Dopachrome tautomerase is a retinoblastoma-specific gene, and its proximal promoter is preferentially active in human retinoblastoma cells

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Purpose: Retinoblastoma (RB) is a malignant childhood intraocular tumor. Current treatment options for RB have undesirable side effects. A comprehensive understanding of gene expression in human RB is essential for the development of safe and effective new therapies.

Methods: We reviewed published microarray and RNA sequencing studies in which gene expression profiles were compared between human RB and normal retina tissues. We investigated the expression of genes of interest using quantitative reverse transcription PCR. We examined the activities of cloned promoter DNA fragments with luciferase assay.

Results: Dopachrome tautomerase (DCT) was among the most overexpressed genes in RB in published studies. We found that DCT was highly expressed in six of 13 samples microdissected from Thai RB tissues. Expression of DCT was absent or barely detected in retina tissues, various human ocular cells, and major organs. We also demonstrated that the −657 to +411 DCT promoter fragment efficiently directs RB cell–specific transcription of the luciferase reporter gene in cell lines.

Conclusions: The present work highlights that DCT is one of the most RB-specific genes. The regulatory elements required for this cell-specific gene expression are likely located within its proximal promoter.

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Retinoblastoma (RB) is the most frequent primary cancer of the eye in childhood and predominantly affects young children under the age of 5 years [1,2]. The global incidence of RB is 1 in 15,000 to 1 in 20,000 live births per year. Most affected individuals live in underdeveloped countries. In Thailand, the estimated incidence of this eye cancer is about 3.1 per 1 million people [3]. The most common initial sign of RB is leukocoria, an abnormal white reflection from the retina in an eye with RB. Other signs of RB include redness of the eye and painful or bulging eye.

Untreated RB can be life-threatening because of its capability to spread outside the eye or to other parts of the body, including lymph nodes, bones, bone marrow, brain, spinal cord, or liver. Early diagnosis and prompt treatment can save the eyes and lives of children with RB. RB-associated mortality rates are relatively high in developing countries [4]. The 5-year overall survival rate is 73% for Thai children with RB [3].

The current standard treatment for RB can cause eye problems and other side effects. Cataracts, dry eye, facial asymmetry, and dental problems may occur after radiotherapy. Patients treated with chemotherapy may develop complications in the eye, kidney, or hearing. Enucleation is considered the primary treatment for advanced RB. Although this surgery is the best way to save these children’s lives, it can be a stressful experience for pediatric patients and their families. The surgery also affects the quality of life of RB survivors [5].

Almost all RB is caused by either germline or somatic mutations in the RB transcriptional corepressor 1 (RB1; Gene ID: 5925, OMIM 614041) gene, which encodes a tumor suppressor protein. Children born with germline mutations tend to have bilateral tumors. RB could also be caused by
amplification of the MYCN proto-oncogene, bHLH transcription factor (MYCN; Gene ID: 4613, OMIM 164840) gene. Other somatic genomic alterations are rare [6].

Genetic changes in RB consequently lead to alteration of the gene expression pattern. Comparisons between the patterns of genes expressed in RB and normal retina tissues have been previously demonstrated with microarray and RNA sequencing analyses [7-12]. Proposed dysregulated pathways in RB include, but are not limited to, the PI3K/AKT/mTOR pathway, DNA damage-response pathways, aryl hydrocarbon receptor signaling, polo-like kinase and mitosis, and purine metabolism pathways.

Studies on RB gene expression profiling not only help us understand RB biology but also allow us to further unravel potential candidate genes that could be therapeutic targets for RB treatment. In this study, we aimed to identify the most RB cell–specific gene. Its promoter may be useful in controlling gene expression in RB cells for expression-targeted gene therapy.

METHODS

Human specimens and cell lines: The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (approval number Si 075/2016). Thirteen retinoblastoma specimens submitted to the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University during the years 2013 to 2018 were included in this study. The data were analyzed anonymously. We excluded samples from analysis based on the availability of an adequate amount of tissue and extracted RNA.

Nine human ocular RNA samples were purchased from 3H Biomedical (Uppsala, Sweden). They were total RNA samples prepared from early passage human corneal epithelial cells, retinal astrocytes, ocular choroid fibroblasts, trabecular meshwork cells, keratocytes, RPE cells, lens epithelial cells, conjunctival fibroblasts, and nonpigment ciliary epithelial cells.

All human cancer cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA). ATCC-formulated RPMI 1640 Medium (Gibco; Waltham, MA) was used to culture the Y79 cell line supplemented with 20% fetal bovine serum (FBS, Biochrom, Berlin, Germany). The NCI-H1975 and HCT-15 cell lines were cultured in 10% FBS-supplemented RPMI-1640 media (Gibco). The ARPE-19 cells (a generous gift from Associate Professor Kanokpan Wongprasert, Faculty of Science, Mahidol University, Bangkok, Thailand), a human RPE cell line, were cultured in Dulbecco’s Modified Eagle Medium: Nutrient Mixture F-12 (DMEM:F12; Thermo Scientific, Waltham, MA). All cell lines were maintained at 37 °C in a humidified incubator in an atmosphere of 5% CO₂ in air. Twenty-five short tandem repeat (STR) loci plus the gender-determining locus, amelogenin, were performed using Investigator 26plex QS kit. Data were analyzed using ATCC STR Profiling Analysis software. Appropriate positive and negative controls were run and confirmed for each sample submitted. The STR analyses are presented in Appendix 1. All cell lines were authenticated using short tandem repeat (STR) analysis with the Investigator 26plex QS kit (QIAGEN, Hilden, Germany).

Bioinformatic analysis: Microarray differential expression profiles for retinoblastoma and normal retina tissue were retrieved from a previous study [8-10]. Sequence read archive (SRA) data for retinoblastoma and retina tissue were collected from previous transcriptomic studies [11,12]. SRA data were converted to FASTQ using the SRA tools (https://github.com/ncbi/sra-tools). The resulting data were then subjected to FASTQC for quality control. Low-quality reads and technical sequences were removed using Trimomatics version 0.39 [13]. The cleaned read sequences were mapped to the human genome sequence (GRCh38) with HISAT2 (v 2.1.0) [14]. Samples with an overall mapping rate of less than 70% were not included in further analysis.

For gene expression quantification, a raw read count was performed using the HTseq Python script for baseline expression. Differential expression analysis of retinoblastoma versus normal retina tissue was calculated using the edgeR R package [15]. Differential expression genes (DEGs) were filtered with the criteria [log2FC] >1 and a false discovery rate (FDR) <0.01. Genes with significant upregulation were further analyzed with Gene Set Enrichment Analysis (GSEA) software [16]. To evaluate gene expression abundance, we calculated the transcript per million (TPM) of each aligned read with TPMcalculator [17].

Laser capture microdissection: The formalin-fixed paraffin-embedded (FFPE) tissues from patients with retinoblastomas were cut in 5 μm thickness using a microtome and placed on the MMI membrane slides. Slides were incubated in xylene and absolute ethanol before cresyl violet staining [18]. Areas of interest were microscopically identified. Target cells from the slides were excised using a laser beam from the MMI CellCut Plus (Molecular Machines & Industries; Eching, Germany) and collected on the MMI isolation caps.

RNA isolation and cDNA synthesis: Total RNA was isolated from microdissected tissues using an FFPE-T RNA extraction kit (Roche, Basel, Switzerland). The RNA concentrations were determined using a spectrophotometer (FLUOstar
Omega, BMG Labtech; Ortenberg, Germany). cDNA was synthesized with the Sensiscript Reverse Transcription Kit (QIAGEN).

The RNeasy Mini Kit (QIAGEN) was used for RNA extraction from the cell lines. Cell line RNA and human ocular RNA samples were converted to cDNA using the Omniscript reverse transcription kit (QIAGEN).

Quantitative PCR (qPCR): Quantitative real-time PCR for the gene expression study was performed on the LightCycler 480 System (Roche) using FastStart SYBR Green Master (Roche) according to the manufacturer's instructions. The qPCR protocol began with one cycle of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The melting curve step (72 °C to 95 °C) occurred after the amplification step. PCR products were used for gel electrophoresis to determine specific product amplification using 2% agarose gel. The 18s rRNA (Gene ID: 106631781, OMIM 180450) gene was used as a housekeeping gene for the gene expression analysis. The delta-deltaCt method was used to normalize the expression levels. Oligonucleotide sequences are shown in Appendix 2.

Plasmid constructs: We amplified human DCT promoter fragments from Y79 genomic DNA using Taq DNA polymerase (Thermo Scientific). The sequences for the amplification primers are shown in Appendix 2. We replaced the EcoRI-BamHI fragment of the pGLuc-Basic vector (New England Biolabs, Ipswich, MA) with the promoter fragments. Plasmid sequences were verified using Macrogen Sanger (Seoul, South Korea) sequencing services.

Luciferase reporter assay: We transiently transfected two plasmid DNA constructs, 210 ng of Gaussia luciferase construct containing the promoter of interest and 40 ng of cytomegalovirus (CMV) promoter-driven Cypridina luciferase construct, to cells in 96-well plates using Lipofectamine 2000 (Life Technologies, Carlsbad, CA). We collected the culture media 48 h after transfection. We then sequentially detected secreted luciferases using Gaussia luciferase and Cypridina luciferase assay kits (New England Biolabs). Luciferase bioluminescence was measured using a FLUOstar Omega plate reader (BMG Labtech). Luciferase expressed from the Gaussia luciferase reporter plasmid was normalized by the activity of Cypridina luciferase.

RESULTS

Commonly upregulated genes in RB are mainly related to the cell cycle: We first performed a literature search in PubMed and found four microarray studies on the gene expression profile of RB published between 2007 and 2013 (Table 1) [7–10]. The number of RB tissues in each study varied from three to 21 samples. Gene expression levels in RB were compared with those in normal retinas (normal retinas in the same patient, normal adult retinas, or fetal retinas). Significant overexpression was seen in 1,004–2,106 genes (2.3–7.3% of genes contained on the microarray chip used in each study). We excluded one microarray data set published by Chakraborty et al. [7] who examined the smallest number of genes (about 19,000 genes), from further analysis.

In addition, we reanalyzed RNA sequencing data from ten RB and six retina samples available in two transcriptome data sets (accession numbers PRJNA436090 and PRJNA517916) deposited in the NCBI Gene Expression Omnibus (GEO; Table 1 and Appendix 3) [11,12]. Four data sets (three RB: SRR8507306, SRR8507307, and SRR8507308; and one normal retina: SRR8507309) were excluded from further analysis because they had a low percentage of reads that mapped to the human reference genome.

Overlap between gene sets is demonstrated in Figure 1A. A total of 116 genes were commonly upregulated in RB samples among the RNA-Seq data and three microarray data sets. To gain mechanistic insight into this gene list, we performed gene ontology (GO) enrichment analysis and found that the top three significant GO terms were related to the cell cycle (Figure 1B), i.e., GO_CELL_CYCLE, GO_CELL_CYCLE_PROCESS, and GO_MITOTIC_CELL_CYCLE.

DCT is the most-expressed gene in RB tissues and is undetectable in normal retinas: To identify genes with high expression in RB and low expression in retina, we first classified 116 commonly upregulated genes based on the level of expression in reanalyzed RNA-Seq data from seven RB samples. None of these genes had a high level of expression (>1,000 TPM). The median expression level of 17 genes in RB was medium (11 to 1,000 TPM), including TMSB15A (Gene ID: 11013, OMIM 300939), MCM7 (Gene ID: 4176, OMIM 600592), TMEM97 (Gene ID: 27346, OMIM 612912), NT5DC2 (N/A), ZWINT (Gene ID: 11130, OMIM 609177), UBE2C (Gene ID: 11065,OMIM 605574), MYCN (Gene ID: 4613 OMIM 164840), FANCQ (Gene ID: 2189, OMIM 602956), CENPV (Gene ID: 20161 OMIM 608139), TYMS (Gene ID: 7298, OMIM 188350), REC8 (Gene ID: 9985 OMIM 608193), DCT (Gene ID: 1638, OMIM 191275), HMGB3 (Gene ID: 3149 OMIM 300193), CDC20 (Gene ID: 991; OMIM 603618), KPNA2 (Gene ID: 3838 OMIM 600685), TCF19 (Gene ID: 6941, OMIM 600912), and RXRG (Gene ID: 6258 OMIM 180247). Interestingly, dopachrome tautomerase (DCT) had an expression level below the 0.5 TPM cut-off value in four of the five retina samples (Figure 2). The TPM for DCT in one
retina sample was 0.85, which is considered a low expression level.

We next examined laser-microdissected formalin-fixed paraffin-embedded tissues collected from 13 Thai patients with RB to investigate whether RB tissues from Thai patients showed similar DCT expression patterns. The characteristics of these patients with RB are described in Table 2. We performed quantitative reverse transcription PCR (qRT-PCR) and demonstrated that six of the 13 RB tissues (46.2%) from the patients had high DCT expression (Figure 3A). The expression levels of DCT varied across these samples. The levels of DCT expression in five RB samples, that is, samples 2, 3, 9, 12, and 13, were higher than those in the Y79 retinoblastoma cell line. Due to the limited amount and quality of extracted FFPE RNA from normal retinas in the same

| Table 1. Summary of published data focused on gene expression profiling in retinoblastoma tissues. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **Microarray**                  | **RNA sequencing**              | **Platform**                    | **Retinoblastoma tissues**      | **Normal control tissues**     |
| Chakraborty S. et al., 2007 [7] | Chai P. et al., 2018 Bioproject number PRJNA436090 [11] | Human 19K cDNA microarray (University Health Network) (19,000 genes) | Number of tissues 3 | Number of tissues 10 (normal retina) |
| Ganguly A. and Shields C., 2010 [8] | Rajasekaran S. et al., 2019 Bioproject number PRJNA517916 [12] | GeneChip Human U133 V2.0 microarray (Affymetrix) (38,500 genes) | Age of patients (years) N/A | Age of donors/patients (years) N/A |
| Kapatai G et al., 2013 [9] | Human Gene 1.0ST (Affymetrix) (28,869 genes) | Human Gene 19K cDNA microarray (University Health Network) (19,000 genes) | Number of tissues 6 (0.9, 1, 1.1, 1.3, 2, 5) | Number of tissues 6 (5 to 22) |
| Nalini V. et al., 2013 [10] | Illumina TruSeq RNA Sample Prep Kit, HiSeq 2000 | Illumina TruSeq RNA Library Prep Kit, HiSeq 2500 | Age of patients (years) N/A | Age of donors/patients (years) N/A |
| **Number of tissues** | **Gene** | **Normal** | **Number of tissues** | **Gene** | **Normal** |
| 3 | DYNC1I2 | 10 | 1,004 | DEPC11, |
| 6 | TOR1AIP1 | 6 (retina in opposite quadrant to RB) | 1,116 | NDC80, |
| 21 | ZNF767 | 96-day human fetal retina | 2,106 | NEIL3, |
| 3 | MBOAT5 | (adult retina) | 1,672 | KIF20A, |
| 3 | RBM5 | (para-tumor) | N/A | GATA4, |
| 3 | LRRCS8 | (frozen retina) | N/A | IGFBL1, |
| 2 | PCSK5 | 1,004 | N/A | CKAP2L, |
| 2 | GNPTAB | 11 | N/A | AL451127.1, |
| 2 | ZNF551 | 11 | N/A | MNX1-AS1, |
| 2 | SPATA21 | 11 | N/A | AC112777.1, |
| **Top ten genes with highest expression in RB tissue** | **Gene** | **Normal** | **Top ten genes with highest expression in RB tissue** | **Gene** | **Normal** |
| DYNC1I2 | DCT, | 1 | DEPC11, |
| TOR1AIP1 | TOP2A, | 1 | NDC80, |
| ZNF767 | DCT, | 1 | NEIL3, |
| MBOAT5 | SNORD41, | N/A | KIF20A, |
| RBM5 | HIST1H3I, | 1 | GATA4, |
| LRRCS8 | DCT, | 1 | IGFBL1, |
| PCSK5 | SNORD29, | 1 | CKAP2L, |
| GNPTAB | SNORDA23, | 1 | AL451127.1, |
| ZNF551 | SNORA7ID, | 1 | MNX1-AS1, |
| SPATA21 | SNORA3, | 1 | AC112777.1, |
Figure 1. Overlap of gene sets and GO enrichment analysis. A: Venn diagram showing overlap in the number of genes identified as upregulated genes in RB from 3 microarray and reanalyzed RNA-seq data. B: Gene/Gene Set Overlap Matrix of 116 commonly upregulated genes. Overlaps between the list of 116 commonly upregulated genes and the top 10 most significantly enriched Gene Ontology terms (FDR q-value less than 0.05) was demonstrated using Gene Set Enrichment Analysis (GSEA) software.
Figure 2. Gene expression levels of 116 commonly upregulated genes. The results are displayed as transcripts per million (TPM) in seven retinoblastoma (RB) and five retina (R) samples, collected from two publicly available RNA-sequencing data sets (designated as A and B).

Medium blue box: medium expression, light blue box: low expression, gray box: expression level below 0.5 TPM.

Table 2. Clinical characteristics of 13 Thai retinoblastoma patients included in gene expression analysis of RB and retina tissues.

| Patient number | Age (months) | Gender | Type | Tumor classification | Eye | Enucleation | RB tissues | Retina tissues |
|----------------|--------------|--------|------|---------------------|-----|-------------|------------|---------------|
| 1              | 14           | Female | Unilateral RB | E     | Right | Primary enucleation | Y          | Y             |
| 2              | 36           | Male   | Unilateral RB | E     | Right | Primary enucleation | Y          | Y             |
| 3              | 6            | Female | Bilateral RB  | E     | Left  | Primary enucleation | Y          | N             |
| 4              | 16           | Male   | Bilateral RB  | D     | Right | Secondary enucleation | Y          | N             |
| 5              | 39           | Male   | Bilateral RB  | E     | Right | Secondary enucleation | Y          | N             |
| 6              | 36           | Female | Bilateral RB  | E     | Right | Primary enucleation | Y          | N             |
| 7              | 5            | Male   | Bilateral RB  | E     | Left  | Secondary enucleation | Y          | N             |
| 8              | 24           | Male   | Unilateral RB | E     | Left  | Primary enucleation | Y          | N             |
| 9              | 24           | Male   | Bilateral RB  | E     | Left  | Secondary enucleation | Y          | N             |
| 10             | 25           | Male   | Unilateral RB | E     | Right | Primary enucleation | Y          | N             |
| 11             | 40           | Male   | Unilateral RB | D     | Left  | Secondary enucleation | Y          | N             |
| 12             | 3            | Female | Bilateral RB  | E     | Left  | Primary enucleation | Y          | N             |
| 13             | 18           | Male   | Unilateral RB | E     | Left  | Primary enucleation | Y          | N             |
patients with RB, we performed qRT-PCR in only two retina tissues (designated as N1 and N2). Expression of the DCT gene was not detected in either retina sample (Figure 3B).

Expression of DCT is barely detectable in various human ocular cells and major organs: We performed RT-qPCR to investigate DCT expression in nine human ocular cells and demonstrated that DCT was minimally detected in the ocular choroid fibroblasts, RPE cells, and nonpigment ciliary epithelial cells (Figure 3C). The expression of DCT in ocular choroid fibroblasts, RPE cells, and nonpigment ciliary epithelial cells was 12.61-, 89.33-, and 86.00-fold lower than in Y79 cells, respectively. No DCT expression was detected in six human ocular cells: retinal astrocytes, lens epithelial cells, corneal epithelial cells, trabecular meshwork cells, keratocytes, and conjunctival fibroblasts.

To further investigate the expression of DCT genes in other cell types in the human body, we explored the large-scale database of gene expression in human tissues from the NIH-funded Genotype-Tissue Expression (GTEx) project [19]. We found that this gene is not detectable (<0.5 TPM) in major organs, including brain, kidneys, liver, pancreas, stomach, intestine, and lungs (Figure 3D,E).

Proximal DCT promoter is active in retinoblastoma cells: To determine whether the proximal promoter of the DCT gene could drive RB-specific transgene expression, we performed luciferase assays in the Y79 and ARPE-19 RPE cell lines. We found that the −657/+411 DCT promoter fragment was active in the Y79 cells (Figure 4A). This promoter activity in the Y79 cells was about ten times higher than that in the ARPE-19 cells (Figure 4B).

Figure 3. DCT gene expression in retinoblastoma (RB) tissues, normal retinas, ocular cells, and other tissues. A: DCT expression normalized to 18s rRNA in 13 Thai RB tissues. The quantitative polymerase chain reaction (qRT)-PCR results are shown as relative expression compared to DCT expression in Y79 cells. B: DCT and 18s rRNA expression in two retinas and two RB tissues from the same patients. The PCR products were demonstrated with gel electrophoresis. C: DCT expression in nine normal human ocular cells. The qRT-PCR results are shown as relative expression compared to DCT expression in Y79 cells. D: Expression of DCT in normal tissues from the GTEx database. E: Relative DCT expression in normal tissues in relation to 16 upregulated genes with medium expression levels in RB.
We also performed a promoter study in NCI-H1975 (non-small cell lung cancer) and HCT15 (colorectal adenocarcinoma) cell lines. The activity of the proximal DCT promoter fragment was not detected by the luciferase assay in these lung and colorectal cancer cells (Figure 4C,D).

Statistical analysis: We presented all data as the mean ± standard deviation (SD) or the standard error of the mean (SEM). We compared sample groups with Student’s two-tailed t test. A p value of less than 0.05 was considered statistically significant.

DISCUSSION

The gene expression profile of retinoblastoma was first reported in 2007. A human 19K cDNA microarray was used to analyze gene expression in the retinoblastoma and normal retina samples at that time. Later, three studies used Affymetrix microarray chips, which cover larger numbers of human genes, to identify the lists of upregulated and downregulated differentially expressed genes and the altered pathways in retinoblastoma [7-10]. It should be emphasized that these studies used different control tissues, including normal retinas from donors’ eyes, normal retinas from the same patients, and fetal retinas. Today, high-throughput sequencing technologies are routinely applied in numerous transcriptome studies. Our reanalysis of two recently published RNA-Seq data sets from RB and normal retina tissues [11,12], in combination with information from three previously reported microarray experiments, suggested that DCT is the most RB-specific gene.

The DCT gene, also known as tyrosinase-related protein 2 (TYRP2), encodes an enzyme required for the melanin biosynthesis pathway. The DCT enzyme catalyzes the tautomerization of dopachrome to 5,5-dihydroxyindole-2-carboxylic acid (DHICA) [20]. DCT expression was previously examined in human melanocytes, melanocyte stem cells, and melanoma cells [21]. To the best of our knowledge, only one study has focused on DCT expression in RB [22]. Japanese researchers performed an RT–PCR experiment and successfully demonstrated that DCT mRNA was highly expressed in three excised RB specimens and Y79 cells in study that has been conducted in 2001. The present results confirmed and extended previous findings regarding DCT expression in RB. The levels of expression in most DCT-expressed RB are greater than those in Y79 cells (Figure 3A). However, DCT was not expressed in all Thai RB tissues in the present study. This finding may be explained in part by retinoblastoma
tumor heterogeneity and heterogeneous study samples. Tumor heterogeneity in retinoblastoma has been reported in several studies [9,23-25]. The microdissected samples used in the present study contained only a small fraction of the tumor and thus, may not be a true representation of all the subclones present in retinoblastoma.

The present study highlighted that the expression levels of DCT in normal human cells and tissues are clearly lower than those in RB cells and tissues, if not undetectable. A previous report showed the presence of DCT expression in ARPE-19 cells [26]. However, the expression levels of DCT in RB appeared much higher than those detected in these cells. Instead of using RNA from the ARPE-19 cell line, in the present qRT-PCR experiments, we used RNA samples prepared from early passage human RPE cells and obtained similar results. DCT mRNA expression in RB cells was much higher than that in RPE cells (Figure 3C). We additionally reported two more human ocular cells with minimal expression of DCT, i.e., ocular choroid fibroblasts and nonpigment ciliary epithelial cells. No DCT expression was detected in the rest of the normal human ocular cells included in the present experiments and in two microdissected normal retina tissues from patients with RB. The RNA-Seq results available from the GTEx database provide more insight into the expression levels of genes in human tissues (Figure 3D,E). These findings underline the fact that DCT is absent or barely expressed in normal tissues.

DCT is also expressed in other cancers derived from melanocytes and neuronal cells, such as melanoma, glioma, and glioblastoma [27,28]. Little is known about the role of DCT in retinoblastoma and these cancers. DCT overexpression in a melanoma cell line resulted in increased activity of the extracellular signal-regulated kinases (ERK)/mitogen-activated protein kinase (MAPK) pathway, which may play an important role in radiation and drug resistance [29]. DCT functions have been investigated in other cell types, including promoting proliferation of neural progenitor cells and regulating reactive oxygen species levels, DNA damage, and the cell cycle in human epidermal keratinocyte cell lines [30,31].

More experimental research is needed to determine whether DCT plays similar roles in retinoblastoma. Cell-specific promoters are beneficial to basic science and gene therapy studies. Use of the RB cell–specific promoter is desirable in the expression cassette to control the expression of the therapeutic gene in RB cells and restrict unwanted expression in other cells. It has been shown that the human DCT promoter construct containing the 3.6 kb 5’-flanking region efficiently directed the expression of the luciferase reporter gene in Y79 cells [22]. Our promoter studies provide preliminary evidence that the −657/411 DCT promoter fragment is likely an RB-specific promoter because it is active in Y79 RB cells, not in normal RPE ARPE-19 cells or other tested cancer cells, that is, NCI-H1975, and HCT15 cells. Further studies on other ocular cells and cancer cells are needed to confirm the specificity of this promoter fragment.

Taken together, the study results suggest that the most RB-specific gene is DCT. Its proximal promoter fragment is possibly useful as an RB cell–specific promoter. These findings enhance the ability to develop novel therapies that can specifically eliminate retinoblastoma cells without many side effects.

APPENDIX 1. STR ANALYSIS
To access the data, click or select the words “Appendix 1.”

APPENDIX 2. OLIGONUCLEOTIDES USED IN THIS STUDY.
To access the data, click or select the words “Appendix 2.”

APPENDIX 3. OVERALL MAPPING RATE OF SEQUENCE READ ARCHIVE (SRA) DATA OF RETINOBLASTOMA AND NORMAL RETINA TISSUE.
To access the data, click or select the words “Appendix 3.”

ACKNOWLEDGMENTS
This work was supported by the Royal Golden Jubilee Ph.D. Scholarship (PHD/0179/2554) from Thailand Research Fund (K. Moolsuwan) and Siriraj research fund (R015934004) from the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. The authors express their gratitude to Pichpisith Pierre Vejvisithsakul and Nonthawut Chat-utchai for their help in optimization of gene expression analysis. Naravat Poungvarin (naravat.pou@mahidol.ac.th) is a co-corresponding author.

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