EXTENDED REPORT

Stem cell markers: ABCG2 and MCM2 expression in retinoblastoma

A Mohan, M Kandalam, H L Ramkumar, L Gopal, S Krishnakumar

See end of article for authors' affiliations

Correspondence to: Dr Subramanian Krishnakumar, Department of Ocular Pathology, Vision Research Foundation, Sankara Nethralaya, 18, College Road, Chennai – 600 006, Tamil Nadu, India; drkrishnakumar_2000@yahoo.com

Accepted for publication 2 March 2006

Br J Ophthalmol 2006;**90**:889–893. doi: 10.1136/bjo.2005.089219

Backgound/aim: The authors studied the expression of cancer stem cell surface marker, ABCG2, and neural stem cell marker, MCM2, in retinoblastoma and correlated clinicopathologically.
Methods: Among 39 retinoblastomas, 18 tumours were not subjected to preoperative/postoperative chemotherapy, 15 tumours underwent postoperative chemotherapy, and six tumours had preoperative chemotherapy. There were 20 tumours with no invasion and 19 tumours with invasion of choroid/optic nerve. ABCG2 and MCM2 expression was studied by immunohistochemistry.
Results: ABCG2 was positive in six of six and MCM2 was positive in five of six tumours that had recurred in the orbit or metastasised. ABCG2 was positive in 15/19 tumours with invasion. MCM2 was positive in 16/19 tumours with invasion. Invasive tumours showed higher expression of ABCG2 ($p<0.01$) and MCM2 ($p<0.01$) proteins. There was no correlation with differentiation and laterality of the tumours. Non-neoplastic retina was positive for ABCG2 and MCM2.
Conclusion: ABCG2 and MCM2 were expressed more in invasive tumours. Further studies are needed to understand the significance of ABCG2 and MCM2 expression in retinoblastoma.

Chemotherapy has an important role in the management of retinoblastoma. To improve treatment outcome, intensive research has focused on clinically relevant mechanisms of chemotherapeutic drug resistance in retinoblastoma.[1] Siegel *et al*[2] observed the presence of a small subpopulation of cancer stem cells (ABCG2 positive) and neural stem cells (MCM2 positive) in tumours from transgenic mice, human retinoblastoma cell lines and a small cohort of archival human retinoblastomas. They concluded that the presence of these cells in retinoblastomas might have significant impact on future treatment strategies.

ABCG2 is a half ATP binding cassette (ABC) transporter expressed on plasma membranes. Overexpression of ABCG2 in cell lines confers resistance on a wide variety of anticancer drugs including mitoxantrone, daunorubicin, doxorubicin, topotecan, and epirubicin.[3–5] MCM2 is one of six members of the family of minichromosome maintenance (MCM) proteins.[6] MCM proteins are components of the prereplicative complex, which binds to replication origins in the G1 phase of the cell cycle and is essential for the initiation of DNA replication.[7] MCM2 is a proved marker for detecting neural stem cells.[8] Since primitive neuroectodermal cells are involved in retinoblastoma tumorigenesis,[9] the presence of these neural stem cells could increase the aggressiveness of the original tumour.

There are no studies on ABCG2 and MCM2 proteins in a large cohort of archival retinoblastoma tumour samples. We investigated the expression of these two proteins and correlated their expression with clinicopathological parameters such as laterality, differentiation, and tumour invasiveness.

MATERIALS AND METHODS

Thirty nine tumours were available from 39 eyes for the study. Among them were tumours from 30 males and nine females. The age ranged from 4 months to 21 years (median 1 year). There were 31 unilateral retinoblastomas and eight bilateral retinoblastomas.

Tumour specimens

The study was reviewed and approved by the local ethics committee at Vision Research Foundation, Sankara Nethralaya, and the committee deemed that it conformed to the generally accepted principles of research, in accordance with the Helsinki Declaration. Tumours enucleated between 1997 and 2002 with a minimum follow up of at least 24 months were included in the study. Paraffin embedded blocks from 39 cases derived from enucleation of retinoblastomas were used for immunohistochemistry. For non-neoplastic retina donor eye balls, which were received in the pathology laboratory, were used.

The tumours were classified into three groups: group 1 (n = 18), tumour samples obtained from enucleated eyes as a part of the treatment and the patients were not subjected to either preoperative or postoperative chemotherapy (table 1); group 2 (n = 15), tumour samples obtained from enucleated eyes as a part of management and then the patients were subjected to postoperative chemotherapy because the histopathological study showed risk factors for metastasis such as invasion of choroids and optic nerve (table 2); and group 3 (n = 6), tumour samples obtained from enucleated eyes of patients who received preoperative chemotherapy (table 3)

Histopathological features

All tumour 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, post-laminar invasion and invasion of the surgical end of the optic nerve were considered.[10]

Invasion of tumours

There were 20 tumours with no invasion of the choroid or optic nerve and 19 tumours with invasion of the choroid, optic nerve, and orbit. Among the 19 tumours with invasion, eight had choroidal invasion (three diffuse and five focal choroidal invasion). There were four tumours with post-laminar optic nerve invasion. There were seven tumours with

Abbreviations: ABC, ATP binding cassette; DLP, diode laser photocoagulation; EBRT, external beam radiation therapy; LSAB, labelled streptavidin biotin; MCM, minichromosome maintenance; PBS, phosphate buffered saline; TCC, transconjunctival cryopexy

Table 1 ABCG2 and MCM2 expression in tumours without preoperative or postoperative chemotherapy

Serial No	Age/sex	Clinicopathological features	% of ABCG2 stained tumour cells	% of MCM2 stained tumour cells	Follow up
1	1y/M	LE: WD No invasion of choroid	40	60	No recurrence in the
2	3y/F	LE: WD and optic nerve	20	30	enucleated eye
3	3m/M	RE: WD	0	60	
4	2y/M	RE: WD	0	70	
5	5y/M	LE: PD	40	70	
6	1y/M	RE: WD	20	50	
7	1y/M	LE: PD	30	40	
8	2m/M	RE: WD,	60	40	
9	5m/M	RE: WD	0	20	
10	2y/M	LE: MD	0	10	
11	2y/M	LE: PD	20	70	
12	2y/M	LE: PD	0	30	
13	2y/M	LE: PD	0	20	
14	9m/M	LE: MD	0	0	
15	2y/M	LE: PD	40	0	
16	4/M	RE: PD, diffuse Ch invasion	70	80	
17	3/F	LE: PD, focal Ch invasion	0	20	
18	3/M	RE: PD, focal Ch invasion	0	70	

M, male; F, female; y, years; m, months; LE, left eye; RE, right eye; PD, poorly differentiated; WD, well differentiated; MD, moderately differentiated; Ch, choroidal; inv, invasion.

invasion of both choroid and optic nerve (five tumours with diffuse choroidal and post-laminar optic nerve invasion and two tumours with focal choroidal and post-laminar optic nerve invasion).

Differentiation of tumours

Retinoblastomas were graded microscopically into three groups according to the predominant pattern of differentiation.[10] There were 11 well differentiated, five moderately differentiated, and 23 poorly differentiated tumours.

Treatment

Groups 1 and 2 tumours were not subjected to preoperative chemotherapy. Group 2 consisted of patients whose tumours were enucleated and then the patients were subjected to postoperative chemotherapy, because of risk factors for metastasis. Group 3 consisted of tumours, which were

Table 2 ABCG2 and MCM2 expression in tumours with postoperative chemotherapy

Serial No	Age/sex	Clinicopathological features	Postoperative chemotherapy	% of ABCG2 stained tumour cells	% of MCM2 stained tumour cells	Follow up
1	5y/F	BE: LE, WD, focal Ch invasion	2 cycles of triple chemo to LE	80	70	No recurrence in the enucleated eye
2	3m/M	LE: PD, post-laminar ON inv	2 cycles of triple chemo to LE	40	0	
3	21m/M	BE: RE, PD, focal Ch invasion	10 cycles of triple chemo to LE	0	30	
4	7y/M	LE: PD, diffuse Ch and post-lam invasion	Triple chemo + EBRT salvage therapy to LE (VP16 + carboplatin)	70	90	Orbital invasion
5	8m/M	BE: RE, WD, focal Ch invasion, and SE end involved	4 cycles of triple chemo to RE	70	80	Child died, Bone marrow met
6	3y/F	RE: PD, diffuse Ch invasion	2 cycles of triple chemo to RE	60	90	Orbital invasion
7	3y/F	RE: PD, focal Ch and post-lam ON invasion	7 cycles of triple chemo to RE	20	80	No recurrence in the enucleated eye
8	2y/F	LE: PD, diffuse Ch inv and post-lam ON invasion	2 cycles of triple chemo to LE	70	70	Orbital invasion
9	4y/F	LE: PD, post-lam ON invasion	4 cycles of triple chemo to LE	40	90	No recurrence in the enucleated eye
10	3y/M	LE: PD, diff Ch and post-lam ON invasion	5 cycles of triple chemo to LE	60	70	Orbital invasion
11	2y/M	BE: RE, MD post-lam ON invasion	9 cycles of triple chemo + TTT + TCC + EBRT to RE	40	90	No recurrence in the enucleated eye
12	14m/M	BE: LE, PD, diffuse Ch, post-lam ON invasion	5 cycles of triple chemo to LE	70	0	Submandibular Met.
13	1m/M	RE: MD, Diff Ch invasion	6 cycles of triple chemo to RE	80	80	No recurrence in the enucleated eye
14	2y/M	LE: PD, diff Ch inv and post-lam ON invasion	6 cycles chemo + 2 EBRT	60	40	
15	2y/F	RE: PD, post-lam ON invasion	4 cycles of triple chemo	0	80	

M, male; F, female; y, years; m, months; LE, left eye; RE, right eye; BE, bilateral; PD, poorly differentiated; WD, well differentiated; MD, moderately differentiated; ON, optic nerve; Ch, choroidal; inv, invasion; post lam, post lamina; diff, diffuse; SE, surgical end; Chemo, chemotherapy; EBRT, external beam radiation therapy; DLP, diode laser photocoagulation; TCC, transconjunctival cryopexy.

Table 3 ABCG2 and MCM2 expression in tumours with pre operative chemotherapy

Serial No	Age/sex	Clinicopathological features	Chemotherapy information	% of ABCG2 stained tumour cells	% of MCM2 stained tumour cells	Follow up
1	3y/M	LE: PD, no invasion	4 cycles of triple chemo	40	0	No recurrence in the enucleated eye
2	2y/M	RE: WD, no invasion	2 cycles of triple chemo	60	0	
3	2y/M	BE: LE, MD, no invasion	6 cycles of triple chemo	20	0	
4	9m/F	RE: PD, no invasion	4 cycles of triple chemo	0	60	
5	2y/M	BE: RE, WD, focal Ch invasion	2 cycles of preop chemo, postop 6 cycles triple chemo + TCC + TTT	40	0	
6	4m/M	BE: RE, PD, no invasion	2 cycles of triple chemo	50	0	

M, male; F, female; y, years; m, months; LE, left eye; RE, right eye; BE, bilateral; PD, poorly differentiated; WD, well differentiated; MD, moderately differentiated; ON, optic nerve; Ch, choroidal; inv, invasion; Chemo, chemotherapy; preop, preoperative; postop, postoperative; TCC, transconjunctival cryopexy.

subjected to preoperative chemotherapy. In bilateral retinoblastomas , the eye where the tumour was small was treated with local therapy. The focal therapies employed were external beam radiation therapy (EBRT), diode laser photocoagulation (DLP), and transconjunctival cryopexy (TCC). The chemotherapy cycles ranged from two to eight cycles and drugs used included carboplatin, vincristine, and etoposide.

Clinical outcome

In group 1, where the tumours did not have any invasion nor had only focal choroidal invasion, there was no recurrence. In group 2, all the 15 tumours were subjected to postoperative chemotherapy because of the risk factors for metastasis. There was orbital invasion in four tumours (cases 4, 6, 8, 10), bone marrow metastasis in one tumour (case 5), and submandibular node metastasis in one tumour (case 12). In group 3, the tumours were subjected to preoperative chemotherapy and then enucleated.

Antibodies and chemicals

Mouse monoclonal anti-ABCG2 (5D3); sc-18841 (200 µg/ml) antibody for the detection of ABCG2 protein was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and also mouse monoclonal antibody MAAB4146 Ms X BRCP (0.25 mg/ml) was obtained from Chemicon International. Primary mouse monoclonal human MCM protein MCM2 (150 µg/ml); clone: CRCT2.1) antibody was obtained from Novocastra, Newcastle, UK. labelled streptavidin biotin (LSAB) kit and developing solution (DAB) were obtained from Dakocytomation Corp, Glostrup, Denmark.

Immunohistochemistry

Paraffin sections of 39 tumour samples of retinoblastoma were washed in phosphate buffered saline (PBS) and incubated for 1 hour with the ABCG2/MCM2 antibody or with 1% BSA-PBS as a negative control. After washing, the slides were incubated with biotinylated anti-mouse immunoglobulin (LSAB) for 30 minutes, washed again, and incubated with horseradish peroxidase conjugated streptavidin for 30 minutes. The reaction was revealed by 3, 3′-diaminobenzidine and counterstained with haematoxylin.

Evaluation of slides

Antigen expression was defined as the presence of membrane staining for ABCG2 and nuclear staining on the tumour cells for MCM2. All stained cells were considered positive, irrespective of staining intensity. Because ABCG2 and MCM2 were expressed heterogeneously, 20 vital tumour fields were evaluated (under 20× magnifications) and a final mean score for each tumour was obtained. The staining was scored as the percentage of positively stained cells. Both ABCG2 and MCM2 were scored on a three tiered scale: – 0 (when ABCG2 or MCM2 negative); 1%–⩽50% cells positive and >50% cells positive for ABCG2 or MCM2. ABCG2 and MCM2 immunoreactivity was correlated with the invasiveness and differentiation of the tumours and in the three groups of tumours.

Statistical analysis

Statistical analyses were performed using the non-parametric Mann-Whitney U test. p Values <0.05 were considered statistically significant. Well differentiated and moderately differentiated tumours were grouped and compared against poorly differentiated tumours.

RESULTS

Tables 1–3 show the clinicopathological information and immunoexpression of ABCG2 and MCM2 in the three groups of tumours. The immunoreactivity data of ABCG2 from two antibodies was concurrent. Figures 1A–C show ABCG2 positivity in the non-neoplastic retina obtained from donor cadaveric eyes and figure 1D–G show ABCG2 immunoreactivity in the retinoblastoma tumour samples. Figures 2A–C show MCM2 positivity in the non-neoplastic retina obtained from donor cadaveric eyes and figure 2D–G show MCM2 immunoreactivity in the retinoblastoma tumour samples.

ABCG2 and MCM2 in non-neoplastic retina

Both ABCG2 and MCM2 were sporadically positive in the ganglion cells, inner nuclear layers, and in the photoreceptor layers in the retina obtained from donor eyes and in the non-neoplastic portion of the retina with retinoblastoma.

ABCG2 expression in retinoblastoma

Among the 20 non-invasive retinoblastomas, ABCG2 was positive in 12 tumours (two tumours with >50% cells positivity and 10 tumours with 1%–⩽50% cells positivity) and negative in eight tumours. Among 19 invasive retinoblastomas , ABCG2 was positive in 15 tumours (10 tumours with >50% cells positivity and five tumours with 1 to ⩽50% cells positivity) and negative in four tumours. Among the six tumours which had either orbital recurrence or metastasis, ABCG2 was positive in six tumours (all >50% cells positivity). The percentage of ABCG2 positive cells was more in the invasive tumours (Mann-Whitney U test; $p<0.01$).

MCM2 expression in retinoblastoma

Among 20 non-invasive retinoblastomas, MCM2 was positive in 14 tumours (six tumours with >50% cells positivity and eight tumours with 1%–⩽50% cells positive) and negative in six tumours Among the 19 invasive retinoblastomas, MCM2 was positive in 16 tumours (13 tumours with >50% cells positivity, three tumours with 1%–⩽50% cells positivity) and negative in three tumours. Among the six tumours, which

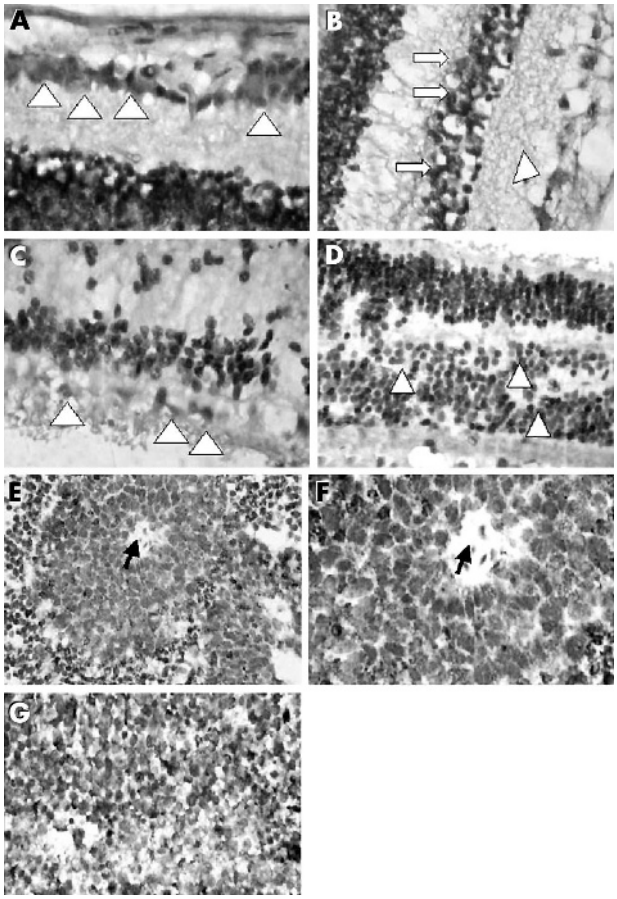

Figure 1 (A) Photomicrograph showing the ABCG2 positivity in the ganglion cells (white arrowhead) in non-neoplastic retina obtained from donor eye tissue (DAB with haematoxylin counterstain, ×100). (B) Photomicrograph showing the ABCG2 positivity in the ganglion cells (white arrowhead) and in the inner nuclear layers (white arrows) of non-neoplastic retina obtained from donor eye tissue (DAB with haematoxylin counterstain, ×100). (C) Photomicrograph showing the sporadic ABCG2 positivity in the photoreceptor cells (white arrowhead) of non-neoplastic retina obtained from donor eye tissue (DAB with haematoxylin counterstain, ×100). (D) Photomicrograph showing the ABCG2 positivity in the inner nuclear layers (white arrowheads) of retina adjacent to retinoblastoma tumour (DAB with haematoxylin counterstain, ×100). (E) Photomicrograph showing the ABCG2 positivity in the tumours cells of a tumour lobule with tumour cells showing no differentiation arranged around a blood vessel (black arrow) (DAB with haematoxylin counterstain, ×40). (F) Photomicrograph showing the ABCG2 positivity in the tumours cells showing no differentiation of a tumour lobule arranged around a blood vessel (black arrow) (DAB with haematoxylin counterstain, ×200). (G) Photomicrograph showing the ABCG2 positivity in majority of the tumours cells showing no differentiation and invading the orbit (DAB with haematoxylin counterstain, ×200).

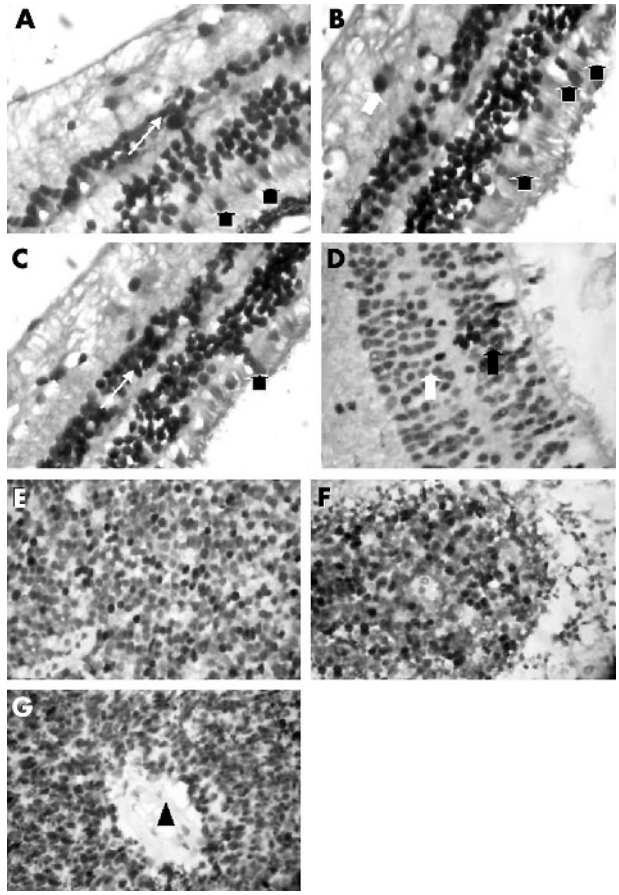

Figure 2 (A) Photomicrograph showing the sporadic MCM2 positivity in the inner nuclear layer (white arrowhead) and in the photoreceptor cells (black arrow) in non-neoplastic retina obtained from donor eye tissue (DAB with haematoxylin counterstain, ×100). (B) Photomicrograph showing the sporadic MCM2 positivity in the ganglion cells (white arrowhead) and in the photoreceptor cells (black arrow) in non-neoplastic retina obtained from donor eye tissue (DAB with haematoxylin counterstain, ×100). (C) Photomicrograph showing the sporadic MCM2 positivity in the inner nuclear layer cells (white arrowhead) and in the photoreceptor cells (black arrow) in non-neoplastic retina obtained from donor eye tissue (DAB with haematoxylin counterstain, ×100). (D) Photomicrograph showing the MCM2 positivity in the inner nuclear layers (white arrow) and outer nuclear layer (black arrow) in the retina adjacent to retinoblastoma tumour (DAB with haematoxylin counterstain, ×100). (E) Photomicrograph showing the 10–20% MCM2 positivity in the tumour cells of retinoblastomas showing differentiation (DAB with haematoxylin counterstain, ×100). (F) Photomicrograph showing the 40–50% MCM2 positivity in the tumours cells showing differentiation in tumour lobule (DAB with haematoxylin counterstain, ×200). (G) Photomicrograph showing the >50% MCM2 positivity in majority of the tumours cells showing no differentiation and arranged around the blood vessel (black arrowhead) (DAB with haematoxylin counterstain, ×200).

had, either orbital recurrence or metastasis MCM2 was positive in five tumours (all >50% cells positivity). The percentage of MCM2 positive cells was more in the invasive tumours (Mann-Whitney U test; $p<0.01$).

ABCG2 and MCM2 expression in tumours not subjected to preoperative chemotherapy

Among 33 tumours (groups 1 and 2) that had no preoperative chemotherapy, ABCG2 was positive in 22 tumours (11 tumours with >50% cells positivity and 11 tumours with 1%–50% cells positivity) and MCM2 was positive in 28 tumours (18 tumours with >50% cells positivity and 10 with 1%–50% cells positivity).

ABCG2 and MCM2 expression in tumours subjected to preoperative chemotherapy

Among the six tumours, ABCG2 was positive in five tumours (four tumours with 1%–0% cells positivity and one tumour with >50% cells positivity) and negative in one tumour. MCM2 was positive in one tumour (>50% cells positivity) and negative in five tumours.

ABCG2 and MCM2 expression and correlation with differentiation and laterality of tumours

There was no correlation observed with MCM2 expression against differentiation and laterality of tumours.

DISCUSSION

The current study compares the immunoexpression of ABCG2 and MCM2 in human archival retinoblastoma tumours with an earlier study by Siegel *et al*[2] where they observed the immunoexpression of ABCG2 and MCM2 in retinoblastoma tumours from mice transgenic for the SV40 T antigen (driven by the β luteinising hormone promoter), cell pellets of human Y79 and WERI-Rb27 retinoblastoma cell lines, and three archival human retinoblastoma pathological specimens. In their study, small numbers of retinoblastoma cells exhibited immunoreactivity to stem cell markers ABCG2 and MCM2. MCM2 positivity was observed in tumour tissue surrounding blood vessels, adjacent to the optic nerve, and in the inner nuclear layer and photoreceptor layers of the retina in eyes with retinoblastoma.

Our results are concurrent with their study in that the human archival retinoblastoma tumour samples showed the immunoexpression of both ABCG2 and MCM2. Both ABCG2 and MCM2 immunoreactivity was observed in both well and poorly differentiated tumours and in tumour cells arranged around blood vessels and also in the invading portion of the tumour cells in the optic nerve and orbit. We also had the opportunity of correlating the expression of these two stem markers with invasiveness and with chemotherapy in our cohort. The percentage of ABCG2 and MCM2 positive cells was more in the invasive tumours (Mann-Whitney U test; $p<0.01$ for both). There was no correlation with respect to laterality and differentiation of the tumours. These data support the earlier observation that stem cells expressed in retinoblastoma could have a role in tumorigenesis.[2]

Among the 33 tumours not subjected to preoperative chemotherapy, ABCG2 was positive in 21 tumours and MCM2 was positive in 29 tumours. This observation suggests that retinoblastoma expresses ABCG2 and MCM2 proteins even previous chemotherapy. This could have implications with regard to chemotherapy response. Among the six tumours subjected to preoperative chemotherapy (table 3) ABCG2 was positive in five tumours and MCM2 was positive in one tumour. The increased percentile of ABCG2 positive cells in tumours, which were enucleated after preoperative chemotherapy, suggests that ABCG2 positive cells could escape the chemotherapy drugs. Regarding the correlation of ABCG2 and MCM2 protein with response to chemotherapy, in the 15 tumours in group 2 (table 2) where the patients were subjected to postoperative chemotherapy, ABCG2 was positive in 12 tumours. In the six patients who had developed orbital recurrence or developed metastasis, ABCG2 was positive in all six tumours and MCM2 was positive in two tumours.

Overexpression of ABCG2 has been observed in several human cancer cell lines selected for drug resistance,[3–5 11] as well as in tumour samples of cancer patients.[12 13] Tumorigenic cells with characteristics similar to neural stem cells with MCM2 positivity have been isolated from paediatric brain tumours.[7] Presence of neural stem cells could increase the aggressiveness of the original tumour. Thus the expression of MCM2 in retinoblastoma is similar to other tumours where MCM2 is seen more in aggressive tumours.[14–18]

We also observed the immunoreactivity of ABCG2 and MCM2 in ganglion cells, inner nuclear layer, and the photoreceptor cells of non-neoplastic retinas obtained from donor eyes. ABCG2 and MCM2 positivity was also observed in the retinal tissue adjacent to the tumour. In normal tissue, high expression of the ABCG2 is found in stem cells, epithelial cells of small and large intestines, ducts and lobules of the breast, endothelial cells of veins and capillaries, and synchitiotrophoblastic cells of the placenta.[19] The localisation of ABCG2 suggests that it could have a potential role in protection against toxins.

In summary, we confirm and expand upon the findings that cancer stem cell and drug efflux protein ABCG2 and neural stem cell and proliferative marker MCM2 are expressed in archival retinoblastoma samples. These could add to the existing drug resistance proteins[20] in retinoblastoma. However, further studies are needed to understand the role of these stem cells in retinoblastoma and their contribution to drug resistance and tumour progression.

ACKNOWLEDGEMENTS

We thank Ms Vaijayanthi Pushparaj, Ms Nalini Venkatesan, and Ms Vanitha K for technical help and Mr J Roy for statistical analysis.

Authors' affiliations

A Mohan, M Kandalam, Department of Ocular Pathology, Vision Research Foundation, Chennai, Tamil Nadu, India
H L Ramkumar, Department of Biological Sciences, Northwestern University, Evanston, IL, USA
L Gopal, Vitreoretina Services, Vision Research Foundation, Chennai, Tamil Nadu, India
S Krishnakumar, Department of Ocular Pathology, Vision Research Foundation, Sankara Nethralaya, Chennai, India

Sponsors: Indian Council of Medical Research, (BMS-80/7/2003-01300) and Vision Research Foundation, Chennai, India.

Commercial interest: none.

REFERENCES

1 **Shields CL**, Meadows AT, Leahey AM, *et al.* Continuing challenges in the management of retinoblastoma with chemotherapy. *Retina* 2004;**24**:849–62.
2 **Seigel GM**, Campbell LM, Narayan M, *et al.* Cancer stem cell characteristics in retinoblastoma. *Mol Vis* 2005;**12**:729–37.
3 **Allen JD**, Brinkhuis RF, Wijnholds J, *et al.* The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. *Cancer Res* 1999;**59**:4237–41.
4 **Scheffer GL**, Maliepaard M, Pijnenborg AC, *et al.* Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. *Cancer Res* 2000;**60**:2589–93.
5 **Litman T**, Brangi M, Hudson E, *et al.* The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J Cell Sci* 2000;**113**:2011–21.
6 **Kearsey SE**, Maiorano D, Holmes EC, *et al.* The role of MCM proteins in the cell cycle control of genome duplication. *Bioessays* 1996;**18**:183–90.
7 **Maslov AY**, Barone TA, Plunkett RJ, *et al.* Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J Neurosci* 2004;**7**:1726–33.
8 **Schlesinger HR**, Rorke L, Jamieson R, *et al.* Neuronal properties of neuroectodermal tumors in vitro. *Cancer Res* 1981;**41**:2573–5.
9 **Dyer M**, Bremner A. The search for the retinoblastoma cell of origin. *Nat Rev Cancer* 2005;**3**:91–101.
10 **Folberg R**, Salomao D, Grossniklaus HE, Association of Directors of Anatomic and Surgical Pathology, *et al.* Recommendations for the reporting of tissues removed as part of the surgical treatment of common malignancies of the eye and its adnexa. *Am J Surg Pathol* 2003;**27**:999–1004.
11 **Maliepaard M**, van Gastelen MA, de Jong LA, *et al.* Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. *Cancer Res* 1999;**59**:4559–63.
12 **Robey RW**, Medina-Perez WY, Nishiyama K, *et al.* Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCrp/ABCP1), in flavopiridol-resistant human breast cancer cells. *Clin Cancer Res* 2001;**7**:145–52.
13 **Diestra JE**, Scheffer GL, Catala I, *et al.* Frequent expression of the multi-drug resistance-associated protein BCRP/MXR/ABCP/ABCG2 in human tumours detected by the BXP-21 monoclonal antibody in paraffin-embedded material. *J Pathol* 2002;**198**:213–19.
14 **Freeman A**, Morris LS, Mills AD, *et al.* Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. *Clin Cancer Res* 1999;**5**:2121–32.
15 **Meng MV**, Grossfeld GD, Williams GH, *et al.* Minichromosome maintenance protein 2 expression in prostate: characterization and association with outcome after therapy for cancer. *Clin Cancer Res* 2001;**7**:2712–18.
16 **Rodins K**, Cheale M, Coleman N, *et al.* Minichromosome maintenance protein 2 expression in normal kidney and renal cell carcinomas: relationship to tumor dormancy and potential clinical utility. *Clin Cancer Res* 2002;**8**:1075–81.
17 **Dudderidge TJ**, Stoeber K, Loddo M, *et al.* Mcm2, Geminin, and KI-67 define proliferative state and are prognostic markers in renal cell carcinoma. *Clin Cancer Res* 2005;**11**:2510–17.
18 **Kato H**, Miyazaki T, Fukai Y, *et al.* A new proliferation marker, minichromosome maintenance protein 2, is associated with tumor aggressiveness in esophageal squamous cell carcinoma. *J Surg Oncol* 2003;**84**:24–30.
19 **Maliepaard M**, Scheffer GL, Faneyte IF, *et al.* Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res* 2001;**61**:3458–64.
20 **Krishnakumar S**, Mallikarjuna K, Desai N, *et al.* Multidrug resistant proteins: P-glycoprotein and lung resistance protein expression in retinoblastoma. *Br J Ophthalmol* 2004;**88**:1521–6.