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Curcumin inhibits proliferation of human lens epithelial cells: a proteomic analysis*

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Abstract: Objective: The incidence of after-cataracts [also known as posterior capsular opacification (PCO)] is between 30% and 50% three years following cataract surgery. Suppressing the proliferation of lens epithelial cells (LECs) is a primary goal in preventing PCO. Here, we investigated the proteomic regulation of the inhibitory effects of curcumin (Cur) on the proliferation of human lens epithelial B3 (HLE-B3) cells. Methods: Recombinant human basic fibroblast growth factor (rhbFGF) was used to induce proliferation of HLE-B3 cells, which were incubated with 20 mg/L Cur in a CO_2 incubator for 24 h. Results: We found that the absorbance (*A*) value of rhbFGF group was significantly higher than the *A* value of the control group. Furthermore, the *A* value of the Cur group was significantly lower compared to the rhbFGF group, with an inhibition of 53.7%. Five different protein spots were obtained from proliferative HLE-B3 cells induced by rhbFGF. Eight different protein spots were obtained in HLE-B3 cells incubated with Cur. There were the common variational protein spots at mass/charge (*m*/*z*) ratios of 8093 and 13767 between rhbFGF group and control group as well as between the Cur group and rhbFGF group. Conclusions: These results show that Cur effectively inhibited HLE-B3 cell proliferation induced by rhbFGF. The protein spots at *m*/*z* of 8093 and 13767 may be the targets of Cur-induced inhibition of HLE-B3 cell proliferation. Cur may be a reliable and effective drug for prevention and treatment of polymerase chain reaction (PCR).

Key words: Curcumin, After-cataract, Posterior capsular opacification (PCO), Proteomics, Lens epithelial cells
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1 Introduction

The after-cataract, also known as posterior capsular opacification (PCO), is one of the major complications inducing long-term disruption in eyesight following extracapsular cataract extraction (ECCE). Previous studies have reported that the average incidence of the PCO is between 30% and 50% three years following cataract surgery, and it is 22%–26% in senile patients (65–80 years old); PCO has also been reported to present in all infants after cataract surgery (Schaumberg *et al*., 1998; Karczewicz *et al*., 2004). PCO is caused by proliferation of the remnant lens epithelial cells (LECs) and their subsequent migration to the posterior capsule after surgery (Marcantonio and Vrensen, 1999). Therefore, effective inhibition of LEC proliferation may prevent the formation of PCO.

Curcumin (Cur) is the principal curcuminoid of the popular Indian spice tumeric, which is a member of the ginger family (Zingiberaceae). Cur has been shown to enhance monokaryon phagocytosis by macrophages, increase immune regulation, inhibit the proliferation, and induce apoptosis of immortal cell lines such as HL-60, K-562, MCF-7, HOS, and VSM cells (Dubey *et al*., 2008; Hussain *et al*., 2008; Miller

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et al., 2008; Lee *et al*., 2009; Chen *et al*., 2010a; 2010b; Giommarelli *et al*., 2010; Watson *et al*., 2010). In our previous studies, we found that Cur effectively inhibited LEC proliferation, and that Cur increased the concentrations of intracellular Ca^{2+} and cyclic adenosine monophosphate and decreased the concentration of cyclic guanosine monophosphate in LECs (Hu *et al*., 2006a; 2006b). To further study the underpinnings of Cur-induced inhibition of the proliferation of human lens epithelial B3 (HLE-B3) cells, we used surface-enhanced laser desorption ionization time of flight mass spectrometry (SELDI-TOF-MS) technology, which allowed us to analyze the protein expression spectrum of HLE-B3 cells to identify possible targets of Cur inhibition.

2 Materials and methods

2.1 HLE-B3 culture

Frozen HLE-B3 cultures were thawed in a water bath at 37 °C. Cells were then diluted 10-fold with Dulbecco's modified Eagle's medium (DMEM), and centrifuged at 1000 r/min at 4 °C for 5 min. The supernatant discarded, and the cells washed three times. The cells were seeded into a 75-ml culture flask at 1×10^6–2×10^6 cells/ml, and incubated at 37 °C with 5% CO_2.

2.2 Detection of HLE-B3 activity by methyl thiazolyl tetrazolium (MTT)

HLE-B3 suspensions (5×10^5 cells/ml) were incubated for 24 h in 96-well tissue culture plates (100 μl per well) with DMEM supplemented with 2.5% newborn calf sera (NCS) to synchronize cell cycles. Three experiment groups included: (1) the recombinant human basic fibroblast growth factor (rhbFGF) group (cells with 10 ng/ml rhbFGF), (2) the Cur group (cells with 10 ng/ml rhbFGF and 20 mg/L Cur), and (3) the control group (cells in DMEM only). After 24 h in culture followed by removal of the supernatant, experimental Groups 1 and 2 were washed with phosphate buffered solution (PBS) containing 100 μl MTT (final concentration of 0.5 mg/ml), incubated in the dark at 37 °C for 4 h, and treated with 100 μl dimethyl sulfoxide (DMSO) per well with gentle shaking. Absorbance (A) values were measured using a microplate reader at 490 nm wavelength. Rates of cell proliferation suppression in the Cur group were determined by the formula: $SR=(A_r-A_c)/A_r\times100\%$, where SR is suppression rate, A_r is the average absorbance value of rhbFGF group, and A_c is the average absorbance value of Cur group.

2.3 SELDI-TOF-MS protein chip array profiling to detect the variation in HLE-B3 proteins

We digested and collected HLE-B3 in DMEM into a cell suspension. HLE-B3 cells were then seeded into a 75-ml culture flask at 1×10^6–2×10^6 cells/ml. After 24 h, rhbFGF was added to the rhbFGF and Cur groups to obtain a final concentration of 10 ng/ml, and Cur was added in the Cur group to obtain the final concentration of 20 ng/ml. An equal volume of DMEM was added to the control group. After 24 h of incubation, the culture medium was discarded. The cultures were washed three times with PBS. Another 1 ml PBS was added to suspend cells, and the suspension was centrifuged at 1500 r/min for 5 min. The supernatants were then discarded, and 1 ml of PBS was added to re-suspend cells. Cell suspension of 10^7 cells/ml was then absorbed into 1.5 ml EP tubes, and centrifuged at 1500 r/m for 5 min. Supernatant was discarded, and 200 μl of cell lysates [8 mol/L urea, 4% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS), 40 mmol/L Tris-HCl] were then added to the suspensions. After agitation in ice for 30 min, the samples were mixed, and centrifuged at 16000 r/min for 30 min, and then the supernatants were discarded. Cell protein concentrations were measured by a biophotometer (Eppendorf® BioPhotometer 6131).

A CM10 protein chip was used to bind to proteins in the cell lysates, and six different sample points from each group were dotted in the chip. Protein chip binding was applied with the normal-position spotting methods specified in the product directions. Each chip sample point was applied three times using 3 μl of integrated buffer (50 mmol/L sodium acetate, pH 4.0) for 5 min. The protein samples were diluted to 1.8 mg/ml and then 5 μl of protein samples were added to each spot and incubated for 1 h at room temperature. A total of 3 μl of high performance liquid chromatography (HPLC) water was then added to each spot, discarded rapidly, and repeated twice. All procedures were performed in a constant humidity chamber. The chip was dried in air

for 20 min and each spot received 1 μl of sinapic acid (SPA) solution, which was produced by combining methyl cyanide solution with 1% trifluoroacetic acid (TFA) and diluting it to 2× volume with HPLC water. The molecular weight calibration error of the SELDI mass spectrometry system is less than 0.1% by using the standard molecular weight protein chip. CM10 chip bind protein was detected by the mass spectrum reader. All the sample points were applied with the following uniform parameters: laser intensity 185, detection sensitivity 7, the upper limit of detection mass/charge (*m*/*z*) 100000, the optimized scope of acquisition data *m*/*z* 2000–20000, the signal collection position 20–80, and each sample calculated mean 144 collected points. Data were displayed in XLM (extensible markup language) format by ProteinChip software 3.2.

2.4 Statistical analysis

MTT data were presented as mean±standard deviation (SD). To fit data to a normal distribution, the multiple groups were analyzed by analysis of variance (ANOVA) and by Dunnett's test for pairwise comparisons. Statistical analyses were performed by SPSS Version 12.0. Statistical significance was defined as $P<0.05$.

The SELDI-TOF-MS data were analyzed by ZUCI-Protein Chip Data Analysis System software. The undecimated discrete wavelet transform was applied to discard any noise and abate the baseline of original data in each sample. Spikes under 2000 Da were filtered. The spike in each sample was found by local extremum value methods, and spikes with a signal-to-noise ratio less than 4 were filtrated. The numbers of spikes from each of the samples as well as the *m*/*z* values themselves differed. The spikes with ≤0.3% *m*/*z* differences in each sample were clustered into one group. After clustering, the spikes in the sample <10% were discarded. Subsequently, the intensities of the spikes in each of the samples were uniformly applied. The characteristic vector was selected by applying filtration-bind, model-dependent screening methods. If experimental data did not fit the normal distribution, the Kruskal-Wallis test was used to test the peak of the *m*/*z* ratio. The Nemenyi test was used to analyze within-group samples of protein peaks with specific *m*/*z* ratios in the control group, rhbFGF group, and Cur group.

3 Results

3.1 Effect of Cur on absorbance value of HLE-B3

MTT analysis showed that the absorbance of HLE-B3 in the rhbFGF group (0.5990±0.0531) was significantly higher than that of the control group (0.4091±0.0422) ($P<0.01$, $n=8$), indicating that the proliferation of HLE-B3 cells in the rhbFGF group had markedly increased. The absorbance of HLE-B3 cells in the Cur group (0.2773±0.0268) was significantly lower than that of the rhbFGF group ($P<0.01$, $n=8$), demonstrating that Cur had effectively inhibited rhbFGF-induced HLE-B3 proliferation. The inhibition rate was 53.70%.

3.2 Effect of rhbFGF on proteome of HLE-B3

There were 235 different protein spots of HLE-B3 bound to the CM10 chip from HLE-B3 cells incubated with rhbFGF. A total of five peak protein spots showed significant differences between rhbFGF and control groups ($P<0.05$). The peak values were up-regulated in two of the five protein spots at *m*/*z* ratios of 8093 and 9516, which showed a 1.53- and 1.33-fold increase compared with the control group, respectively. The peak values were down-regulated in three of the five protein spots at *m*/*z* ratios of 5361, 9666, and 13767 (Table 1).

Table 1 Comparison of five protein mass peaks between the rhbFGF and control groups

Protein mass peak *m*/*z*	Mean peak value*		P ($\times10^{-4}$)
	Control group	rhbFGF group	
5361	1124.06±57.59	837.87±60.77	0.078799
8093	443.59±20.71	677.46±100.97	7.207700
9516	991.63±116.38	1320.91±175.64	0.630471
9666	526.81±44.58	267.38±39.65	0.008885
13767	4060.17±882.82	1940.63±485.25	4.291229

* Values are expressed as mean±SD ($n=6$)

3.3 Effect of Cur on proteome of rhbFGF-induced HLE-B3 proliferation

There were 222 different protein spots of HLE-B3 bound to the CM10 chip from rhbFGF-induced HLE-B3 cells incubated with Cur. Of these, eight peak values of protein spots showed significant differences between the rhbFGF and Cur groups ($P<0.05$). Compared with the rhbFGF group, peak

values in the Cur group were down-regulated in four of the eight protein spots at *m*/*z* of 4582, 7272, 8093, and 14263. Peak values in the Cur group were up-regulated in the other four spots at *m*/*z* ratios of 2996, 8189, 13767, and 13994, compared with the rhbFGF group, which showed 5.79, 1.51, 2.40, and 1.33 times the normal proliferation, respectively (Table 2).

Table 2 Comparison of eight protein mass peaks between the Cur and rhbFGF groups

Protein mass peak *m*/*z*	Mean peak value*		*P* ($\times10^{-6}$)
	rhbFGF group	Cur group	
2996	198.77±67.74	1151.79±88.19	0.0013
4582	1286.72±41.65	722.28±19.65	0.0000
7272	2168.05±255.16	1151.95±104.77	4.0396
8093	677.46±100.97	246.32±34.57	2.4766
8189	2517.72±201.41	3799.11±159.71	0.2479
13767	1940.63±485.25	4655.30±670.53	4.0396
13994	900.66±290.42	2420.45±332.52	7.4061
14263	619.65±27.40	405.43±15.93	0.0135

* Values are expressed as mean±SD (n=6)

There were the common variational protein spots at *m*/*z* of 8093 and 13767 between the rhbFGF and control groups as well as the Cur and rhbFGF groups. In the protein spot at *m*/*z* 8093, the peak value in the rhbFGF group was up-regulated compared with the control group. The same protein spot in the Cur group, however, was down-regulated in relation to the rhbFGF group. In the protein spot at *m*/*z* of 13767, the peak value in the rhbFGF group was down-regulated relative to the control group, whereas the peak value in Cur group was up-regulated compared with the rhbFGF group.

4 Discussion

Proteome, a term coined by Wilkins in 1994, is a blend of the terms protein and genome (Wasinger *et al*., 1995), and refers to the entire complement of proteins, including the modifications made to a particular set of proteins produced by an organism or system. In our study, proteomics referred to our analysis of cellular proteins by mass SELDI-TOF-MS technology, a novel proteome research method, which includes the separation and purification of proteins as well as mass spectrometric detection. Based on the different modified characteristics of a protein chip, the different protein was selectively acquired in the sample, which greatly reduced the complexity of proteins in the sample while retaining the simultaneous analysis of various samples and proteins. Using this method, we could detect proteins at an order of magnitude of fmol (10^{-15} mo1) levels, allowing thousands of proteins to be analyzed from each sample, making it a powerful tool to analyze the cell proteome.

Based on previous studies (Ge *et al*., 2006; Hu *et al*., 2010), we applied CM10 protein chips to bind to proteins in the specimen. We found two important protein spots at *m*/*z* of 8093 and 13767. In HLE-B3 proliferation induced by rhbFGF, the protein spot at *m*/*z* of 8093 showed up-regulation expression, whereas expression was down-regulated after Cur inhibited HLE-B3 proliferation. However, in cultures of HLE-B3 proliferation induced by rhbFGF, the protein spot at *m*/*z* of 13767 showed down-regulation of expression, whereas the expression was up-regulated after Cur inhibited HLE-B3 proliferation. This indicated that when Cur inhibited HLE-B3 cell proliferation induced by rhbFGF, HLE-B3 cells expressed a distinct protein, which may be the critical target of Cur inhibition.

Further identifying these proteins and their functions will help us further understand the pathogenesis of PCO, determine novel therapy targets, as well as develop effective targeted agents. From the *m*/*z* provided by our mass spectrum and the isoelectric point provided by the chip, we were able to retrieve the matching protein information from the Swiss-Prot database, which identified eight matching proteins in the protein spot at *m*/*z* of 8093: chemotatic factor A17, chemotatic factor A22, enteroglucagon, interleukin-8 (IL-8), 60S ribosomal protein, protein FAM24B, nicotinamide adenine dinucleotide (NADH) dehydrogenase 1α subunit, and neutrophil active peptide 2. In addition, we found ten types of matching proteins in the protein spot at *m*/*z* of 14767 retrieved from the Swiss-Prot database: histone H2A, histone H2B, glycosylated protein, meta tetraiodothyronine protein, atypical protein C5orf23, atypical protein CXorf52, cartilage regulin-1, V-I domain IG H-chain SIE, eosinophil granule major basic protein, and T cell leukocythemia virus I type binding protein. These proteins may play a critical role in the Cur-induced

inhibition of HLE-B3 proliferation induced by rhbFGF, and have the potential to be therapy targets for the treatment of PCO.

We classified these proteins by five different functions: (1) mediators of inflammatory protein such as chemotatic factor A17, chemotatic factor A22, IL-8, enteroglucagon, neutrophil active peptide 2; (2) proteins related to cell energy metabolism (NADH dehydrogenase 1α subunit); (3) proteins related to cell synthetic protein (60S ribosomal protein); (4) proteins related to DNA synthesis (histone H2A, histone H2B, etc.); (5) other proteins, the functions of which have not been identified.

The failure of the blood-aqueous humor barrier after cataract surgery, the release of inflammatory mediators, as well as complement activation all play roles in the formation of PCO. Nishi *et al*. (1999) studied in vitro cultures using LEC anterior lens capsules during cataract surgery, and found that LECs could synthesize transforming growth factor α, transforming growth factor β, as well as IL-8 mRNA, all of which play critical roles in LEC proliferation after cataract surgery, and induce the inflammatory reactions that produce the PCO. Our results confirmed that Cur was able to reduce the expression of mediators of inflammation such as HLE-B3 cellular chemotatic factor, IL-8, and neutrophil active peptide 2, and that the reduction of these inflammatory mediators may play a key role in Cur-induced inhibition of HLE-B3 proliferation that prevents PCO.

NADH-CoQ reducase, also called NADH dehydrogenase or Complex I, is an enzyme in the mitochondrial respiratory chain that catalyzes the transfer of two electrons from NADH to coenzyme Q. In our study, we found that Cur was able to reduce the activity of NADH dehydrogenase in HLE-B3 cells, which may induce HLE-B3 mitochondria dysfunction, thereby affecting cell proliferation and metabolism. Our findings point to NADH dehydrogenase as the specific target of HLE-B3 proliferation inhibited by Cur.

A quantity of repeated sequences in the genomes encoding histones also point to a mechanism of action for PCO formation. As DNA is copied, histone duplication increases. Moreover, after DNA synthesis of a small section, histones bind rapidly, which means a large quantity of histones were synthesized in a short time. Therefore, genomes involved in histone production were also involved in Cur-induced proliferation inhibition. Sidjanin *et al*. (1996) found that the ultraviolet irradiation inhibited LEC cell cycle processing, which correlated with a reduction of histone transcript accumulation. Our study showed that Cur was able to up-regulate expressions of histones H2A and H2B in HLE-B3 cells. We also found that Cur was able to inhibit the cell cycle processing of HLE-B3 thereby inhibiting HLE-B3 proliferation. Accordingly, we suggest that histones H2A and H2B may not be the action targets of HLE-B3 proliferation inhibited by Cur. However, this finding may be due to the differences in histone subtypes between our study and those in Sidjanin *et al*. (1996)'s work.

Ribosomes are a critical part of the molecular machine for the synthesis of cell proteins. Zhang *et al*. (2002) compared the expression disparity of ribosomal proteins in normal lens and the lens of age-related cataract patients. They found that expressions of L21, L15, L13a, and L7a genes that encoded the ribosomal protein showed significant decreases in the lens of age-related cataract patients compared with the normal lens, indicating that ribosomal proteins regulating synthesis of cell proteins and mediating other cell functions correlated with the genesis of age-related cataracts. We reported that Cur was able to down-regulate the expression of 60S ribosomal protein in HLE-B3, affecting the protein synthesis in HLE-B3 and inhibiting HLE-B3 cell proliferation. For this reason, the 60S ribosomal protein may also be an additional target of HLE-B3 proliferation inhibited by Cur.

Compared with other proteome research technologies, SELDI-TOF-MS showed higher discrimination for molecules with low molecular weights (2000 to 20000 Da). The majority of the discovered proteins were the low-molecular-weight proteins generated in the specific environment, which may include metabolites, modified proteins generated by abnormal dissection, neurotransmitters, polypeptides, or cytokines. Therefore, a large number of different proteins detected in our study may be restricted to the micromolecules involved in the onset and prevention of PCO, which to our knowledge, have not been previously reported. Identifying and studying these proteins may further elucidate the effect of micromolecular substances in the onset and prevention of PCO, which also further defines the specific effects of known, related proteins.

5 Conclusions

Cur significantly inhibited HLE-B3 cell proliferation induced by rhbFGF. The protein spots at *m*/*z* of 8093 and 13767 might be the targets of Cur-induced inhibition of HLE-B3 cell proliferation. Cur may be a reliable and effective drug for prevention and treatment of PCO. The different proteins expressed during the inhibition of HLE-B3 proliferation by Cur are the key to understanding the pathogenesis of PCO as well as identifying novel targets for PCO therapy. Further studies of these proteins and their effects in PCO formation are needed.

References

Chen, Q.Y., Wang, Y.Y., Xu, K.D., Lu, G.H., Ying, Z., Wu, L.J., Zhan, J.W., Fang, R., Wu, Y.Q., Zhou, J.Y., 2010a. Curcumin induces apoptosis in human lung adenocarcinoma A549 cells through a reactive oxygen species-dependent mitochondrial signaling pathway. *Oncol. Rep*., **23**(2):397-403. [doi:10.3892/or_00000648]

Chen, Q.Y., Lu, G.H., Wu, Y.Q., Zheng, Y., Xu, K., Wu, L.J., Jiang, Z.Y., Feng, R., Zhou, J.Y., 2010b. Curcumin induces mitochondria pathway mediated cell apoptosis in A549 lung adenocarcinoma cells. *Oncol. Rep*., **23**(5): 1285-1292. [doi:10.3892/or_00000762]

Dubey, S.K., Sharma, A.K., Narain, U., Misra, K., Pati, U., 2008. Design, synthesis and characterization of some bioactive conjugates of curcumin with glycine, glutamic acid, valine and demethylenated piperic acid and study of their antimicrobial and antiproliferative properties. *Eur. J. Med. Chem*., **43**(9):1837-1846. [doi:10.1016/j.ejmech.2007.11.027]

Ge, Z., Zhu, Y.L., Zhong, X., Yu, J.K., Zheng, S., 2006. Proteomic analysis of human gastric adenocarcinoma epithelial AGS cells following transfection of *Helicobacter pylori* cytotoxin-associated gene A. *Chin. J. Cell Biol*., **28**(4):603-610 (in Chinese).

Giommarelli, C., Zuco, V., Favini, E., Pisano, C., Dal Piaz, F., de Tommasi, N., Zunino, F., 2010. The enhancement of antiproliferative and proapoptotic activity of HDAC inhibitors by curcumin is mediated by Hsp90 inhibition. *Cell Mol. Life Sci*., **67**(6):995-1004. [doi:10.1007/s00018-009-0233-x]

Hu, Y.H., Qi, M.X., Huang, X.R., Ma, L., Yan, J., Wu, Z.Z., 2006a. Inhibitory effect of curcumin on proliferation of lens epithelial cells. *Chin. Ophthalmic Res*., **24**(2):122-124 (in Chinese).

Hu, Y.H., Qi, M.X., Huang, X.R., Ma, L., Yan, J., Wu, Z.Z., 2006b. Signal transduction mechanism of curcumin in inhibiting the proliferation of bovine lens epithelial cell induced by recombinant human epidermal growth factor. *J. Chin. Integr. Med*., **4**(1):39-42 (in Chinese). [doi:10.3736/jcim20060111]

Hu, Y.H., Huang, X.R., Qi, M.X., Hou, B.Y., 2010. Study on proteomics of inhibitory effects of elemene on proliferation of human lens epithelial cell. *Chin. J. Ophthalmol*., **46**(5):427-431 (in Chinese). [doi:10.3760/cma.j.issn.0412-4081.2010.05.011]

Hussain, A.R., Ahmed, M., Al-Jomah, N.A., Khan, A.S., Manogaran, P., Sultana, M., Abubaker, J., Platanias, L.C., Al-Kuraya, K.S., Uddin, S., 2008. Curcumin suppresses constitutive activation of nuclear factor-κB and requires functional Bax to induce apoptosis in Burkitt's lymphoma cell lines. *Mol. Cancer Ther*., **7**(10):3318-3329. [doi:10.1158/1535-7163.MCT-08-0541]

Karczewicz, D., Pienkowska-Machoy, E., Modrzejewska, M., Gronkowska, J., Sylwestrzak, Z., 2004. Posterior capsule opacification as a complication of the posterior chamber intraocular lens implantation. *Klin. Oczna*, **106**(1-2): 19-22.

Lee, D.S., Lee, M.K., Kim, J.H., 2009. Curcumin induces cell cycle arrest and apoptosis in human osteosarcoma (HOS) cells. *Anticancer Res*., **29**(12):5039-5044.

Marcantonio, J.M., Vrensen, G.F., 1999. Cell biology of posterior capsular opacification. *Eye*, **13**(3b):484-488. [doi:10.1038/eye.1999.126]

Miller, M., Chen, S.L., Woodliff, J., Kansra, J., 2008. Curcumin (diferuloylmethane) inhibits cell proliferation, induces apoptosis, and decreases hormone levels and secretion in pituitary tumor cells. *Endocrinology*, **149**(8): 4158-4167. [doi:10.1210/en.2007-1760]

Nishi, O., Nishi, K., Wada, K., Ohmoto, Y., 1999. Expression of transforming growth factor (TGF)-α, TGF-β2 and interleukin 8 messenger RNA in postsurgical and cultured lens epithelial cells obtained from patients with senile cataracts. *Graefe's Arch. Clin. Exp. Ophthalmol*., **237**(10): 806-811. [doi:10.1007/s004170050316]

Schaumberg, D.A., Dana, M.R., Christen, W.G., Glynn, R.J., 1998. A systematic overview of the incidence of posterior capsule opacification. *Ophthalmology*, **105**(7):1213-1221. [doi:10.1016/S0161-6420(98)97023-3]

Sidjanin, D., Grdina, D., Woloschak, G.E., 1996. UV-induced changes in cell cycle and gene expression within rabbit lens epithelial cells. *Photochem. Photobiol*., **63**(1):79-85. [doi:10.1111/j.1751-1097.1996.tb02995.x]

Wasinger, V.C., Cordwell, S.J., Cerpa-Poljak, A., Yan, J.X., Gooley, A.A., Wilkins, M.R., Duncan, M.W., Harris, R., Williams, K.L., Humphery-Smith, I., 1995. Progress with gene-product mapping of the Mollicutes: *Mycoplasma genitalium*. *Electrophoresis*, **16**(1):1090-1094. [doi:10.1002/elps.11501601185]

Watson, J.L., Hill, R., Yaffe, P.B., Greenshields, A., Walsh, M., Lee, P.W., Giacomantonio, C.A., Hoskin, D.W., 2010. Curcumin causes superoxide anion production and p53-independent apoptosis in human colon cancer cells. *Cancer Lett*., **297**(1):1-8. [doi:10.1016/j.canlet.2010.04.018]

Zhang, W.Y., Hawse, J., Huang, Q.L., Sheets, N., Miller, K.M., Horwitz, J., Kantorow, M., 2002. Decreased expression of ribosomal proteins in human age-related cataract. *Invest. Ophthalmol. Vis. Sci*., **43**(1):198-204.