Spontaneous Ultraweak Photon Emission during the Growth of the Cell Population of Cultured HeLa Cell Line

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We analyzed the change in the intensities of spontaneous ultraweak photon emission during the growth of the cell population of HeLa cell line using a cell culture system. Comparisons of the temporal change of intensities of spontaneous ultraweak photon emission with that of the growth curve showed that the intensity followed almost the same course with the change rate kinetics of cell population. The analysis of cell viability during the growth of the cell population indicated that there is a close relationship between the intensities of photon emission and proliferative rate of viable cells. The flow-cytometric analysis indicated that the intensities of the photon emission are related primarily to the 2n diploid cell equivalent populations. We suggest that the spontaneous ultraweak photon emission is mainly involved in the changes of the ploidy number during the proliferative process of the cancer cell line. The therapeutic effects of acupuncture may be explained scientifically by the spontaneous ultraweak photon emissions from the cells during the changes of its ploidy number in the anatomical structure of acupuncture meridians.

Key words —— spontaneous ultraweak photon emission, cancer cell line, cell population, ploidy number, viability, acupuncture

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INTRODUCTION

The phenomena of spontaneous ultraweak light emissions of various biological systems, which were also referred to as biophoton emissions, have been observed in biological systems from the cellular levels to whole bodies of living organisms in their physiological and pathological states.[1–3] A biophoton in connection with inter-cellular communication is introduced with its anatomical source, which can explain scientifically the therapeutic effects of acupuncture.[4,5] Even though there was still controversy over the origins of biophoton emissions, it was widely accepted that several metabolic reactions including free radical reactions might play an important role for these very weak photon emissions.[1–3]

Cell culture models have been previously examined in relation to clinical applications, and few studies on biophoton emission from the normal and malignant cell culture in physiological conditions or in harvested cell suspension have been reported because of its extremely weak intensity.[6–9] The intensity of biophoton emission from an esophageal carcinoma cell line has been reported to mainly depend on the cell population during the proliferation of a human esophageal cancer cell line in a flow culture system.[9]

We have recently reported that spontaneous ultraweak photon emission and delayed luminescence are involved in cellular process of death and/or proliferation in excised mouse liver after acute exposure to carbon tetrachloride with sublethal doses.[10]

In the present study, we analyze the changes in the intensities of spontaneous ultraweak photon emission along with the growth of the cell population of cultured HeLa cell line. We show that the temporal changes in the intensities of spontaneous ultraweak photon emission depends on the change rate kinetics of cell population in cultured HeLa cells and suggest that the spontaneous ultraweak photon emission is mainly related to proliferative process of the cell.

MATERIALS AND METHODS

Cell Line and Chemicals —— Human cervical carcinoma cell line, HeLa cells were from Ameri-

can Type Culture Collection. RPMI 1640 medium without phenol red, fetal bovine serum (FBS) and trypsin-EDTA solution, which were used to detach cells from the bottom of a culture vessel, were purchased from Invitrogen (Grand Island, NY, U.S.A.). Other chemicals were of the best grade commercially available.

Cell Culture —— HeLa cells were grown as a monolayer at 37°C and 5% CO_2 in a culture vessel in RPMI 1640 medium without phenol red supplemented with Antimycotic-Antibiotic (Invitrogen; 100 U/ml penicillin, 100 mcg/ml streptomycin, 0.25 mcg/ml amphotericin B) and 10% FBS. Two sets of HeLa cells, in which the average initial cell population was 1×10^4 cells, were seeded and allowed to adhere to the bottom of 35 mm disposable plastic petri dishes and incubated for the indicated time period of 24, 48, 72 and 96 hr in a CO_2 incubator. After incubation, cultured HeLa cells in one set were briefly exposed to trypsin and dispersed in fresh medium to restore cell concentration to starting level and the cell number of the cell suspension was counted with a hemocytometer (Improved Neubauer).

Cell Viability Assay —— Cells from one 100 mm dish were harvested by trypsinization, an aliquot of cells was mixed with 0.1% trypan blue at a 1:1 ratio, and viable cells, which exclude trypan blue dye, were counted with a hemocytometer.

Cell Cycle Analysis —— Both adherent cells and detached cells from one 100 mm dish were scraped off plates, harvested by centrifugation at $500 \times g$ for 5 min, and fixed at −20°C for 4 hr in 2 ml of 70% ethanol. Cells were pelleted, re-suspended in 0.1 ml of phosphate-citrate buffer containing 0.192 M Na_2HPO_4 and 4 mM citric acid, and incubated for 30 min at room temperature. Cells were then pelleted, treated with 1 ml phosphate-buffered saline (PBS) containing 10 mcg/ml RNase A (DNase-free, Sigma, St. Louis, MO, U.S.A.) and 10 mcg/ml propidium iodide and incubated for 20 min at room temperature. Subsequent flow-cytometric analysis, in which at least 5000 cells from each sample were analyzed for DNA content, was performed with a FACS Vantage flow cytometer (BD, Franklin Lakes, NJ, U.S.A.). Fluorescence data were displayed as dot plots and percentages of cells in G1, S, and G2/M phases were determined by using the Cell Quest software (BD).

Measurement of Ultraweak Photon Emission —— The HeLa cells in a petri dish were cultivated inside a CO_2 incubator for specified time periods. Then, each of cultured cells was placed in the measurement apparatus and the biophoton emissions from the cells in the medium were counted along with the medium itself which was incubated for the indicated time period of the same condition. As shown in Fig. 1, the cells in the petri dish were moved to the dark chamber where emitted photons were counted by the photomultiplier tube (PMT, R331-05 selected, Hamamatsu, Japan). The numbers of emitted photons from the samples were measured by the PMT and associated data acquisition systems. The PMT operated at room temperature had a spectral response in the range of 300 to 650 nm and the wavelength of maximum response was 420 nm. Proper housing and a magnetic shield case for the PMT were used to ensure complete light-shielding and magnetic-shielding measurements. The distance between the PMT and the sample was 5 cm. Actual photon detections were made within the dark chamber in a dark room. In the dark chamber a petri dish was put in the sample holder. The sample holder was maintained at constant temperature (37°C) by the temperature controller with a thermocouple and a heater. The dark chamber and the sample holder were made of anodizing aluminium.

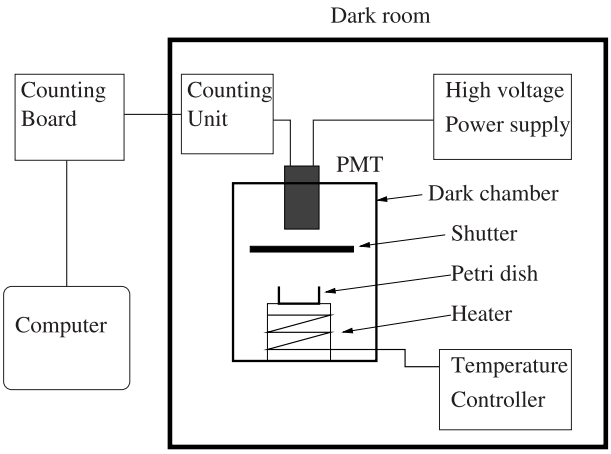

Fig. 1. Schematic Diagrams of the Measurement Apparatus for Ultraweak Photon Emission from the Sample of the Cultured Cell Line in a Dark Chamber

RESULTS AND DISCUSSION

The growth curve of the total cells in the cultured HeLa cells displayed a lag phase and a log phase (Fig. 2). The increases in the cell population were 4.5, 9.5, 35.5 and 58.0 times the initial pop-

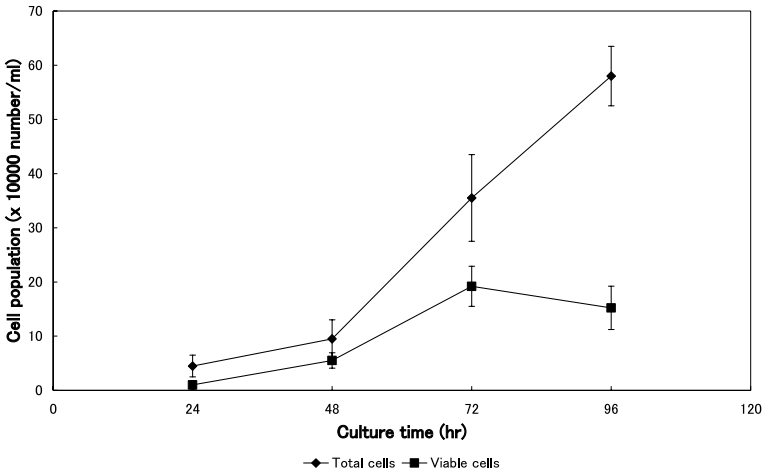

Fig. 2. Growth Curves of the Total Cells and the Viable Cells in the Cultured HeLa Cells

Each cultured cells was incubated for the indicated time period of 24, 48, 72 and 96 hr, and the total cells was counted with a hemocytometer. Each cultured cells incubated for the indicated time period of 24, 48, 72 and 96 hr was mixed with trypan blue, and the viable cells was counted with a hemocytometer. The data displayed were calculated from three independent experiments, and mean ± S.D. values are shown.

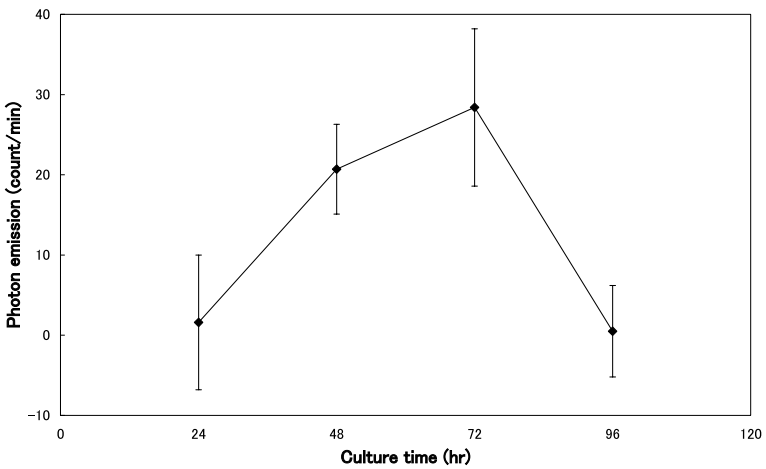

Fig. 3. Changes in the Intensity of Spontaneous Ultraweak Biophoton Emission Exclusively Radiated from the Cultured HeLa Cells

Each cultured cells incubated for the indicated time period of 24, 48, 72 and 96 hr was placed in the measurement apparatus and the photon emissions from the cells in the medium was counted along with the medium itself. The data for the spontaneous ultraweak biophoton emissions exclusively radiated from the cells was calculated by subtracting the reference counts by the medium itself from the emission intensities by the cells in the medium. The data displayed were calculated from three independent experiments, and mean ± S.D. values are shown.

ulation at 24, 48, 72 and 96 hr, respectively, and the doubling time of the cultured HeLa cell line in RPMI 1640 medium was calculated as 11.3 hr. The assay of cell viability using trypan blue dye during the growth of the cultured HeLa cell line indicated that the temporal change of population of viable cells were proportional with the change rate kinetics of cell population (Fig. 2).

Changes in the emission intensities of ultraweak biophoton emission exclusively radiated from the cultured HeLa cells were obtained during the growth of the cell population (Fig. 3). The dark counts showed that the photoemission intensity was the level of *ca* 135 counts/min. The photon emission from the medium itself was counted as the reference counts and the emission intensity was the level of *ca* 240 counts/min with long-term stability. A low intensity of emission during the initial log phase was observed in the cultured HeLa cells, and the emission intensity at 24 hr was 2 counts/min. Then, the emission intensity followed a drastic increase until 72 hr of the log phase and reached a maximum level of 28 counts/min at 72 hr, the log phase in which the cell population is most reproducible. After this

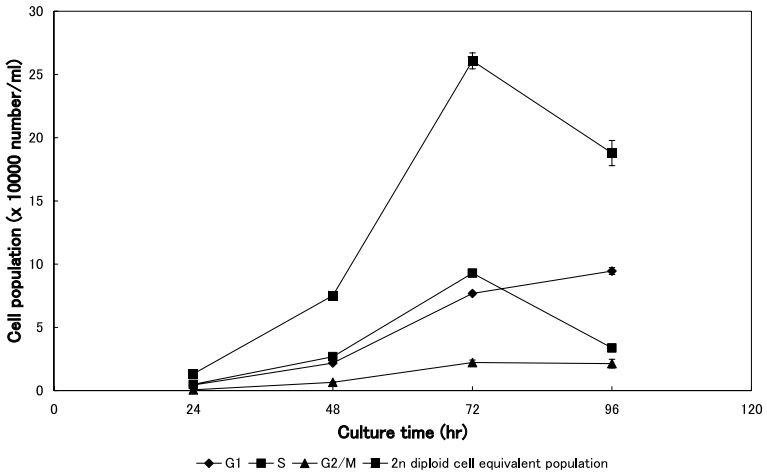

Fig. 4. Cell Cycle Analysis of the Cultured HeLa Cells

Each cultured cells incubated for the indicated time period of 24, 48, 72 and 96 hr were fixed, stained with propidium iodide, and analyzed by flow cytometry. The data for the cell populations in each phase of cell cycle were calculated from the populations of total viable cells and the proportions of cells in each phase of the cell cycle. The data for the diploid 2n cell equivalent populations of total viable cells were calculated from the populations of the cells in each phase of the cell cycle and the average ploidy numbers of cells in each phase of the cell cycle. The data displayed were calculated from two independent experiments, and mean ± S.D. values are shown.

increase, the emission intensity from the cultured HeLa cells was significantly decreased until the end of the log phase, in which the emission intensity of the cells continuously decreased to the level of 1 counts/min at 96 hr. In this study, the emission intensity from the cultured HeLa cells was low at 96 hr of a plateau phase as well as 24 hr of a lag phase.

The cultured HeLa cells have been shown to produce a spontaneous ultraweak photon emission, which was not due to the delayed luminescence, since the photoemission from the samples was measured after long time of dark adaptation. The intensities of spontaneous ultraweak photon emission of the HeLa cells were significantly higher than that of RPMI 1640 medium. Comparisons of the change of emission intensities of spontaneous ultraweak biophoton with that of the growth curve showed that the intensity followed almost the same course with the change rate kinetics of cell population. This comparison indicates that there is a close relationship between the biophoton emission intensity and the change rate of HeLa cell population. Thus, the temporal changes in the intensities of spontaneous ultraweak photon emission mainly depends on the change rate kinetics of cell population in this cell culture, in which the change rate kinetics of cell population is reflective of the proliferative rate of cells. Also, the assay of cell viability using trypan blue dye during the growth of the cell population indicated that there is a close relationship between the intensity of photon emission and the proliferative rate of viable cells.

The flow-cytometric analysis was carried out to clarify the relationship between the emission intensity and the proliferative process of the cells during the growth of the cell population (Fig. 4). The flow-cytometric analysis indicated that the proliferative rate of viable cells was consistent with the proportion of cells in various phases of the cell cycle during the time period of cell culture. Both the populations of HeLa cells in G1 and G2/M phases had increased during the time period of cell culture from 24 to 96 hr. However, the population of cells in S phase had increased from 24 to 72 hr, and decreased from 72 to 96 hr. The flow-cytometric analysis indicated that primarily the change in the 2n diploid cell equivalent populations is related to the change in intensities of the spontaneous ultraweak photon emission. This data indicated that there is a close relationship between the intensity of the spontaneous ultraweak photon emission and the ploidy number of each cells.

In conclusion, these observations collectively indicate that the spontaneous ultraweak photon emission from cultured HeLa cells was closely related to the proliferative process of the living cells. We suggest that the spontaneous ultraweak photon emission is mainly involved in the changes of the ploidy number during the proliferative process of the cancer cell line. The therapeutic effects of acupuncture may be explained scientifically by the spontaneous ultraweak photon emissions from the

cells during the changes of its ploidy number in the anatomical structure of acupuncture meridians. The present approach by means of the biophoton measurement may be useful for the analysis of growth activity such as viability and proliferation of cancer cells.

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