Original Article

Plasma Membrane Calcium ATPase Expression in Human Lens Epithelium Cell Lines

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Abstract

Purpose: To study the expression of four plasma membrane calcium ATPase (PMCA) isoforms in human lens epithelium cell lines (HLE-B3 cells) both on mRNA and protein levels.

Methods: Both total mRNA and membrane protein samples were collected, after HLE-B3 cells were cultured to 90% confluency. Reverse Transcription Polymerase Chain Reaction (RT-PCR) were used to detect mRNAs of PMCA isoform 1, 2, 3, and 4 by using corresponding PMCA isoform 1, 2, 3, and 4 primers. Western Blot analysis was employed to detect PMCA isoform 1, 2, 3, and 4 protein using corresponding anti-PMCA1, 2, 3, and 4 antibodies.

Results: A 420 bp fragment was amplified with PMCA1 primer. A 550 bp fragment was amplified with PMCA2 primer. A 840 bp fragment was amplified with PMCA4 primer. No fragment was amplified with PMCA3 primer. Western Blotting confirmed that the expected ~153 kDa, ~125 kDa and ~147 kDa protein were recognized by anti PMCA1, 2 and 4 antibodies respectively. No protein was recognized by PMCA3 antibody.

Conclusion: This is the first study showing only PMCA1, 2, and 4 gene are expressed in HLE-B3 cells on both mRNA and protein level. PMCA3 is not expressed in HLE-B3 cells. The PMCA isoforms expression pattern in HLE-B3 cell lines is different from that in the lens of other species. PMCA2 may play a more important role over other isoforms.

Keywords: plasma membrane calcium ATPase; gene expression; reverse transcription-polymerase chain reaction (RT-PCR); HLE-B3 cells; Western Blot

Introduction

It is well known that Ca^{2+} regulates numerous physiological cellular phenomena as a second messenger as well as triggering pathological events such as cell injury and death. The maintenance of normal Ca^{2+} homeostasis in lens is very important. Cortical cataracts in the human lens have been shown to involve a selective increase in calcium[1,2], increase intracellular Ca^{2+} and induce cortical opacification in the human lens[3].

The free intracellular calcium level in the lens is of 30 uM, while the extracellular calcium level is close to 2000 uM[4]. A huge transmembrane calcium gradient leads to a passive inward leak of calcium. This passive inward leak is balanced by extruding the Ca^{2+} outward to the extracellular space by plasma membrane calcium ATPase (PMCA) and Na^{+}–Ca^{2+} exchanger, or sequestering into sarcoplasmic reticulum (SR) by sarcoplasmic/endoplasmic calcium ATPase (SERCA), or binding to proteins by calcium binding protein (Hightower, 1985; Duncan, et al., 1993; Churchill GC and Louis, 1999) Among them, Plasma membrane calcium ATPase (PMCA) plays a pivotal role in keeping a low intracellular Ca^{2+} concentration because lens epithelial cells are non-muscle cells which have an extremely low resting intracellular calcium concentration[5,6]. Na^{+}/Ca^{2+} exchange plays a relatively minor role in calcium regulation, at least at resting calcium levels[7].

Plasma membrane calcium ATPase (PMCA) is a plasma membrane protein family composed of four isoforms and their respective alternative splice variants. Their molecular weight is about 125–150 kDa[8,9]. Four genes for the PMCA pump have been found in mammals, PMCA1, 2, 3 and 4[9]. Not surprisingly, the different isoforms have distinct patterns of tissue distribution and have different functional properties. The four PMCA genes are not equally expressed in all tissues. PMCA1 and PMCA4 are putative "housekeeping gene"[10], as they are widely expressed in ap-

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proximately equivalent amounts in all tissues analyzed so far. PMCA2 expression is mainly restricted to brain and heart, while PMCA3 expression is restricted in brain and skeletal muscle[11]. PMCA originally represent the gene coding plasma calcium ATPase, but recently this term has been used to refer both the protein and the genes. In this article, we use "PMCA gene" to represent PMCA DNA, "PMCA mRNA" for the mRNA and "PMCA isoforms" for PMCA protein.

The activity of PMCA is about four times higher in lens epithelium than in cortex, but can't be demonstrated in the nucleus of human lens[12], rabbit lens and bovine lens[13]. Ca^{2+}-ATPase activity was approximately 50% less in membranes prepared from cataractous lenses in comparison to clear lenses[14]. To reverse the decreased PMCA activity and restore calcium homeostasis in cataractous lens, the expression of PMCA and the regulation of its expression and activity in the lens are currently being investigated.

Although the expression patterns of PMCA have been investigated in bovine lens[15], rat lens[16] and porcine lens[17], and the PMCA pump activity has been detected in human lens, no information about the PMCA isoform expression pattern in human lens has been reported.

This study investigates the PMCA isoform expression pattern from HLE-B3 cell lines. The highest PMCA activity in human lens has been found in lens epithelium, But primary HLE cell culture is very difficult to acquire due to limited sources of the cells, low viability and delicacy of the cells. Even worse is that the amount of PMCA protein expressed on HLE membrane is very small[18]. Human Lens Epithelium B3 cell lines (HLE-B3 cells) are created by infecting human lens epithelium cells with Adeno12-simian virus 40 (Ad12-SV40)[19]. In this way, human lens epithelium cells have an extended life span while maintaining lens specific properties, such as the capability to express crystallins αA, αB and βB2[20]. It has been a good model for investigating the physiology of human lens epithelium cells. The possible role of the PMCA expression in HLE-B3 cell lines is also discussed here.

Materials and Methods

HLE-B3 cell culture

Human Lens Epithelium-B3 cells (a transformed human lens epithelial cell line kindly provided by Dr. Usha Andley) were cultured in MEM medium (Sigma, St. Louis, MO) with 50 μg/ml gentamycine and glutamine and 10% fetal bovine serum. Cells were incubated under 5% CO_2 and 95% O_2 at 37℃.

Cell membrane protein preparation

When the cells were 90% confluent, the culture medium was discarded and the cells washed three times with phosphate buffer (1 mM sodium phosphate and 0.09% NacL, pH7.4). Cell growth and activity were stopped by adding cold buffer A (150 mM sucrose, 10 mM HEPES, 0.9 mM dithiothreitol and 0.05 mM EGTA) containing the protease inhibitors antipain (16.5 mM), leupeptin (21 mM), pepstatin A (14 mM), PMSF (40 mM) and aprotinin (0.027 trypsin inhibitor units/ml).

Scraped off the cells from the flask and put them into centrifuge tube. Dispersed and homogenized the cells by ultrasonifiction (branson, Ultrasonics Co., Danbury, CT, USA.) at medium power for 3 × 5 sec with a 3 min ice-cooling interval. The homogenate was centrifuged at 115 000 g for 60 min. Discard the supernate and the resulting pellet was resuspended in buffer A containing protease inhibitors and briefly sonicated at a low power setting for 3 × 5 sec with a 3 min ice-cooling interval. The resulting membrane protein sample was then quick-frozen in liquid nitrogen and stored at −70℃. The protein concentration was determined using the Biorad protein assay kit (Biorad, Hercules, CA, USA).

Electrophoresis and protein transfer (comparison of 20 gel with 8 cm gel)

Membrane proteins and a rat brain microsome preparation (positive control) were solubilized in SDS reducing sample buffer containing 125 mM Tris (pH 6.8), 4% (w/v) SDS, 0.1% (v/v) Bromophenol blue, 5% (v/v) glycerol, and 10% β-mercaptoethanol seperately. Membrane proteins were separated by electrophoresis on 4% and 7.5% sodium dodecylsulfate-polyacrylamide gels in buffer systems containing 25 mM Tris and 192 mM glycine at pH 8.3. Approximately 40 μg of membrane protein from each sample was added to specific lanes. Prestained molecular-weight markers (Biorad, CA, USA.) were also

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used. Using PROTEAN II xi 20 mm Cell (Biorad, Hercules, CA, USA), sample proteins were separated at constant current of 16 mA/each gel until the front edge pass the stacking gel, which took about 1 h and 45 min. Then the current was changed to 24 mA/each gel until the front edge was 3 cm to the bottom of the gel, which took about 4–5 h.

The separated proteins were fixed in transfer buffer containing 25 mM Tris, 192 mM glycine and 10% methanol at pH 8.3 for approximately 30 min, then transferred to nitrocellulose membranes (Amersham Life Sciences, NJ, USA) at a constant voltage of 25 V overnight at 4℃ using the Tansblot Cell (Biorad, Hercules, CA, USA).

For the purpose of comparison, same membrane protein samples were also separated by traditional 8 cm gel SDS-PAGE. The equipment used was Mini-PROTEAN 3 Cell, with a separating area of 8 cm × 7.3 cm. The stacking gel is 4% and separating gel is 7.5%. Approximately 40 μg of membrane protein was loaded in each lane and prestained high-range molecular-weight markers (Biorad, CA, USA) were also loaded. The samples were separated at 200V for 45 min. Then, the separated proteins were fixed in transfer buffer containing 25 mM Tris, 192 mM glycine and 10% methanol at pH 8.3 for approximately 30 min. After that, the proteins were transferred to nitrocellulose membranes (Amersham Life Sciences, NJ, USA) at a constant voltage of 25 V at 4℃ using the Transblot Cell (Biorad, Hercules, CA, USA).

Western blot

For immuno-detection of the PMCA1, PMCA2, PMCA3 and PMCA4 proteins, the nitrocellulose was blocked for 1 h with 5% milk in Tris-buffered saline containing 25 mM Tris, 137 mM NaCl and 0.5% Tween at pH 7.4 (TBS-T). The nitrocellulose membranes were washed in Tris-buffered saline once for 15 min and twice for 5 min. They were then incubated with polyclonal anti-PMCA, anti-PMCA4 or monoclonal anti-PMCA3, PMCA2 and PMCA1 antibodies (ABR riffinity bioreagents, Inc., Golden, CO, USA) for 1 h. After incubation with primary antibody, the nitrocellulose was then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h. The nitrocellulose was again washed with TBS-T, once for 15 min and four times for 5 min before visualization by Enhanced Chemi Luminescence (Amersham, England, UK).

RT-PCR (Analysis of PMCA1 PMCA2 PMCA3 and PMCA4)

Total RNA extraction

Total RNA were extracted from HLE-B3 cells using Rneasy Mini Kit (Qiagen, Valencia, CA). When the cells are 90% confluency, discarded the culture medium and washed cells with PBS buffer once. Trypsin 0.10–0.25% (3–4 ml) was added to trypsinize the cell. After the cells detached from the flask, about 10 fold (30–40 ml) of culture medium was added to inactivate the trypsin.

The cells were transferred to RNase-free centrifuge tube and centrifuged for 300 g for 5 min. All the supernatant was completely aspirated. The cells were then disrupted by adding Buffer RLT, which contained guanidine isothiocyanate. The sample was then homogenized by pipetting the transparent lysate onto a QIAshredder and centrifuged for 2 min at the maximum speed (8000 g). One volume (usually 350 μl or 600 μl) of 70% ethanol was added to the resulting aqueous phase of the cell lysate and mixed well with the cell lysate by pipetting. 700 μl of this ethanol treated solution was taken to an RNeasy mini column and centrifuged for 15 s at the maximum speed. The remaining RNA pellet on the Rneasy mini column silica-gel membrane were rinsed with Buffer RW1 once and then rinsed with Buffer RPE twice. Finally, the total RNA was eluted out with water by pipeting 30–50 μl RNase-free water directly into the RNeasy silica-gel membrane and centrifuged for 1 min at 8000 g. The resulting RNA solution was stored under –20℃ or –70℃. The concentration of the RNA solution was detected by Cary 50 UV-Vis Spectrophotometer (Alltech Associates Inc., NSW, Australia).

Primers: The primers used were the same as those described in previous study[15].

Reverse transcription

For cDNA synthesis, 5 μg total RNA from HLE-B3 with random hexamer (50 ng per 1 μg RNA sample) was denatured at 65℃ for 5 min followed by at least 1 min of inubation on ice. The RNA was mixed

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Table 1 Primers

PMCA1	5′-TAGGCACTTTTGTGGTACAG-3′
pimer	5′-GCTCTGAATCTTCTATCCTA-3′
PMCA2	5′-AGATCCACGGCGAGCGCAAT-3′
primer	5′-CGAGTTCTGCTTGAGCGCGG-3′
PMCA3	5′-AGCTCAAGTGCCTGAAGGAAG-3′
primer	5′-CTGAAGAGGTAGCTGACTTGG-3′
PMCA4	5′-CATTCACCACCCAGCCAGCACTAT-3′
primer	5′-CGGTGAAAAGTCCCATCATCACC-3′

in 20 μl of PCR buffer containing 10 mM Tris-HCl (pH 8.3), 25 mM $MgCl_2$ and 5 mM dNTP mixture, and then incubated at 25℃ for 2 min. Two hundred units of SuperScript Ⅱ reverse transcriptase were then added. Control groups were prepared without adding reverse transcriptase. The reaction mixture was incubated at 42℃ for 50 min. The reaction was terminated at 70℃ for 15 min and treezed on ice. The reaction mixture was collected by brief centrifugation. Then 1 ml of Rnase H and Dnase was added, which was followed by 20 min of incubation at 37℃. In cDNA synthesis, a negative control containing no SuperScript Ⅱ reverse transcriptase was applied to demonstrate the absence of genomic DNA contmination. (The RT-PCR kit was purchased from Life Technology, Rockville, MD, USA) For amplification of the target cDNA, 50 μl of the PCR reaction solution containing 2.5 μl of 10 μm 5′ and 3′primer of PMCA1, 2, 3 or 4 seperately, 1 μl of first strand cDNA, 1.5 μl of 50 mM $MgCl_2$, and 1 μl of 10 mM dNTP were denatured for 5 min at 94℃.

Polymerase chain reaction

The PCR reaction was initiated by adding 1 to 3 U of Taq DNA polymerase. Using a PCR thermocycler (Perkin Elmer, Foster City, CA, USA), samples underwent 28 programmed cycles at 94℃ for 1 min (denaturing), 58℃ for 1 min (annealing), 72℃ for 2 min (extention), and 72℃ for 10 min (final extention). PCR products were visualized by ethidium bromide staining on agarose gel. (PMCA1, 2, 3 and 4 primers are listed in Table 1).

Results

RT-PCR analysis

RT-PCR was used to identify the expression of PMCA gene on mRNA level in HLE-B3 cells. Figure 1 showed a photograph of an ethidium bromide-stained agarose gel with our RT-PCR reaction products using four primer pairs. These primer pairs had been successfully used to identify expression of PMCA isoform gene on mRNA level[15]. In lane A, a 420 bp product was produced by using PMCA1 specific primer. The band was very clear and was the only band produced. In lane B, a 550 bp product was produced by using PMCA2 primer. But the amount of PMCA2 RT-PCR product was much smaller than PMCA1. When loading the original product 20 ul, the band was very faint.The band showed in Figure 1 was the effect of loading 20 μl of the 200% concentration of the original RT-PCR product.In lane C, primers that were specific for PMCA3 produced no bands at all. In lane D, a 840 bp product was observed. It is also the only RT-PCR product generated by PMCA4 primer in HLE-B3 cells. Samples without Reverse Transcriptase (RT) or cDNA template showed no bands (Fig 1 lane E and lane F).

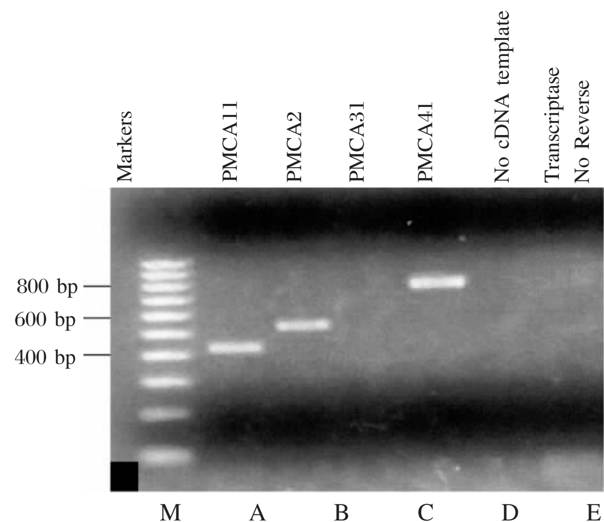

Figure 1 RT-PCR analysis of PMCA gene expression in HLE B-3 cells.Total RNA form HLE-B3 cells was reverse-transcribed, and then amplified by PCR using four pairs of primer flanking PMCA transcript splice site C.The PCR products were separated by agarose gel electrophoresis and visualized with ethidium bromide and photographed. Each lane was loaded with 20 ul of the PCR reaction mixture, except that the lane of PMCA2 was loaded with 20 μl 200% concentration of the original PCR reaction mixture.The molecular weight marker was a 100-bp DNA ladder.

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The expression of PMCA1, 2, 3, 4, by Western blot analysis

Upon establishing that HLE-B3 cells have PMCA1, 2, 4 mRNA expression, we then detected the PMCA1, 2, 4 isoforms and PMCA3 isoform as well in those cells by Western blot. We subjected the membrane protein sample prepared from HLE-B3 cells to Western blot with isoform-specific antibodies. A rat brain microsome preparation was used as a positive control. For PMCA1, an immunoreactive band at ~153 kDa was displayed when probed with PMCA1 specific antibody. At almost the same molecular weight position, a much thicker and broader band is observed in rat brain. For PMCA2, a very clear immunoreactive band at ~125 kDa was displayed when probed with PMCA2 specific antibody. Same as PMCA1, the signal of PMCA2 in rat brain positive control is much stronger. For PMCA3, no band was displayed at the expected molecular weight range when probed with PMCA3 isoform specific antibody. But in rat brain positive control, a weak but clear band at ~145 kDa was displayed. For PMCA4, an immunoreactive band at ~ 147 kDa was clearly displayed when probed with PMCA4 specific antibody. At the same molecular weight position, a much thicker and broader band is observed in rat brain.

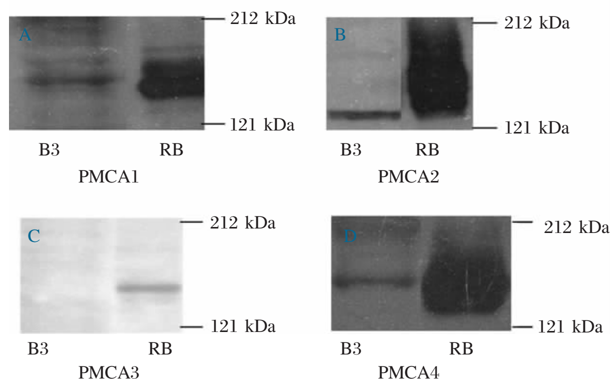

Figure 2 Western blot of PMCA isoform peptides determined in membrane protein prepared from HLE-B3 cells (left lanes labeled as B3), and a rat brain microsome preparation (right lanes labeled as RB), which was used as a positive control. The SDS-PAGE electrophoresis was done by a 20 cm^2 size gel. Samples were probed with PMCA isoforms specific antibodies, anti-PMCA1 to PMCA4. Anti-PMCA1, 2and 4 antibody were diluted to 1:2000, while Anti-PMCA3 antibody was diluted to 1:500. Right: Molecular weight markers (in kilodaltons).

Comparison of the Western blot result between 20 cm gel and 8 cm gel

In this study, the main electrophoresis method adopted is 20 cm gel electrophoresis, because this is the one we were going to use in two-dimensional electrophoresis study. In this way, the experiment will provide us with some preliminary information about in what molecular weight ranges will the PMCA isoform proteins be located. Since the 20 cm gel is much longer than the traditional 8 cm gel, it took much longer time (5–6 h) to finish than the 8 cm gel (0.5 h) did. In order to observe how these difference between two kinds of gels influence the electrophoresis behavior of the PMCA isoforms, the same protein samples were also subjected to Western blot analysis with 8 cm gel. Then the image of the 20 cm gel was shrinked proportionally to the size of 8 cm gel to make a comparison between them.

PMCA1, PMCA2 and PMCA4 isoforms protein were all recognized with 8 cm gel too. Before shrinking the image, the bands on 20 cm gel were generally more diffused and broader than in 8 cm gel. In Figure 3A, the relative migration distances of protein molecular weight marker 212 kDa, 121 kDa and 96 kDa were quite identical with each other between the 20 cm gel and 8 cm gel. Figure 3B showed in 20 cm gel and 8 cm gel, the apparent molecular weight of PMCA1 isoforms were both 153 kDa. Figure 3C showed in 20 cm gel, the apparent molecular weight of PMCA2 was just 125 kDa, but in 8 cm gel, the apparent molecular weight is as high as 153 kDa. So the PMCA2 isoform detected in 20 cm gel is about 28 kDa smaller in molecular weight than that in 8 cm gel. Figure 3D showed that PMCA4 isoform migrated to 147 kDa in 20 cm gel, and to 152 kDa in 8 cm gel. They were not exactly identical but very similar to each other.

Discussion

Different cell types have different Ca^{2+} handling mechanism which is achieved by specific expression of the various PMCA isoforms. A striking finding in this study is that PMCA isoforms expressed in HLE-B3 cells were PMCA1, 2 and 4, whereas PMCA3 was absent in HLE-B3 cells. This is the first study about the different PMCA isoforms expression in hu-

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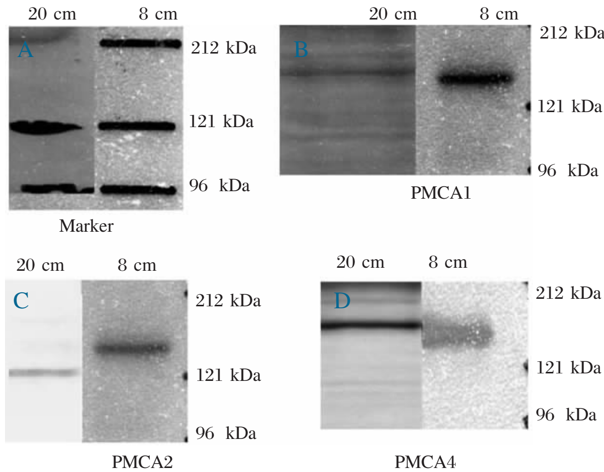

Figure 3 Comparison of the Western blot results between 20 cm gel and 8 cm gel. Left lanes were Western Blot result from 20 cm gel, and right lanes were from 8 cm gel. A. The relative migration distance of the same molecular weight markers was identical between 20 cm gel and 8 cm gel, 153 kDa. B. The relative migration distance of PMCA1 was the same between 20 cm gel and 8 cm gel. C. PMCA2 migrate to a apparent MW of 125 kDa in 20 cm gel, while in 8 cm gel it migrated to an apparent MW of 153 kDa. D. PMCA4 migrated to 147 kDa in 20 cm gel, and to 152 kDa in 8 cm gel.

man lens epithelium cell lines both on mRNA level and protein level.

Our finding that PMCA3 is not expressed in HLE-B3 cells is confirmed on both mRNA and protein levels. Besides the pair of PMCA3 primer mentioned in Materials and methods, another 2 pairs of PMCA3 primers were designed and used. However, the result was still negative in HLE-B3 cells.The RT-PCR conditions were changed, such as the annealing temperature, Mg^{2+} concentration in reaction mixture and the number of reaction circles.PMCA3 mRNA was still not found. In Western Blot, using the same anti-PMCA3 antibody, PMCA3 protein was detected in the rat brain control sample, but not in the HLE-B3 sample. All the above evidence confirmed that PMCA3 was missing in HLE-B3 cells. This study provides support to the previous finding that PMCA3 has a restricted distribution, mainly in brain and skeletal muscle[11].

PMCA isoform expression pattern in the lens may vary among different species (Table 2 summarizes the inter-species differences in PMCA isoform expression in the lenses). In porcine lens epithelium, Western Blot analysis indicates the presence of only two PMCA isoforms, PMCA2 isoform and PMCA4 isoform[17], whereas the house keeping PMCA1 isoform is missing. In normal rat lens, only isoform PMCA1b is expressed[16]. However, our previous study has demonstrated that all four PMCA mRNAs are expressed in bovine lens and PMCA3 mRNA is the most abundant form[15]. This phenomemon is also seen in other organs.In rat liver, no PMCA4 is found[21,22], whereas in human liver, PMCA4 is the most abundant form[23]. Investigation of the physiological properties of different PMCA isoforms should provide more insight into the role of different PMCA isoform in regulating calcium homeostasis in the lens tissue of different species.

Now PMCA1, PMCA2 and PMCA4 are all detected in HLE-B3 cells. PMCA1 and PMCA4 are widely expressed in approximately equivalent amounts in all tissues analyzed so far, so it was not surprising for them to be detected here. But PMCA2 was different; it only appeared in restricted tissues. The most significant difference among the PMCA isoforms was the stimulation of their activity by calmodulin[18]. PMCA2 has the highest affinity to calmodulin (5nM) 24.When there is only limited amount of free calmodulin, PMCA2 will still be able to interact with calmodulin and be activated by it, in contrast to the PMCA4 and PMCA1 pumps, whose affinity to calmodulin (Kd around 50 nM)is likely to be too low[18].Since high affinity to calmodulin signifies

Table 2 PMCA expression in lens of different species

Species	PMCA1		PMCA2		PMCA3		PMCA4		Reference
	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	
Bovine	?	+	?	+	?	+++	?	+	15
Rat	?	+	?	−	?	−	?	−	16
Porcine	−	?	+	?	−	?	+	?	17
Human HLE-B3	+	+	+	+	−	−	+	+	This study

Note: "+"=detected, "−"=not detected , "? "=unkown

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higher sensitivity to low Ca^{2+} concentrations, the PMCA2 pump will probably start to pump out Ca^{2+} before the others[18]. Therefore we hypothesize that PMCA2 may play a more important role of plasma calcium pumps in maintaining lens calcium homeostasis in the human lens. Kozel et al, reported that a defect in the PMCA2 isoform of PMCA resulted in a loss of hearing in mice, but he didn't mention whether there is any change in the lens[25].

We have also found two distinctive features of PMCA2 in this study. Although the amount of mRNA used and the RT-PCR condition were the same for all the PMCA isoform mRNA, the amout of PMCA2 production is the smallest. The band for PMCA2 is so faint that the PMCA2 RT-PCR product had to be concentrated before loading for agarose gel electrophoresis. In Western Blot, however, the band of PMCA2 isoform protein is not the faintest. Since the relative sensitivity among all these antibodies is unknown, it is in need of further investigation whether or not this means a higher translation rate for PMCA2 than for other isoforms. It has been reported that the molecular weight of PMCA2 is usually 3~8 kDa higher than PMCA1 and PMCA4[26]. But in our study, PMCA2 is ~20 kDa smaller than that of PMCA1 and PMCA4. One possible explanation is that since the gel we used for SDS-PAGE electrophoresis is as big as 20 cm, which took much longer for the proteins to migrate, PMCA2 might have some kind of proteolysis. PMCA2 is probably more sensitive to this kind of proteolysis effect that is associated with long electrophoresis time. When the SDS-PAGE electrophoresis is performed using a 8 cm gel, which takes at least 5 h less than the 20 cm gel does, the apparent molecular weight of these three PMCA isoforms are very similar, ~152–153 kDa.

In this study, we adopted the 20 cm gel electrophoresis as the main electrophoresis method, because this is the one we planned to use in two-dimensional electrophoresis study. In this way, the experiment will provide us with some preliminary information about in what molecular weight ranges will the PMCA isoform proteins be located. Since the 20 cm gel is much longer than the traditional 8 cm gel, it took much longer (5–6 h) to finish than the 8 cm gel (0.5 h). Through Figure 3A, it was found that the relative migration distance of different proteins from each other is quite consistent between these two gels. The apparent molecular weight detected by 20 cm gel was quite agreeable between two gels for PMCA1 and PMCA4. However, by using 20 cm gel, PMCA4 isoform was found ~ 5 kDa smaller than PMCA1. This is very likely because the much larger migration space in 20 cm gel allowed the proteins to migrate from each other and therefore small MW difference was magnified. For PMCA2 isoform, however, the difference is so great that even the 8 cm gel electrophoresis can detect. So we suspect it was due to some kind of proteolytic effect.

HLE-B3 cell line should be a good model for studying real human lens epithelium cells in terms of PMCA expression. Continuous cell culture has a number of advantages over in vivo tissues. It affords improved reproducibility, ease of application of quantitative techniques, and controlled experimental conditions, especially when primary HLE cell culture is very difficult due to limited sources, low viability and delicacy of the cells. Even worse is that the amount of PMCA protein expressed on HLE membrane is very small[18]. But still, studies on human lens tissue should further provide valuable information on the difference in gene expression between the cell lines and lens tissue[27].

Cataract is one of the most common eye diseases. Although cataract surgery is almost always successful there would be tremendous benefits from developing medical therapy to retard cataract development or prevent its onset. Calcium plays a vital role in normal lens cell function and that loss of calcium homeostasis might be one of the vital components in the cascade of events leading to cataract development. This study should provide some insight of the calcium homeostasis in the human lens. Studies reported that changes in cytosolic Ca^{2+} control not only the activity of the Ca^{2+} pumps but also their expression[18]. This allows the cells to express the best suitable set of Ca^{2+} pumps in response to long-term changes in cytosolic Ca^{2+} concentrations. As it has been found before, Ca^{2+}-ATPase activity was approximately 50% less in membranes prepared from cataractous lenses in comparison to clear lenses[14]. By

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observing different effects of cataractogenous factors on PMCA expression, some explanation about this reduction of PMCA activity in cataractous lens can be achieved.

In conclusion, this is the first study demonstrating that only PMCA1, 2 and 4 are expressed in HLE-B3 cells. PMCA3 is not expressed in HLE-B3 cells. The expression pattern for PMCA isoforms in HLE-B3 cell lines is different from that in the lens of other species. PMCA2 may play a more important role over other isoforms. Future studies will use HLE-B3 cell line as a model to test the relative sensitivity of different isoforms to the changes of calcium homeostasis and other cataractogenous factors.

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Declaration

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