve. Radiographics 27: 1159-1186.
2. Krishnakumar S, Mohan A, Mallikarjuna K, Venkatesan N, Biswas J et al. (2004) EpCAM expression in retinoblastoma: a novel molecular target for therapy. Invest Opthalmol Vis Sci 45: 4247-4250.
3. Chantada G, Fandiño A, Manzitti J, Urrutia L, Schvartzman E (1999) Late diagnosis of retinoblastoma in a developing country. Arch Dis Child 80: 171-174.
4. Kivela T (2009) The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. Br J Ophthalmol 93: 1129-1131.
5. Deepa PR, Nalini V, Mallikarjuna K, Vandhana S, Krishnakumar S (2009) Oxidative stress in retinoblastoma: correlations with clinicopathologic features and tumor invasiveness. Curr Eye Res 34: 1011-1018.
6. Suzuki S, Kaneko A (2004) Management of intraocular retinoblastoma and ocular prognosis. Int J Clin Oncol 9: 1-6.
7. Dong M, Ma G, Tu W, Guo KJ, Tian YL et al. (2005) Clinicopathological significance of p53 and Mdm2 protein expression in human pancreatic cancer. World J Gastroenterol 11: 2162-2165.
8. Marques YM, de Lima Mde D, de Melo Alves Sde M Jr, Soares FA, de Araujo VC et al. (2007) Mdm2, p53, p21 and pAKT protein pathways in benign neoplasms of the salivary gland. Oral Oncol 44: 903-908.
9. Dong M, Dong Q, Zhang H, Zhou J, Tian Y et al. (2007) Expression of Gadd45a and p53 proteins in human pancreatic cancer: potential effects on clinical outcomes. J surg Oncol 95: 332-336.
10. Meeran SM, Katiyar SK (2008) Cell cycle control as a basis for cancer chemoprevention through dietary agents. Front Biosci 13: 2191-2202.
11. Selivanova G, Wiman KG (1995) p53: a cell cycle regulator activated by DNA damage. Adv Cancer Res 66: 143-180.
12. Sa CT, Fonseca LMS, Cardoso SV, Aguiar MCF, Carmo MAV (2006) p53 immunoexpression in oral squamous cell carcinomas from different anatomical sites: a comparative study. Int J Morphol 24: 231-238.
13. Bode-Lesniewska B, Zhao J, Speel EJ, Biraima AM, Turina M et al. (2001) Gains of 12q13-14 and overexpression of Mdm2 are frequent findings in intimal sarcomas of the pulmonary artery. Virchows Arch 438: 57-65.
14. Chene P (2004) Inhibition of p53-MDM2 interaction: targeting a protein-protein interface. Mol Cancer Res 2: 20-28.
15. Hunziker A, Jensen MH, Krishna S (2010) Stress specific response of the p53-Mdm2 feedback loop. BMC Syst Biol 4: 94.
16. Mansouri SE, Martin A, Mercadier A, Capoulade C, Marechal V et al. (1999) High expression of MDM2 protein and low rate of p21(WAF1/CIP1) expression in SCID mice epstein barr virus induced lymphoproliferation. J Histochem Cytochem 47: 1315-1322.
17. Giovannati E, Backus HH, Wouters D, Ferreira CG, van Houten VM et al. (2007) Changes in the status of p53 affect drug sensitivity to thymidylate synthase (TS) inhibitors by altering TS levels. Br J Cancer 96: 769-775.
18. Asomaning K, Reid AE, Zhou W, Heist RS, Zhai R et al. (2008) Mdm2 promoter polymorphism and pancreatic cancer risk and prognosis. Clin Cancer Res 14: 4010-4015.
19. Shwe M, Chiguchi G, Yamada S, Nakajima T, Maung KK et al. (2001) p53 and Mdm2 co-expression in tobacco and betel chewing-associated oral squamous cell carcinomas. J Med Dent Sci 48: 113-119.
20. O'Keefe K, Li H, Zhang Y (2003) Nucleocytoplasmic Shuttling of p53 is essential for Mdm2-Mediated Cytoplasmic Degradation but Not Ubiquitination. Mol Cell Biol 23: 6396-6405.
21. Esteller M, Cordon-Cardo C, Corn PG, Meltzer SJ, Pohar KS et al. (2001) p14ARF silencing by promoter hypermethylation mediates abnormal intracellular localization of Mdm2. Cancer Res 61:2816-2821.
22. Guo Y, Pajovic S, Gallie BL (2008) Expression of p14ARF, Mdm2 and Mdm4 in human retinoblastoma. Biochem Biophys Res Commun 375: 1-5.
23. Foulkes WD, Stamp GW, Afzal S, Lalani N, McFarlane CP et al. (1995) Mdm2 overexpression is rare in ovarian carcinomas irrespective of TP53 mutation status. Br J Cancer 72: 883-888.
24. Tyagi N, Hameed S, Gogi R, Ahuja OP, Nath K (1978) Degenerative lesions in retinoblastoma and their significance. Indian J Ophthalmol 26: 23-25.