Effect of Superposed Electromagnetic Noise on DNA Damage of Lens Epithelial Cells Induced by Microwave Radiation

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PURPOSE. To investigate the influence of the 1.8-GHz radiofrequency fields (RFs) of the Global System for Mobile Communications on DNA damage, intracellular reactive oxygen species (ROS) formation, cell cycle, and apoptosis in human lens epithelial cells (hLECs) and whether the effects induced by RF could be blocked by superposing of electromagnetic noise.

METHODS. After 24-hour intermittent exposure at the specific absorption rate of 1 W/kg, 2 W/kg, 3 W/kg, and 4 W/kg, the DNA damage of hLECs was examined by alkaline comet assay and immunofluorescence microscope detection of the phosphorylated form of histone variant H2AX (γH2AX) foci, respectively. ROS production was quantified by the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Cell cycle and cell apoptosis were determined by flow cytometry.

RESULTS. DNA damage examined by alkaline comet assay was significantly increased after 3 W/kg and 4 W/kg radiation (P < 0.05), whereas the double-strand breaks (DSBs) evaluated by γH2AX foci were significantly increased only after 4 W/kg radiation (P < 0.05). Significantly elevated intracellular ROS levels were also detected in the 3-W/kg and 4-W/kg groups (P < 0.05). After exposure to 4 W/kg for 24 hours, hLECs exhibited significant G1/G0 arrest (P < 0.05). There was no detectable difference in cell apoptosis between the microwave radiation and sham exposure groups (P > 0.05). All the effects mentioned were blocked when the RF was superposed with 2 μT electromagnetic noise.

CONCLUSIONS. Microwave radiation induced hLEC DNA damage after G1/G0/G1 arrest does not lead to cell apoptosis. The increased ROS observed may be associated with DNA damage. Superposed electromagnetic noise blocks microwave radiation-induced DNA damage, ROS formation, and cell cycle arrest. (Invest Ophthalmol Vis Sci. 2008;49:2009–2015) DOI: 10.1167/iovs.07-1333

The ever-increasing applications of radiofrequency (RF) electromagnetic fields (EMFs; 10–300 GHz) in, for example, mobile phones, microwave ovens, radios, and radar, have raised public concerns about the potential health hazards associated with nonionizing radiation. The microwave radiation (300 MHz-300 GHz) included in RF EMFs is also widespread, especially the 1.8-GHz microwaves of the Global System for Mobile Communications (GSM) used in mobile phones.

Given that positive13,14 and negative15 results are published in the literature, whether microwave radiation does induce DNA damage is still controversial. Previously, we reported that 1.8-GHz RF EMF (specific absorption rate [SAR], 3 W/kg) radiation could induce repairable DNA damage in hLECs after 2-hour exposure8 and DNA damage in Chinese hamster lung cells after 24-hour exposure.9 However, exposure to 1.8-GHz RF EMF (SAR, 3 W/kg) for 2 hours did not induce human lymphocyte DNA damage in vitro but could enhance DNA damage induced by mitomycin C (MMC; DNA cross-linker) and 4-nitroquinoline-1-oxide (4NQO; UV-mimetic agent).10 It may be associated with the different sensitivity of cell line exposure to EMF. Because the energy of microwaves is ostensibly too weak to break DNA directly, the mechanism is unclear. Previous studies have shown that EMF increased the formation of reactive oxygen species (ROS).11,12 which have been shown to induce DNA damage. It is possible the RF-induced DNA damage may be associated with the overproduction of ROS.

Eyeballs are hot spots of radiofrequency field radiation (RFR).13 Many investigations have demonstrated that microwave radiation can induce cataracts.14,15 The ocular lens is sensitive to microwave radiation because of its nonvascularity, noninnervation, and high percentage of water. The oxidative damage of hLECs is the main mechanism of cataract formation;16 DNA damage to hLECs is also associated with cataracts.17

The biophysical mechanisms by which cells respond to electromagnetic exogenous fields are still unknown. These fields are several orders of magnitude weaker than the random thermal noise fields generated by the thermal motion of the ions in and around the cells. To resolve the signal-to-noise problem, Litovitz et al.18-21 proposed that living cells were affected only by EMF that were temporally and spatially coherent, whereas endogenous thermal noise fields are normally temporally and spatially incoherent. They suggested that the bioeffects induced by EMF would be interfered with when superposed with spatially coherent but temporally incoherent “noise” fields. This suggestion has been subsequently supported by findings from several experiments. Simultaneous exposure to noise significantly attenuated the bioeffects caused by microwave radiation, such as enhancement of ornithine decarboxylase activity in 1929 cells22 and spatial learning deficits in rats.23 In our previous studies, superposition of noise magnetic field (MF) could inhibit epidermal growth factor (EGF) receptor clustering in Chinese hamster lung fibroblasts induced by GSM 1800 MHz RF EMF24 and could block MF-induced gap junction intercellular communication suppression in mouse fibroblast cells.25

A localized specific absorption rate (head and trunk) of 2 W/kg is recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) as the basic restriction in terms of RF EMF (10 MHz-10 GHz) for general public

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exposure. According to these criteria, the safety limit for mobile phones in most European countries is 2 W/kg, though the safety limit for mobile phone emissions in the USA is 1.6 W/kg according to the Institute of Electrical and Electronics Engineers standard established in 1999. Furthermore, in the 1998 guidelines of the ICNIRP, the available experimental evidence indicated that the threshold for irreversible effects in even the most sensitive tissues was greater than 4 W/kg, which is recommended as the occupational whole-body exposure restriction with a traditional safety factor of 10, indicating 0.4 W/kg. Therefore, in this experiment, we used an sXc-1800 RF exposure system to investigate whether exposure to 1.8 GHz RF (217 Hz amplitude-modulated) at the SAR of 1, 2, 3, and 4 W/kg for 24 hours intermittently (5 minutes fields on/10 minutes fields off) would cause DNA damage or ROS formation.

Cell cycle arrest and cell apoptosis, the events that follow DNA damage, were examined by flow cytometry. The effects of microwave radiation superposed with a noise MF or noise MF alone were also examined.

MATERIALS AND METHODS

Cell Culture

Human lens epithelial cell line SRA01/04 was purchased from the Riken Cell Bank (Ibaraki, Japan) and was cultured in Dulbecco modified Eagle medium (DMEM; Gibco, Grand Island, NY) with 20% heat-inactivated fetal bovine serum (HIFBS; Hyclone Laboratories Inc., Logan, UT) at 37°C in an atmosphere of 95% air and 5% CO2. The cells were divided into four groups: a sham exposure group; microwave radiation group at the SAR of 1, 2, 3, or 4 W/kg; a 2 μT noise MF group; and a microwave radiation superposed of noise MF. For 24 hours, respectively. Exponentially dividing cells were cultured in 35-mm diameter dishes (Nunc, Roskilde, Denmark) in a total volume of 2 mL for radiation or sham exposure.

Exposure Systems

sXc-1800 equipment producing a GSM signal, designed by the Foundation for Information Technologies in Society (Zurich, Switzerland) and described in detail by the designer and other groups. was used as a microwave source. It consists primarily of an RF generator, an arbitrary function generator, a narrow band amplifier, and two rectangular waveguides operating at a frequency of 1.8 GHz. The two waveguides, one for exposure and the other for sham exposure, are placed inside a conventional incubator to ensure constant environmental conditions (37°C, 5% CO2, 95% air atmosphere). The increased temperatures of the cells within the culture dish exposed to the SAR of 1 W/kg, 2 W/kg, 3 W/kg, and 4 W/kg were 0.27°C, 0.05°C, 0.08°C, and 0.10°C, respectively. A dish holder inside the waveguide guarantees that the dishes are placed exactly in the field maximum of the standing wave and are exposed simultaneously in E polarization inside a waveguide. The system enables the exposure of a monolayer of cells with less than 30% nonuniformity of SAR. Six Petri dishes can be exposed simultaneously in one exposure waveguide. The entire setup is computer controlled, enabling the automated control of the exposure parameters, including exposure strength (SAR), exposure time, and exposure pattern. The RF EMF-simulating GSM 1.8 GHz signal is amplitude modulated by a rectangular pulse with a repetition frequency of 217 Hz and a duty cycle of 1:8. The hLECs were irradiated (5 minutes fields on/10 minutes fields off) exposed or sham-exposed to RF EMF for 24 hours at an average SAR of 1, 2, 3, or 4 W/kg, respectively.

To generate a noise MF, both sides of the waveguides of sXc-1800 system were wrapped with two rectangular Helmholz coils. The center distance of two Helmholz coils is 24 cm. The direction of the coils is the same as the circular wires in the RF waveguides, and the direction of the noise MF is consistent with the magnetic field of microwave radiation. The coils were provided with a 30- to 90-Hz white noise signal (generated through software designed by Luis M. Penafiel, Catholic University of America). The amplitude of the noise MF was 2 μT in the experiment.

Comet Assay

The alkaline (pH >13) single-cell gel electrophoresis assay was performed by a modified method of Singh et al. The solution, 0.65% normal melting agarose (NMA) and 0.65% low melting agarose (LMA), was prepared in Ca2+-, Mg2+-free phosphate-buffered saline (PBS). Cells were suspended in LMA, and 75 μL LMA-cell suspension were pipetted onto a frosted glass microscope slide precoated with a 100-μL layer of 0.65% NMA. The third layer of 75 μL of 0.65% LMA was finally added. Then the slides were immersed in freshly prepared ice cold lysis solution (1% N-lauroylsarcosine sodium salt, 2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris-HCl, 1% Triton X-100, and 10% DMSO [pH 10]) to lyse the cell proteins and allow DNA unfolding. After at least 1 hour at 4°C in the dark, the slides were covered with fresh buffer (1 mM Na2EDTA, 500 mM NaOH [pH 5.1]) in a horizontal electrophoresis unit. The slides were allowed to sit in this buffer for 20 minutes to allow for DNA unwinding. Then the DNA was electrophoresed at 20 V and 300 mA for 20 minutes. Unwinding and electrophoresis were performed at 4°C. The slides were washed gently to remove alkali and detergent in a neutralization buffer (0.4 M Tris HCl [pH 7.5]) and fixed in methanol for 5 minutes, and then were stained with 50 μL ethidium bromide (20 μg/mL). By all the steps described were conducted under yellow light or in the dark to prevent additional DNA damage. Pictures of 300 cells per treatment sample (100 cells/slide, three replicate slides per experiment per SAR group) were taken individually at 400× magnification under a fluorescence microscope (BX51; Olympus, Tokyo, Japan) equipped with a 530-nm excitation filter, a 590-nm emission filter, and a digital camera (DPS0, Olympus). Nuclear width and extent of migration of DNA fragments, mean tail length, and mean tail moment were analyzed using the Image-Pro Plus program (Media Cybernetics Inc.).

Immunofluorescence Microscope Detection of γH2AX Foci

After radiation, cells were fixed in 4% paraformaldehyde for 15 minutes, washed with PBS, and permeabilized in 0.2% Triton X-100. After treatment with blocking serum (Zhongshan Biotechnology Co., Beijing, China) for 2 hours, samples were incubated with a 1:1000 mouse monoclonal anti-γH2AX antibody (Upstate Technology, Lake Placid, NY) for 2 hours, followed by 1:500 FITC-conjugated goat anti-mouse IgG secondary antibody (Zhongshan Biotechnology Co.,) for 1 hour. To stain the nuclei, DAPI was added to the cells and incubated for another 15 minutes. The coverslip was then removed from the plate, mounted onto a glass slide, and observed under a fluorescence microscope (AX70; Olympus), and the γH2AX foci in each cell were counted (Image Pro Plus; Media Cybernetics).

Intracellular ROS Detection

ROS production was quantified by the DCFH-DA method based on the ROS-dependent oxidation of DCFH-DA to DCF. Cells were incubated for 30 minutes at 37°C with DCFH-DA solution with a final concentration of 50 μM. After incubation, cells were rinsed twice with PBS, collected with trypsin-EDTA solution (0.25% trypsin-0.02% ethylenediamine tetra acetic acid solution; Gibco, Grand Island, NY). After centrifugation at 1500 rpm for 5 minutes, the supernatant was discarded. The pellet was suspended with PBS. Fluorescence of the samples was monitored at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. The ROS level was expressed as OD per milligram protein.

Cell Cycle Assay

After treatment, approximately 2 × 106 cells were washed twice with PBS, then fixed in 75% ethanol for 24 hours at 4°C. After three washes with cold PBS, cells were stained with solution containing 50 μg/mL...
propidium iodide (Sigma, St. Louis, MO) and 10 μg/mL RNase (Sigma). After incubation at 37°C for 30 minutes, cells were analyzed by flow cytometry (FACSCalibur; Becton Dickinson, Mountain View, CA) using specialized software (CellQuest 3.1f; BD Biosciences, San Jose, CA).

Cell Apoptosis Assay
Apoptosis assay was performed by flow cytometry (FACSCalibur; Becton Dickinson), using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) kit (Sigma) in which Annexin V bound to the exposed phosphatidylserine on the plasma membrane of the apoptotic cells. Statistical analysis was performed using specialized software (CellQuest 3.1f; BD Biosciences). The percentage of the early apoptosis was estimated by counting cells that were Annexin V positive but PI negative, whereas the percentage of late apoptosis plus necrosis was estimated by counting cells that were both Annexin V and PI positive.

Statistical Analysis
Statistical comparisons were conducted with one-way ANOVA multiple-comparison followed by the Dunnett test. P < 0.05 was considered statistically significant.

RESULTS
DNA Damage Induced by Microwaves in Comet Assay
Figure 1 shows the mean tail length and mean tail moment results of the alkaline comet assay on hLECs after exposure to 1.8 GHz microwave radiation for 24 hours. No significant difference in DNA damage was observed between 1 W/kg or 2 W/kg microwave exposure and sham exposure (P > 0.05). However, 1.8 GHz microwave exposure at the SAR of 3 W/kg and 4 W/kg for 24 hours induced significantly increased DNA damage compared with sham exposure (P < 0.05). Moreover, DNA damage induced by 4 W/kg microwave radiation was significantly higher than that induced by 3 W/kg microwave radiation (P < 0.05). DNA damage induced by 3 W/kg and 4 W/kg microwave radiation was blocked by superposing of electromagnetic noise (P > 0.05).

DSBs Induced by Microwaves in γH2AX Foci Formation Test
Statistically significant difference of DSBs in the γH2AX foci formation test was observed between the sham exposure and 4 W/kg microwave exposure (P < 0.05). No significant increase in γH2AX foci (P > 0.05) was found at the SAR of 1-, 2-, or 3-W/kg groups and also not in the microwave radiation superposed with the noise MF group (Fig. 2A).

Figure 2B shows that 70% to 80% of cells in sham exposure and microwave radiation at the SAR of 1, 2, or 3 W/kg did not contain any γH2AX foci, whereas the percentage of cells lacking γH2AX foci in the 4-W/kg group was 57.34% ± 5.73% (P < 0.05). The percentage of cells with 1 to 10 γH2AX foci was 36.65% ± 4.90%, and the percentage with more than 20 γH2AX foci was 4.58% ± 1.28% in 4-W/kg group; these were significantly higher than the percentages in the sham exposure group (21.42% ± 2.98% and 1.75% ± 1.00%, respectively; P < 0.05).

Intracellular ROS Increase after Microwave Radiation
ROS production in exposed samples was expressed as a percentage of the sham-exposed ones. SAR of 3 W/kg and 4 W/kg microwave radiation induced significantly increased intracellu-
lar ROS \( (P < 0.05) \), whereas no significant changes were found at the SAR of the 1-W/kg and 2-W/kg groups compared with sham exposure \( (P > 0.05) \). Electromagnetic noise blocked the increased ROS induced by microwaves \( (P > 0.05; \text{Fig. 3}) \).

Microwave Radiation Blocks hLEC Cell Cycle in the G0/G1 Phase

Figure 4 shows the effects of 3 W/kg and 4 W/kg microwave radiation on the cell cycle, as determined by flow cytometry. The hLECs were arrested in the G0/G1 phase of the cell cycle after microwave radiation at the SAR of 4 W/kg after S-phase and G2/M-phase decreases \( (P < 0.05) \). Changes in the cell cycle induced by 4 W/kg microwave radiation were blocked by superposition of a noise MF compared with sham exposure \( (P > 0.05) \). There was no significant modification on the cell cycle in 3-W/kg radiation-treated cells \( (P > 0.05) \).

Microwave Radiation Does Not Induce Significant Cell Apoptosis

Percentages of early apoptosis were estimated by counting cells that were Annexin V positive but PI negative, whereas the percentage of late apoptosis plus necrosis was estimated by counting cells that were both Annexin V and PI positive. There were no significant differences in cell apoptosis between the microwave radiation and sham exposure groups \( (P > 0.05) \).

Electromagnetic Noise Does Not Induce Bioeffects

Exposure to electromagnetic noise alone did not lead to DNA damage, intracellular ROS formation, cell cycle arrest, or cell apoptosis compared with sham exposure \( (P > 0.05) \).

DISCUSSION

The eyes, despite their small volume, absorb a considerable amount of electromagnetic radiation. A specific absorption rate map of a versatile eccentric-sphere model of the human head, designed by Moneda et al.,\textsuperscript{13} revealed the existence of hot spots in the eyes and near the center of the brain. The RFR emitted by mobile phones, which are held close to the head and thus are in close proximity to eyeballs when in use, has provoked concern about the potential cataractogenesis that develops as a result of such exposure. The lens is an encapsulated, avascular, transparent tissue susceptible to damage. It contains a single layer of epithelial cells, and their functional disorders often lead to the formation of cataracts. In this study, we used a human lens epithelial cell line to investigate the mechanism of microwave radiation-induced cataracts. Although the immortalized human lens epithelial cells SRA 01/04 retain some of the human lens-specific characteristics,\textsuperscript{31} they do not undergo differentiation into lens fibers in long-term culture.\textsuperscript{32,33}

The assessment of direct and indirect effects on DNA is one of the worldwide subjects of interest in terms of the RFR domain.\textsuperscript{34} The alkaline comet assay \( (\text{pH} > 13) \) is considered a
sensitive assay for detecting DNA damage, such as single-strand breaks (SSB), double-strand breaks (DSB), and alkali labile sites (ALS), and incomplete excision repair sites, especially the SSB. The phosphorylated form of histone variant H2AX (γH2AX) plays an important role in the recruitment of DNA repair and checkpoint proteins such as the Mre11/Rad50/Nbs1 complex, BRCA1, and 53BP1, to sites of DNA damage, particularly at DSBs. A few minutes after DSB formation, H2AX is phosphorylated by members of the phosphatidylinositol 3-kinase family and forms localized foci at DSB sites. The number of γH2AX foci, which is quantitatively the same as that of DSBs, has been used as an indicator for DSBs in many studies.

The results of alkaline comet assay on hLECs in the present study indicated that DNA damage induced by microwave exposure SAR; both 3 W/kg and 4 W/kg was significantly higher than that induced by sham exposure. On the other hand, there was a significant difference in DNA damage examined by comet assay between the 4-W/kg group and 3-W/kg group; DSBs detected by the γH2AX foci formation test only were observed in the 4-W/kg group. Results of the comet assay and the γH2AX foci formation test showed that DNA damage induced by 4-W/kg microwave radiation was significantly higher than that induced by 3-W/kg microwave radiation. We suppose that the G0/G1 phase arrest in the 4-W/kg radiation-treated cells may be related to higher DNA damage, which needs more time for repair. Although 3 W/kg and 4 W/kg microwave radiation could induce DNA damage of hLECs, the results of cell apoptosis indicated that DNA damage induced by microwave radiation did not result in apoptosis; it is possible that DNA damage is repairable.

The mechanism by which microwaves induce DNA damage is still unclear. As is well known, ROS are reactive and readily damage biological molecules, including DNA. ROS are generated as a byproduct of normal mitochondrial activity in aerobic cells. The overproduction of ROS reportedly causes severe damage to cellular macromolecules, especially the DNA. Stoczyk et al. found that oxidative stress after exposure to microwaves may be the reason for many adverse changes in cells. The study of Moustafa et al. indicated that acute exposure to the radiofrequency fields of commercially available cellular phones may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing the

Figure 1. Flow cytometry analysis of the cell cycle of hLECs after 1.8 GHz microwave radiation with or without the superposition of electromagnetic noise. *P < 0.0001; #P = 0.002.

Figure 2. Intracellular ROS formation in hLECs induced by 1.8 GHz microwave radiation with or without the superposition of electromagnetic noise. Significant increases in intracellular ROS induced by 3 W/kg and 4 W/kg microwave radiation were blocked by the superposition of electromagnetic noise: *P = 0.035; #P = 0.036.

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References

1. Garaj VV, Horvat D, Koren Z. The relationship between colony-forming ability, chromosome aberrations and incidence of micro-
nuclei in V79 Chinese hamster cells exposed to microwave radi-
2. Lai H, Singh NP. Acute low-intensity microwave exposure in-
creases DNA single-strand breaks in rat brain cells. Bio-
3. Lai H, Singh NP. Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radi-
4. Diem E, Schwarz C, Adikofe F, Jahn O, Rudiger H. Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human 
5. Malaya PS, Ahern EW, Bi C, et al. DNA damage in rat brain cells 
after in vivo exposure to 2450 MHz electromagnetic radiation and 
and apoptosis in Molt-4 cells after in vitro exposure o radio-
915 MHz microwave waves induces changes in gene expression 
but not double stranded DNA breaks or effects on chromatin 
field on DNA damage and expression of heat shock protein 70 in 
microwave electromagnetic fields on DNA damage in Chinese hamster lung cells. Zhonghua Yu Fang Yi Xue Za Zhi. 
effect of 915 MHz microwave on the expression of apoptosis- 
related genes and protein in C6 rat glioma cells. J Huazhong Uni 
11. Stopczyk D, Gnitecki W, Buczynski A, Kowalski W, Buczynska M, 
Kowalski W. Exposure of human lens epithelial cells to 
ELF- and VLF- frequency electromagnetic fields induced cluster 
ing of membrane surface receptors and interference by noise magnetic fields. Zhong 
guha Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2006;24(8):461– 
464.
effects induced by exposure to microwaves are mitigated by 
450.
13. Lai H. Interaction of microwaves and a temporally incoherent 
magnetic field on spatial learning in the rat. Physiol Behav. 
frequency electromagnetic fields induced clustering of membrane 
surface receptors and interference by noise magnetic fields. Zhong 
ghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2006;24(8):461– 
464.
junction intercellular communication suppression induced by 50 
Guidelines for limiting exposure to time-varying electric, mag-
netic, and electromagnetic fields (up to 300 GHz). Health Phys. 
17. IEEE Standard for Safety Levels with Respect to Human Exposure 
to Radio Frequency Electromagnetic Fields, 3 kHz to 300 
Hz. Std C95.1. IEEE. 1999.
18. Schunborn F, Pokovic K, Burkhartt M, Kuster N. Basis for optimi-
ization of in vitro exposure apparatus for health hazard evaluations 
550.
19. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for 
quantitation of low levels of DNA damage in individual cells. 
20. Lawler JM, Song W, Demaree SB. Hindlimb unloading increases 
oxidative stress and disrupts antioxidant capacity in skeletal mus-
22. Arita T, Jan LR, Susan SR, Reddy VN. Enhancement of differentia-
tion of human lens epithelium in tissue culture by changes in cell-
2395–2404.
capsule in long-term culture of human lens epithelial cells. Invest 
quency radiation: DNA/Genetox Expert Panel. Environ Mol Mu-
25. Fairbairn DW, Olive PL, O'Neill KL. The comet assay: a compre-
26. Fairbairn DW, Olive PL. Frequency radiation induced early changes in 
427.
27. Chen SC, Lin LR, Okamoto H, Pipas JM, Reddy VN. Enhancement 
2003;278(22):20303–20312.
28. Li WC, Chen SP, Chiang H, et al. Enhancement of differentia-
tion of human lens epithelium in tissue culture by changes in cell-
3525–3530.