SHORT REPORT OPEN ACCESS

Comparison of mature retinal marker expression in Y79 and WERI-RB27 human retinoblastoma cell lines

Linda L Cassidy, Anthony Bejjani, Meerim Choi, Gail M Seigel

ABSTRACT

Introduction: Cancer stem cells are thought to be responsible for chemotherapy resistance in retinoblastoma and other cancers, but the lineage of retinoblastoma remains unclear. In previous work, we reported on the presence of stem cell markers in retinoblastoma cells. We hypothesized that human retinoblastoma cell lines would differentially express mature retinal markers, in addition to stem cell markers. In this report we compare the expression of the mature retinal markers CRX, recoverin, Tuj-1, PKCa and MAP2 in the most commonly studied Y79 and WERI-RB27 human retinoblastoma cell lines. Methods: RNA expression of mature retinal markers CRX, recoverin, Tuj-1, PKCa and MAP2 was measured using qPCR and protein expression was examined by quantitative immunocytochemistry. Results: Both RB cell lines expressed all mature markers tested at the protein and mRNA levels. WERI-RB27 displayed higher levels of recoverin and MAP2 expression than Y79 using immunocytochemistry. CRX, Tuj-1, and PKCa showed similar levels of protein expression between the two cell lines. qPCR analysis indicated that relative levels of recoverin expression were similar in both cell lines. MAP2 exhibited the greatest difference in RNA expression between the two cell lines, with levels in WERI-RB27 being much higher than in Y79. Conclusion: CRX, recoverin, Tuj-1, PKCa, and MAP2 mature retinal markers are all expressed at both the protein and RNA levels in WERI-RB-27 and Y79 RB cell lines. Recoverin and MAP2 showed the highest protein expression in WERI-RB27 cells. RNA expression differed from the pattern of protein expression for some markers, implying post-transcriptional modification of expression.

Keywords: Retinoblastoma, Retinal markers, Stem cells

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Cassidy LL, Bejjani A, Choi M, Seigel GM. Comparison of mature retinal marker expression in Y79 and WERI-RB27 human retinoblastoma cell lines. Oncyocytology 2012;2:1–5.

Article ID: 100002OCYLLC2012

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doi:10.5348/ocyj-2012-2-SR-1

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Received: 02 May 2012
Accepted: 02 June 2012
Published: 09 July 2012

INTRODUCTION

Retinoblastoma is an intraocular cancer occurring in early childhood, caused by a disruption in the tumor suppressor RB gene [1]. The Y79 and WERI-RB27 cell lines are established retinoblastoma cell lines containing deletions in the RB gene [2–5].

There is considerable debate as to the origin of retinoblastoma cells. One hypothesis implicates cancer stem cells, which are thought to cause chemotherapy

resistance. We previously documented the expression of stem cell markers in retinoblastoma, providing evidence in support of the stem cell hypothesis [6, 7].

In this report, we sought to compare the expression of mature retinal markers MAP2, PKCalpha, CRX, recoverin and Tuj1 (beta tubulin) in commonly studied retinoblastoma cell lines Y79 and WERI-RB27. We tested the hypothesis that these two cell lines would differentially express mature retinal markers, in addition to the previously reported stem cell markers.

MATERIALS AND METHODS

Cell culture

The retinoblastoma cell lines Y79 and WERI-RB27 were grown in suspension in Dulbecco's Modified Eagle Medium (DMEM) with 10% calf serum (Life Technologies, Grand Island, NY) in a 37°C incubator with 5% CO2. MDA-MB-231, a human breast adenocarcinoma cell line, was grown as a control in Leibovitz's L15 medium (ATCC, Manassas, VA) with 10% calf serum at 37°C in 100% room air.

Immunocytochemistry

Immunostaining was performed as previously described [6, 7]. Cells were harvested, resuspended in cytospin solution (72% isopropanol, 19% acetone, 7.6% glycerol) and were spun onto slides using a Shandon Cytospin II. Slides were blocked with enzyme/protein block (Abcam, Cambridge, MA), followed by one hour incubation in primary antibody at the appropriate concentration (Table 1) or isotype control antibody. Slides were washed with PBS and incubated in secondary polymer (Syd Laboratories, Malden, MA). Staining with DAB (diaminobenzidine) was performed for five minutes, and the slides were washed with water before being covered with cover slip. Three groups of 100 cells were counted as immunopositive for each slide. Slides were photographed using a SONY ICX 285AL SPOT camera (Diagnostic Instruments, Sterling Heights, MI). Each experiment was repeated three times and graphed as shown in Figure 1.

Table 1: Primary antibodies used for immunocytochemistry.

Marker	Cell type	Company, cat. no.	Concentration (ug/ml)
CRX	Earliest photoreceptor [13, 14, 20]	Sigma Aldrich HPA036762	0.4
Recoverin	Intermediate photoreceptor [18, 19]	Abgent AP1565b	2.5
MAP2	Neuronal [12]	Abcam Ab11267	5
Tuj1	Ganglion/ amacrine cells [21]	Sigma Aldrich T3952	3.33
PKC alpha	Bipolar cells [22, 23]	Sigma Aldrich HPA006563	0.5

Abbreviations: CRX: Cone Rod Homeobox, MAP2: Microtubule Associated Protein 2, Tuj1: Beta tubulin, PKC alpha: Protein Kinase C alpha

q RT PCR of mature retinal markers

RNA from Y79 and WERI-RB27 cells was prepared using the RNeasy Plus kit (Qiagen, Valencia, CA). One µg of RNA was reverse transcribed using the iScript cDNA synthesis kit (BioRad, Hercules, CA). qPCR was performed using Sybr Green Master mix (Qiagen) and the primers listed in Table 2 (IDT, Coralville, IA). The reactions were run in a BioRad My iQ cycler using the following conditions: MAP2, Tuj1: 40 cycles; 95°C 15 sec, 60°C 1 min. CRX, recoverin, PKCa: 35 cycles; 95°C 30 sec, 60°C 30 sec, 72°C 30 sec.

Statistical analysis

The percentage of immunoreactive cells for three repeated experiments for each marker was graphed using Prism software (Graphpad, La Jolla, CA). For qPCR analysis, relative expression was calculated with the 2–delta, delta Ct equation using GAPDH as the reference gene. Three repeats of each experiment were performed using different cell preparations and the results were graphed using Prism software (Graphpad, La Jolla, CA).

Table 2: qPCR primers.

Gene		Primer sequence (5' to 3')	Product size
GAPDH	F	TGCACCACCAACTGCTTAGC	87 bp
	R	GGCATGGACTGTGGTCATGAG	
CRX	F	CCACTATTCTGTCAACGC	232 bp
	R	CCAAACCTGAACCCTGG	
Recoverin	F	CCAGAGCATCTACGCCAAGT	196 bp
	R	CACGTCGTAGAGGGAGAA	
Tuj1	F	CTCAGGGGCCTTTGGACATC	160 bp
	R	CAGGCAGTCGCAGTTTTCAC	
PKCalpha	F	GTGGCAAAGGAGCAGAGAAC	151 bp
	R	TGTAAGATGGGGTGCACAAA	
MAP2	F	CCAATGGATTCCCATACAGG	179 bp
	R	CTGCTACAGCCTCAGCAGTG	

Abbreviations: CRX: Cone Rod Homeobox, MAP2: Microtubule Associated Protein 2, Tuj1: Beta tubulin, PKCalpha: Protein Kinase C alpha

RESULTS

Retinoblastoma cells express mature markers at the protein level

Cells were tested for CRX, Tuj1, recoverin, MAP2 and PKC alpha expression by immunocytochemsitry. WERI-RB27 showed high levels of recoverin and MAP2 expression with 35% and 25% of cells staining positive respectively. Two and 10% of Y79 cells were immunopositive for recoverin and MAP2 respectively (Figure 1). CRX, Tuj-1 and PKCa showed similar levels of protein expression between the two cell lines, with 5–10% of the cells being immunoreactive. Controls were immunonegative.

Retinoblastoma cells express mature markers at the RNA level

For qPCR analysis, mature retinal marker expression was compared in Y79, WERI-RB27 and MDA-MB231, a breast cancer control cell line. Y79 expression was set at one and the other cell types were compared to it. MDA-MB231 cells exhibited low levels of all mature retinal markers except for PKCa, as expected, as these cells are known to contain high levels of PKCa [8]. Relative levels of recoverin expression were similar in both cell lines using qPCR. RNA expression levels were about two times higher in Y79 for PKCa, Tuj-1, and CRX. MAP2 RNA expression was 7–10 times higher in WERI-RB27 cells than in Y79 cells (Figure 2).

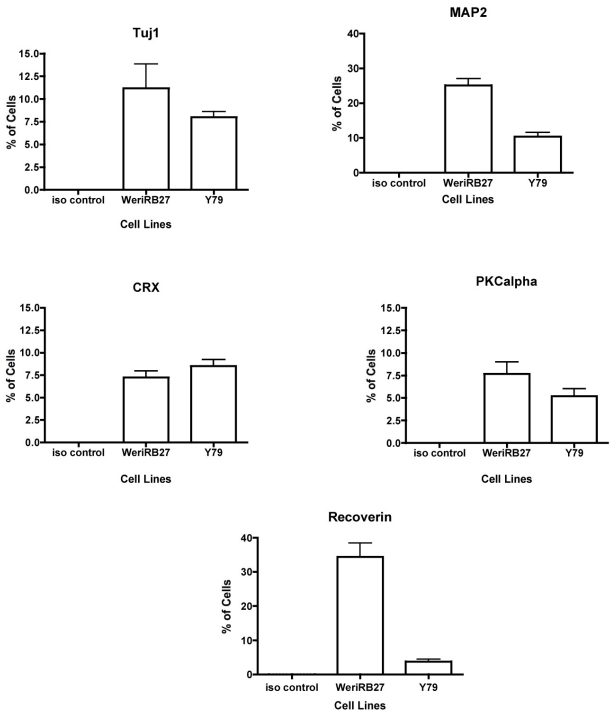

Figure 1: Quantitative immunocytochemistry of mature retinal markers Tuj1, MAP2, CRX, PKC alpha and recoverin Y79 and WERI-RB27 cells were analyzed by immunocytochemistry and found to contain subpopulations of cells immunoreactive to Tuj1, MAP2, CRX, PKCalpha, and recoverin. Three groups of 100 cells were counted for each marker and the percentage of immunopositive cells was graphed. Negative controls consisted of isotype control antibody instead of primary antibody.

DISCUSSION

We previously noted the presence of stem cell markers in retinoblastoma cell lines [6, 7]. Here we report on the comparative levels of mature retinal

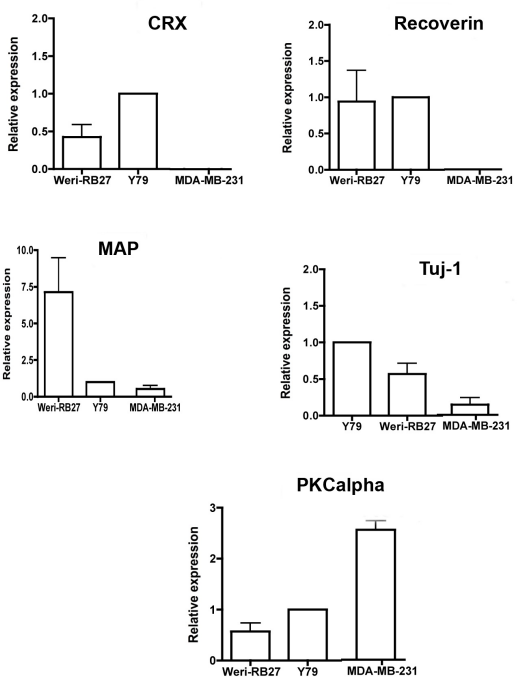

Figure 2: q RT PCR of mature marker expression: qPCR was performed using GAPDH as the reference control. Y79 was set at one and Weri-RB27 and MDA-MB231 were compared to it to determine relative expression. Y79 and WERI-RB27 cells showed expression of all mature markers, with MAP2 expressed at a 7–10 times higher level in WERI-RB27. MDA-MB231 control cells showed very little or no expression of mature markers at the RNA level, except for PKCalpha, which displayed high levels as previously reported.

markers in these cells. Y79 and WERI-RB27 cell lines both exhibit expression of mature markers CRX, PKCa, recoverin, Tuj1 and MAP2 at the protein and mRNA levels.

The origin of retinoblastoma is controversial, with some evidence implying a neuronal lineage, as indicated by expression of neuronal markers MAP2 (Microtubule-associated protein- 2) and beta tubulin [9–12]. The data regarding MAP2 expression in the present study corroborate this conclusion, as MAP2 was found to be expressed at high levels in WERI-RB27 at both the protein and mRNA levels.

Recoverin is a photoreceptor marker [13, 14] and was found to be present in high levels in WERI-RB27 using immunocytochemistry. The discrepancy between

protein and RNA expression of recoverin may be explained by post-transcriptional processing.

Lower levels of CRX, a photoreceptor homeobox transcription factor [15–17], and Tuj1, a retinal ganglion cell marker [18], were expressed by both cell types at the protein and mRNA levels.

MDA-MB231 cells expressed high levels of PKCa, a bipolar cell marker in the retina [19, 20], as the marker also labels aggressive breast cancer cells [8].

CONCLUSION

WERI-RB27 and Y79 retinoblastoma cell lines express a variety of mature markers representing several retinal cell types. This observation reinforces the potential capacity for differentiation into more mature cells. Future studies into the nature of these cells are warranted, as these markers may potentially be used as target endpoints for differentiation therapies.

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Acknowledgements
This work was supported by the Cornell Center on the Microenvironment and Metastasis through Award Number U54CA143876 from the National Cancer Institute, R21CA127061 and NYSTEM C026412. We thank Dr. Catherine Foster and Dr. Senthil Manohar for technical advice and assistance.

Author Contributions
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Anthony Bejjani – Acquisition of data, analysis and interpretation of data, Critical revision of the article, Final approval of the version to be published
Meerim Choi – Acquisition of data, analysis and interpretation of data, Critical revision of the article, Final approval of the version to be published
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Guarantor
The corresponding author is the guarantor of submission.

Conflict of Interest
Authors declare no conflict of interest.

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