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Cell-Specific Differences in the Processing of the R14W CAIV Mutant Associated With Retinitis Pigmentosa 17

Aisha Pandor,[1] Rajkumar Ramesar,[1] and Sharon Prince[2*]

[1] *Division of Human Genetics, Department of Clinical and Laboratory Sciences, University of Cape Town, Cape Town, South Africa*

[2] *Faculty of Health Sciences, Department of Human Biology, University of Cape Town, Cape Town, South Africa*

ABSTRACT

Retinitis pigmentosa is a highly heterogeneous form of inherited blindness which affects more than 1.3 million individuals worldwide. The RP17 form of the disease is caused by an arginine to tryptophan (R14W) mutation in the signal sequence of carbonic anhydrase IV (CAIV). While CAIV is expressed in the choriocapillaries of the eye and renal epithelium, the R14W mutation results in an exclusively ocular phenotype in affected individuals. In order to investigate the mechanism of disease in RP17 and the lack of kidney phenotype, we compared the subcellular localization and post-translational processing of wild-type (WT)- and mutant-CAIV in three cell types. We show using immunocytochemistry that unlike WT CAIV which is transported to the plasma membrane of transfected COS-7 and HT-1080 cells, the R14W mutant CAIV is retained in the endoplasmic reticulum. Western blot analyses further reveal that whereas the WT CAIV is processed to its mature form in both these cell lines, significant levels of the R14W mutant protein remain in its immature form. Importantly, flow cytometry experiments demonstrate that compared to WT CAIV protein, expression of specifically the R14W CAIV results in an S and G2/M cell-cycle block, followed by apoptosis. Interestingly, when the above experiments were repeated in the human embryonic kidney cell line, HEK-293, strikingly different results were obtained. These cells were unaffected by the expression of the R14W mutant CAIV and were able to process the mutant and WT protein equally effectively. This study has important implications for our understanding of the RP17 phenotype. J. Cell. Biochem. 111: 735–741, 2010.

KEY WORDS: RETINITIS PIGMENTOSA 17; CARBONIC ANHYDRASE IV; PROTEIN MISFOLDING; APOPTOSIS

Retinitis pigmentosa (RP) describes a heterogeneous group of inherited ocular disorders, which are characterized by progressive degeneration of photoreceptor cells in the retina [Farrar et al., 2002; Hims et al., 2003; Daiger et al., 2007]. RP is a major cause of blindness, affecting approximately 1 in 3,000 to 1 in 5,000 people worldwide [Kalloniatis and Fletcher, 2004]. We have previously identified an arginine to tryptophan (R14W) amino acid change in the signal sequence of the carbonic anhydrase IV (CAIV) protein as the defect underlying a dominant form of RP, RP17 [Rebello et al., 2004]. Although this mutation appears to be the major contributing factor to RP17, subsequent work has also identified additional causative mutations in the mature portion of the CAIV protein [Yang et al., 2005; Alvarez et al., 2007; Tian et al., 2010].

CAIV is a glycosylphosphatidylinositol membrane-anchored zinc metalloenzyme, which is localized on the luminal surface of microcapillaries and is highly expressed in the choriocapillaris of the human eye and in segments of the proximal renal tubular epithelium [Alvarez et al., 2007]. CAIV catalyzes the reversible hydration of carbon dioxide, and supplies oxygen to the retinal pigment epithelium (RPE), which in turn provides nutrients to the photoreceptor cells. Similar to other membrane-bound proteins, CAIV is translated with a hydrophobic signal sequence which directs the protein to the endoplasmic reticulum (ER). In the ER, the signal sequence is cleaved from the protein, and the mature protein is subsequently modified, packaged, and sorted for transfer to the plasma membrane [Ng et al., 1995].

There is a controversy in the literature surrounding the molecular mechanisms underpinning the RP17 phenotype. Several groups have reported that transfected R14W CAIV mutant protein leads to an increase in markers of ER stress and apoptosis in COS-7 cells [Bonapace et al., 2004; Rebello et al., 2004]. The observed apoptosis

Abbreviations used: RP17, retinitis pigmentosa 17; CAIV, carbonic anhydrase IV protein; WT, wild-type; RP, retinitis pigmentosa; ER, endoplasmic reticulum; RPE, retinal pigment epithelium; NBC1, sodium bicarbonate co-transporter 1; PS, phosphatidylserine.

*Correspondence to: Dr. Sharon Prince, Faculty of Health Sciences, Department of Human Biology, Anzio Road, Observatory, Cape Town 7925, South Africa. E-mail: sharon.prince@uct.ac.za

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was speculated to result from ER-retention due to misfolding and impaired trafficking of mutant CAIV. Yang et al. [2005] on the other hand suggested an alternative molecular pathology based on similar studies using HEK-293 cells. They showed that the R14W mutant CAIV does not bind effectively to transfected sodium bicarbonate co-transporter 1 (NBC1) protein. Since CAIV and NBC1 normally form a transporter system complex on the plasma membrane of choriocapillaris cells which acts to remove H^+ ions, Yang et al. [2005] proposed that the R14W mutation compromises this system in these cells. They therefore contend that levels of CAIV protein are important, and that haploinsufficiency of functional CAIV protein contributes to disease pathogenesis. The above studies together with the reported lack of kidney phenotype in RP17 affected individuals emphasize the need to re-examine the molecular mechanisms underlying RP17.

In this study, we compare the sub-cellular localization and post-translational processing of transfected R14W mutant- and wild-type (WT)-CAIV protein in COS-7 monkey kidney epithelial, HT-1080 human fibroblast and HEK-293 human embryonic kidney cells. Our results show that mutant CAIV is retained within the ER in an unprocessed, immature form in COS-7 and HT-1080 cells. Importantly, when these cells are transfected with mutant CAIV they undergo a dramatic S and G2/M cell-cycle arrest, a condition which if not ameliorated, has been linked to apoptosis [Evan et al., 1995; King and Cidlowski, 1995; Maddika et al., 2007]. These cell-cycle arrests were indeed shown to correlate with apoptosis when analyzed for Annexin V staining using flow cytometry. In contrast, the HEK-293 cells appear to process and traffic the mutant protein efficiently, with no consequence on the apoptotic pathway. These results have important implications for the understanding of the molecular basis of how the R14W mutation in CAIV causes RP17, and the processing of mutant CAIV protein in different cell types.

MATERIALS AND METHODS

PLASMID CONSTRUCTS

Wild-type and R14W mutant full-length human CA4 cDNA sequences in the pCXN vector were a kind gift from Professor William S. Sly, Saint Louis School of Medicine, Saint Louis, MO. Full-length CA4 sequences were also digested out of the pCXN mammalian expression vector using *Hin*dIII and *Sac*II and cloned into the fluorescent pEGFP-N1 vector (Clontech) at the *Hin*dIII (+622) and *Sac*II (+649) sites.

CELL CULTURE

SV40 transformed monkey kidney epithelium COS-7 cells (ATCC CRL-1651), human fibroblast epithelial HT-1080 cells (ATCC CCL-121) and human embryonic endothelial kidney HEK-293 cells (ATCC CRL-157) were cultured in Dulbecco's modified Eagle's medium (DMEM, D7777-1L; Sigma) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in an atmosphere of 5% CO_2.

TRANSIENT TRANSFECTION ASSAY

Cells were plated at a density of 1.5×10^5 cells/ml in 3.5 cm plates 1 day prior to transfection. Non-liposomal mediated gene transfer was performed using FuGENE®6 (Roche Applied Science) according to the manufacturer's instructions using 1 μg of DNA consisting of the full-length WT- or R14W-CAIV sequences cloned into pEGFP-N1 or full-length WT- or R14W-CAIV in pCXN vector. Cells were harvested 72 h after transfections and fixed using ice-cold (−20°C) methanol or room temperature 4% paraformaldehyde as a fixative agent, or viewed live in 1× phosphate-buffered saline (PBS).

WESTERN BLOT ANALYSES

Protein was prepared from cells 72 h after transfection. Cells were harvested and solubilized at 4°C with lysis buffer (40 mM Tris–HCl, 150 mM NaCl, 0.5% sodium deoxycholate, 1% Nonidet P40, 0.1% SDS and protease inhibitors), incubated on ice for 30 min and centrifuged at 12,000 rpm for 20 min at 4°C. Protein concentrations in lysates were determined using the BCA (bicinchoninic acid) protein assay kit (Thermo Scientific) with bovine serum albumin as the standard. Two micrograms of protein extract were separated on a 12% SDS–PAGE gel under both non-reducing and reducing conditions and then transferred onto nitrocellulose Hybond-C membrane (Amersham). Following blocking for 1 h at room temperature, the membranes were probed with rabbit monoclonal anti-human CAIV primary antibody (1:5,000; gift from Professor William S. Sly, Saint Louis School of Medicine, Saint Louis, MO). Immunoreactive bands were visualized with a horseradish peroxidase-conjugated secondary goat anti-rabbit serum (1:5,000; Biorad) and detected with enhanced chemiluminescence (ECL; Pierce).

FACS ANALYSIS

Cells were collected by trypsinization 72 h after transfection, washed twice with cold PBS, suspended in 2 ml of cold 1× PBS, and fixed in 8 ml of 70% ice cold ethanol for a minimum of 30 min at −20°C. Ethanol was removed by pelleting of cells by centrifugation. Cells were then washed twice with PBS and treated for 15 min at 37°C in 50 μg/ml RNase A. Twenty minutes before processing, cells were stained with propidium iodide (PI) solution (2 mM $MgCl_2$, 10 mM Pipes buffer, 0.1 M NaCl, 0.1% Triton X-100, 0.01 mg/ml PI). FACS analysis was performed in a Beckman Coulter Cytomics FC500 flow cytometer using the BD CellQuest™ Flow Cytometry Software (BD Biosciences).

APOPTOSIS ANALYSIS

Cells were collected by trypsinization 72 h after transfection and washed twice with cold PBS. Cells were harvested by centrifugation at 3,000 rpm for 5 min. The supernatant was discarded, and the pellet was resuspended in 1× binding buffer at a density of 1.0×10^5 cells/ml. One hundred microliters of the sample was transferred to a 10 ml culture tube, and incubated with Annexin V-FITC/PI (Sigma) as per the manufacturer's instructions. Annexin V conjugated to FITC was used to quantitatively determine the percentage of cells in a population that are undergoing apoptosis and PI was used to stain all dead cells. Four hundred microliters of 1× binding buffer was added to each sample, and analysis was performed by FACS in a Beckman Coulter Cytomics FC500 flow cytometer using the BD CellQuest™ Flow Cytometry Software (BD Biosciences).

FLUORESCENCE MICROSCOPY

Cells grown on glass coverslips were fixed in ice-cold (−20°C) methanol or 4% paraformaldehyde at room temperature for 20 min and permeabilized in 0.2% Triton X-100 in 1× PBS for 10 min. Cells were incubated overnight with rabbit monoclonal anti-human CAIV antibody at a dilution of 1:5,000 followed by incubation with Cy3 donkey anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, Inc.). Cells were subsequently incubated in the dark for 10 min with 1 μg/ml 4′,6-Diamidino-2-phenylindole (DAPI) in 1× PBS, mounted on slides and visualized by fluorescence microscopy. Cells transfected with pEGFP-N1 CAIV were incubated in a 1:10,000 dilution of ER-Tracker™ Blue-White DPX (E12353 Molecular Probes, Invitrogen, The Scientific Group-Adcock Ingram, SA) in 1× Hank's buffered salt solution for 30 min in order to specifically label the ER. These cells were then viewed live in 1× PBS.

RESULTS

EXPRESSION OF R14W MUTANT CAIV IN COS-7 CELLS, BUT NOT HEK-293 CELLS, LEADS TO S AND G2/M ARRESTS

Previous reports have suggested that the R14W mutation in CAIV may be causing RP17 by inducing ER-stress and apoptosis [Rebello et al., 2004; Bonapace et al., 2004; Datta et al., 2009]. To investigate this further, COS-7 and HEK-293 cells were transfected with the pCXN expression vector encoding either WT- or R14W-CAIV and their cell-cycle profiles were analyzed using flow cytometry. Our results showed that while the COS-7 cells expressing WT CAIV protein have a cell-cycle profile typical of normal dividing cells, ectopic expression of R14W mutant CAIV in these cells resulted in an S and G2/M cell-cycle arrest (Fig. 1A–C). HEK-293 cells expressing either the WT- or R14W mutant CAIV, however, displayed comparable cell-cycle profiles and had similar numbers of cells at S and G2/M (Fig. 1D–F). These results suggested that whereas the R14W mutant CAIV protein induced cell cycle arrests in COS-7 cells, it had no effect on the HEK-293 cells.

EXPRESSION OF R14W MUTANT CAIV INDUCES APOPTOSIS IN COS-7 CELLS

Since conditions of stress that trigger cell-cycle arrest can induce apoptosis, we next compared the effect of expressing the WT- and R14W mutant CAIV protein on apoptosis in COS-7 and HEK-293 cells. To this end, cells were transfected with the same expression constructs used in Figure 1 above and stained with PI and Annexin

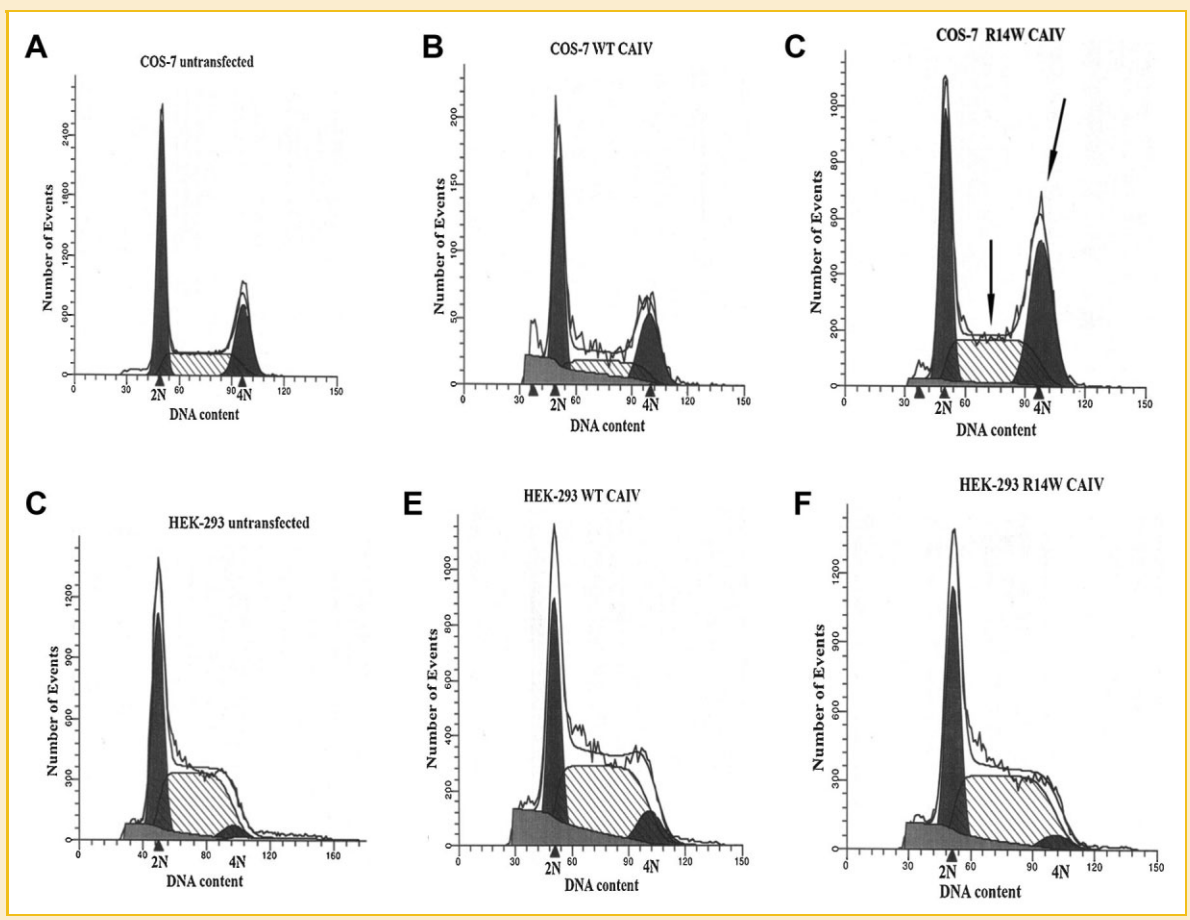

Fig. 1. Expression of R14W mutant CAIV in COS-7 cells, but not HEK-293 cells, leads to S and G2/M cell-cycle arrests. The cell-cycle status of untransfected cells (A,D) or cells transfected with pCXN-WT-CAIV (B,E) or pCXN-R14W-CAIV (C,F) was determined by measuring their DNA content using fluorescence-activated cell sorting analyses. Peaks at 2N and 4N DNA content represent the number of cells in G1 and G2/M phases, respectively, and the hatched areas represent the number of cells in S-phase. The light gray shaded areas represent cellular debris. Arrows in (C) point to S and G2/M cell-cycle arrests.

V-FITC for flow cytometry. Annexin V is an impermeable dye which binds to phosphatidylserine on the cell membrane when cells undergo apoptosis and apoptotic cells were therefore measure by positive staining for both PI and FITC. The results for the COS-7 cells showed that whereas 20.7% of R14W mutant CAIV transfected cells underwent apoptosis, this was the case for only 5.8% of cells expressing the WT protein (Fig. 2A,B). In contrast, the numbers of HEK-293 cells that stained positive for FITC and PI were similar for cells expressing either the WT or the R14W mutant CAIV protein (Fig. 2C,D). When the numbers of Annexin V-FITC/PI positive cells transfected with the R14W CAIV were expressed relative to that of cells expressing WT CAIV protein it was shown that at least 3.5 times more COS-7 cells underwent apoptosis (Fig. 2E). Consistent with the cell-cycle analyses shown in Figure 1, the number of apoptotic HEK-293 cells expressing either WT- or R14W mutant was comparable (Fig. 2E).

R14W MUTANT CAIV PROTEIN IS NOT PROCESSED TO ITS MATURE FORM IN COS-7 CELLS

Taken together, the above results provided additional evidence that expression of the R14W mutant CAIV protein causes apoptosis, which begged the question as to how this occurs. The mature CAIV protein results from post-translational processing which includes the formation of two disulfide linkages which act to compact the protein, resulting in its migration as a single 35 kDa band during

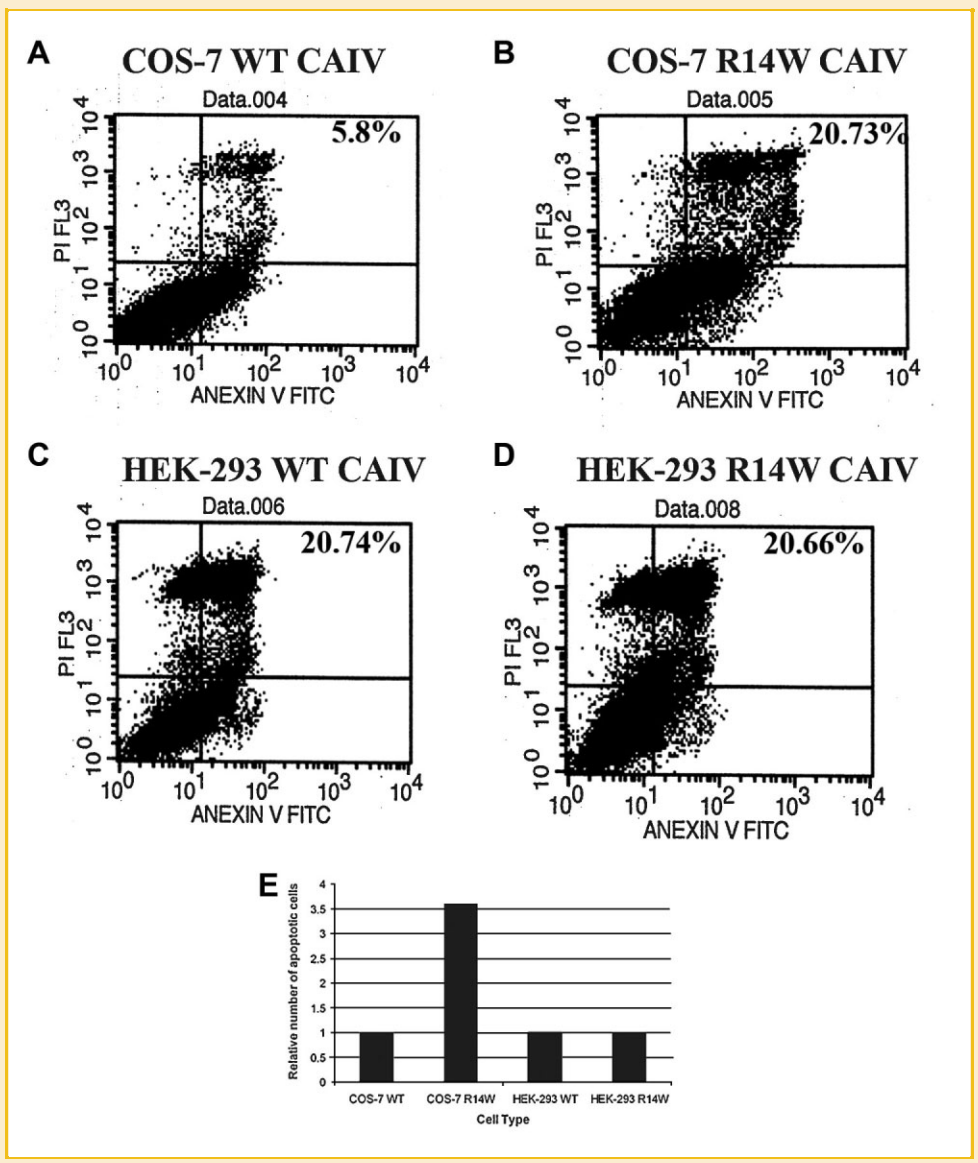

Fig. 2. Expression of R14W mutant CAIV induces apoptosis in COS-7 cells. (A,B) COS-7 and (C,D) HEK-293 cells expressing either pCXN-WT CAIV or pCXN-R14W CAIV were stained with Annexin V-FITC and propidium iodide (PI) in order to quantify apoptosis by flow cytometry. In the histograms, values indicate the percentages of cells that stain positive for FITC/PI that are undergoing apoptosis (upper right-hand quadrant). The graph (E) shows the numbers of Annexin V-FITC/PI positive cells transfected with the R14W CAIV relative to Annexin V-FITC/PI positive cells expressing WT CAIV protein which was set to 1.

non-reducing SDS–PAGE, whereas the misfolded precursor protein migrates at 37 kDa [Stams et al., 1996; Rebello et al., 2004]. It was previously speculated that expression of the R14W mutant CAIV protein in the COS-7 cells induced apoptosis due to improper post-translational processing and folding. To test this in our system, protein from cells transfected with either WT- or mutant CAIV in the pCXN expression vector was analyzed by Western blot analyses under reducing and non-reducing conditions.

Our results for COS-7 cells showed that while the R14W mutant protein migrated as a diffuse band, indicative of more than one form of the protein, the WT CAIV protein migrated as a single compact band (Fig. 3A). The same results were obtained when these experiments were performed in the HT-1080 human fibrosarcoma cells (Fig. 3A). To confirm that the difference in mobility of WT- and R14W mutant CAIV protein is indeed due to disulfide bond formation, protein samples were also analyzed by Western blotting using reducing conditions. As seen in Figure 3B, the WT- and R14W mutant CAIV protein migrated at the same rate under these conditions. These results thus provide support for the hypothesis that the R14W mutation prevents the proper processing and folding of the CAIV protein. Interestingly, and in line with the flow cytometry findings, the HEK-293 cells were able to process the WT- and R14W mutant CAIV equally efficiently, as evidenced by the respective proteins migrating as single integral bands (Fig. 3A).

R14W MUTANT CAIV FORMS ENDOPLASMIC RETICULUM (ER) ASSOCIATED AGGREGATES IN COS-7 CELLS

To further elucidate the mechanism by which the R14W mutant CAIV-induced apoptosis we tested the hypothesis that it was due to the ER-retention of the misfolded and incompletely processed protein. The subcellular localization of transfected WT- and R14W mutant CAIV protein in COS-7, HT-1080, and HEK-293 cells was therefore investigated by immunocytochemistry. As expected, fluorescent imaging of all cells expressing the WT CAIV revealed that the protein was distributed throughout the cell and in particular along the plasma membrane (Fig. 4A). In contrast, the R14W mutant CAIV protein aggregated in regions around the cell nucleus in COS-7 and HT-1080 cells (Fig. 4A). Interestingly, expression of R14W CAIV in HEK-293 cells resulted in subcellular localization similar to that of WT CAIV. Importantly, when COS-7 cells were transfected with WT- or R14W mutant-GFP-tagged CAIV and the cells stained with an ER-tracker dye and analyzed by immunofluorescence, only the GFP-tagged mutant CAIV co-localized with the ER (Fig. 4B). These results show that the R14W mutant CAIV protein is indeed retained in the ER in COS-7 cells.

DISCUSSION

We have previously identified an R14W mutation in the CAIV gene of all affected individuals in a cohort comprising 101 individuals from two large South African families affected with autosomal dominant RP (RP17) [Rebello et al., 2004]. There is, however, a controversy regarding how this mutation leads to the RP17 pathology. Data presented by Rebello et al. [2004] suggest that misfolding of the R14W mutant CAIV protein, and its retention in the ER, lead to apoptosis of choriocapillaris cells and consequently, the RP17 phenotype. In contrast, Yang et al. [2005] have suggested that the R14W mutation leads to an incapacity for CAIV to form a functional membrane complex with the NBC1 protein, compromising pH buffering, and leading to cell death. The results from the present study provide compelling evidence to support the hypothesis suggested by Rebello et al. [2004]. Firstly, we show that COS-7 cells expressing a transfected R14W mutant CAIV, experience S and G2/M cell-cycle arrests and apoptosis. Secondly, Western blot analysis shows that whereas the WT CAIV protein is predominantly present in its mature form, the turnover of precursor to mature enzyme is impeded in the R14W mutant CAIV. These results suggest that the R14W mutation affects correct folding of CAIV which explains the retention of the mutant in the ER as observed by fluorescence microscopy.

The observation that HEK-293 cells are able to process, fold and traffic the R14W mutant CAIV comparably to the WT protein is significant, especially as they do not undergo a cell-cycle block and apoptosis. These data raises the interesting possibility that different cell types may have different mechanism(s) for processing the CAIV protein to its mature form and that some cells have mechanisms of bypassing the deleterious effect of the R14W mutation. Furthermore, this differential processing of a mutant protein by different cell types, may explain the lack of "penetrance" of the pathogenic effect of mutant CAIV in different tissues, where it is known to be expressed and functional. For example, CAIV is expressed in the renal tubules but RP17 patients show no discernable kidney

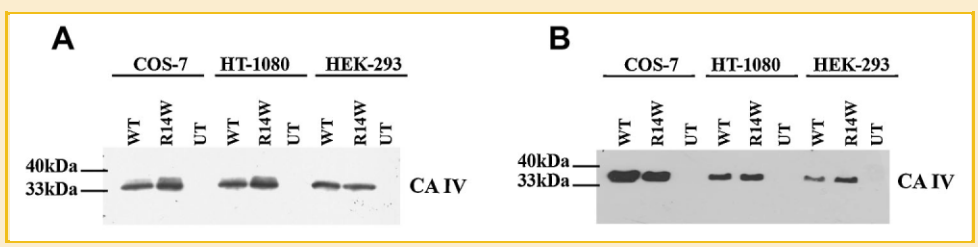

Fig. 3. R14W mutant CAIV protein is not processed to its mature form in COS-7 and HT-1080 cells. Western blot analyses under non-reducing conditions (A) and reducing conditions (B) were used to compare expression of CAIV from total protein (2 μg) isolated from COS-7, HT-1080 and HEK-293 cells transfected with pCXN-WT CAIV or pCXN-R14W CAIV. Protein blots were probed with an antibody specific for CAIV and detected by chemiluminescence. The retarded mobility of CAIV under non-reducing conditions in (A) is a measure of the immature protein.

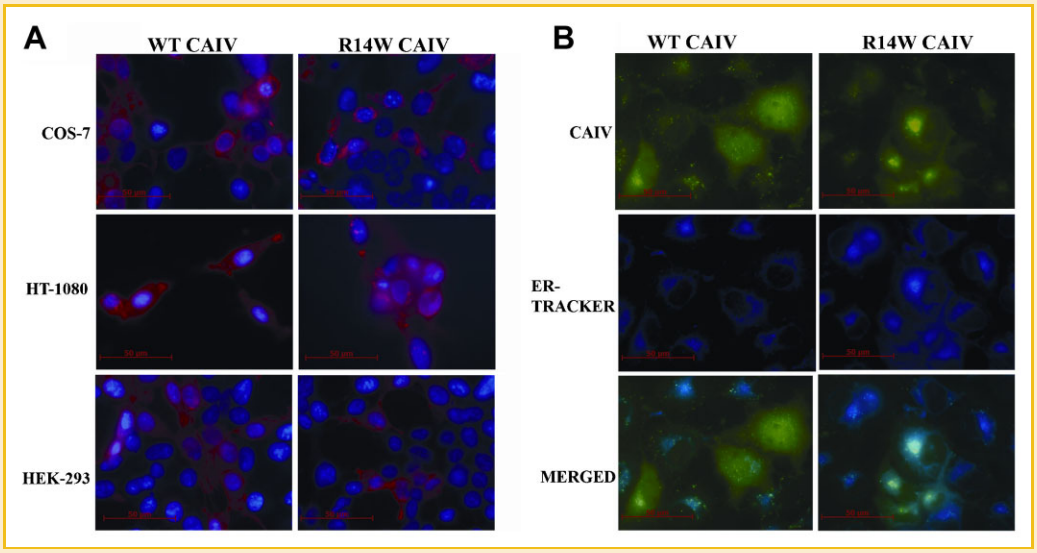

Fig. 4. The R14W mutant CAIV protein forms endoplasmic reticulum (ER)-associated aggregates in COS-7 cells. (A) R14W CAIV aggregates in a region corresponding to the ER in COS-7 and HT-1080 cells. COS-7, HT-1080 and HEK-293 cells expressing either pCXN-WT CAIV or pCXN-R14W were analyzed by immunohistochemistry to determine the cellular localization of WT- and R14W-CAIV protein. Cells were fixed, permeabilized, and treated with anti-CAIV antibody, followed by Cy3-conjugated secondary antibody and visualized by fluorescence microscopy for DAPI (blue nuclear stain) and CAIV (red stain). Scale bar represents 50 μm. (B) The R14W mutant CAIV protein co-localizes with the ER in COS-7 cells. In order to determine whether R14W mutant CAIV protein is retained in the ER, COS-7 cells were transfected with either pEGFPN1-WT CAIV or pEGFPN1-R14W CAIV and incubated in medium containing an ER-tracker dye. Live cells were visualized by fluorescence microscopy and the green stain shows localization of WT- and R14W mutant-CAIV protein while the ER stains blue. Scale bar represents 50 μm.

phenotype [Yang et al., 2005; Alvarez et al., 2007]. This phenomenon has previously been attributed to the high-metabolic activity of the retina, and possible lower tolerance of retinal tissue to metabolic imbalance. In addition, it has been suggested that the presence of CAIV isozymes in renal tissue may compensate for mutant CAIV and counter its deleterious effect [Yang et al., 2005; Alvarez et al., 2007]. It is of course also possible that the detrimental effect of the R14W mutation is not seen in renal tissue because proximal tubule cells express specific chaperones, that allow them to correctly fold R14W mutant CAIV, and therefore evade apoptosis. This is a particularly interesting possibility in light of a recent report that transgenic mice expressing R14W CAIV have no retinal phenotype but experience progressive renal damage which is enhanced by haploinsufficiency of an ER co-chaperone ($p58^{IPK}$) [Ogilvie et al., 2007; Datta et al., 2010]. The study by Datta et al. [2010] provides additional evidence for cell-specific differences in CAIV processing but further suggests that this may also be species specific and highlights the need for cautious interpretation when using mouse models for human diseases.

Interestingly, recent findings have also shown that in northern Sweden, the R14W mutation is present in 4% of the population as a benign polymorphism [Köhn et al., 2008]. Due to the pathogenicity of the mutation being confirmed in studies with patients of both South African Caucasian and Chinese ethnicity [Rebello et al., 2004; Yang et al., 2005; Alvarez et al., 2007], we propose that the lack of penetrance of the RP17 phenotype in the Swedish group with the R14W mutation is either as a result of modifier genes which switch off/decrease mutant CAIV expression, or other factors which allow cells to resist the apoptotic effect of the mutant CAIV.

This study provides an explanation for how the R14W mutant CAIV induces apoptosis in cells of the choriocapillaris, but not kidneys, of RP17 patients and opens up exciting possibilities of approaches that could be employed in the therapy of, or treatment for, RP17.

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REFERENCES

Alvarez BV, Vithana EN, Yang Z, Koh AH, Yeung K, Yong V, Shandro HJ, Chen Y, Kolatkar P, Palasingam P, Zhang K, Aung T, Casey JR. 2007. Identification and characterization of a novel mutation in the carbonic anhydrase IV gene that causes retinitis pigmentosa. Invest Ophthalmol Vis Sci 48:3459–3468.

Bonapace G, Waheed A, Shah GN, Sly WS. 2004. Chemical chaperones protect from effects of apoptosis-inducing mutation in carbonic anhydrase 4 identified in retinitis pigmentosa 17. Proc Natl Acad Sci USA 101:12300–12305.

Daiger SP, Bowne SJ, Sullivan LS. 2007. Perspective on genes and mutations causing retinitis pigmentosa. Arch Ophthalmol 125:151–157.

Datta R, Waheed A, Bonapace G, Shah GN, Sly WS. 2009. Pathogenesis of retinitis pigmentosa associated with apoptosis-inducing mutations in carbonic anhydrase IV. Proc Natl Acad Sci USA 106:3437–3442.

Datta R, Shah GN, Rubbelke TS, Waheed A, Rauchman M, Goodman AG, Katze MG, Sly WS. 2010. Progressive renal injury from transgenic expression of human carbonic anhydrase IV folding mutants is enhanced by deficiency of p58IPK. Proc Natl Acad Sci USA 107:6448–6452.

Evan GI, Brown L, Whyte M, Harrington E. 1995. Apoptosis and the cell cycle. Curr Opin Cell Biol 7:825–834.

Farrar GJ, Kenna PF, Humphries P. 2002. On the genetics of retinitis pigmentosa and on mutation-independent approaches to therapeutic intervention. EMBO J 21:857–864.

Hims MM, Daiger SP, Inglehearn CF. 2003. Retinitis pigmentosa: Genes, proteins and prospects. Dev Ophthalmol 37:109–125.

Kalloniatis M, Fletcher EL. 2004. Retinitis pigmentosa: Understanding the clinical presentation, mechanisms, and treatment options. Clin Exp Optom 87:65–80.

King KL, Cidlowski JA. 1995. Cell cycle and apoptosis: Common pathways to life and death. J Cell Biochem 58:175–180.

Köhn L, Burstedt MSI, Jonsson F, Kadzhaev K, Haamer E, Sandgren O, Golovleva I. 2008. Carrier of R14W in carbonic anhydrase IV presents Bothnia dystrophy phenotype caused by two allelic mutations in RLBP1. Invest Ophthalmol Vis Sci 49:3172–3177.

Maddika S, Ande SR, Panigrahi S, Paranjothy T, Weglarczyk K, Zuse A, M.Eshraghi M, Manda K, Wiechec E, Los M. 2007. Cell survival, cell death and cell cycle pathways are interconnected: Implications for cancer therapy. Drug Resist Updat 10:13–29.

Ng TWD, Brown JD, Walter P. 1995. Signal sequences specify the targeting route to the endoplasmic reticulum membrane. J Cell Biol 134:269–278.

Ogilvie JM, Ohlemiller KK, Shah GN, Ulmasov B, Becker TA, Waheed A, Hennig AK, Lukasiewicz PD, Sly WS. 2007. Carbonic anhydrase XIV deficiency produces a functional defect in the retinal light response. Proc Natl Acad Sci USA 104:8514–8519.

Rebello G, Ramesar R, Vorster A, Roberts L, Ehrenreich L, Oppon E, Bardien S, Greenberg J, Bonapace G, Waheed A, Shah GN, Sly WS. 2004. Apoptosis-inducing signal sequence mutation in carbonic anhydrase IV identified in patients with the RP17 form of retinitis pigmentosa. Proc Natl Acad Sci USA 101:6617–6622.

Stams T, Nair SK, Okuyama T, Waheed A, Sly WS, Christianson DW. 1996. Crystal structure of the secretory form of membrane-associated human carbonic anhydrase IV at 2.8-Å resolution. Proc Natl Acad Sci USA 93: 13589–13594.

Tian Y, Tang L, Cui J, Zhu X. 2010. Screening for the carbonic anhydrase IV gene mutations in Chinese retinitis pigmentosa patients. Curr Eye Res 35:440–444.

Yang Z, Alvarez BV, Chakarova C, Jiang L, Karan G, Frederick JM, Zhao Y, Sauvé Y, Li X, Zrenner E, Wissinger B, Den Hollander AI, Katz B, Baehr W, Cremers FP, Casey JR, Bhattacharya SS, Zhang K. 2005. Mutant carbonic anhydrase 4 impairs pH regulation and causes photoreceptor degeneration. Human Mol Genet 14:255–265.