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A novel method of assessing carcinoma cell proliferation by biophoton emission

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Abstract

Changes in the emission intensities of ultraweak biophoton emission during the cell proliferation of human carcinoma cell culture (TE9 cell line) were detected using a highly sensitive and low noise measurement apparatus coupled with a flow culture system. In the sampling period of 93 h, the biophoton emission intensity from the culture followed a similar course as that of the growth curve. Spectral analysis of the biophoton emission from the cell culture demonstrated a significant peak at around 530 nm. Our results suggest that the emission intensity mainly depends on the cell population and that this non-invasive technique has a potential role in cancer diagnosis. © 1998 Elsevier Science Ireland Ltd.

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1. Introduction

Ultraweak biophoton emission is the spontaneous light emission radiated from various biological systems derived from chemiexcitation of biological molecules during oxidative metabolic processes [1]. Therefore, this weak light is a possible representation of physiological or pathological conditions of cells and tissues. Detection of biophoton emission makes it possible to acquire immediate information on biological activities non-invasively, as this phenomenon occurs without any external enhancement [1]. Thus, extension of biophoton measurement to clinical areas has been proposed for brain and cancer research.

Cancer is a major cause of mortality and several diagnostic methods have been developed to ascertain pathologic conditions of cancer. Trials on ultraweak biophoton measurement of serum or urine from cancer patients have also been performed in relation to diagnostic applications [2,3]. The elevation of the emission intensity from serum or urine was attributed to metabolic changes in patients. Therefore, it is naturally expected that measurement of carcinoma lesions or cells would provide more accurate information on the pathologic conditions of cancer.

Mamedov et al. [4] measured ultraweak light emission from several cancer cell lines and normal cells by

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static cell culture and presented a difference in emission intensities between cell lines. This study suggested the application of biophoton emission from cell culture for biological or medical applications, but the measurements were not made in continuous cell cultures and the results did not connect biophoton emission to physiological changes. van Wijk and van Aken [5] also measured biophoton emission using human hepatoma cells and hepatocytes in static cell cultures. No elevation of the emission intensity from hepatoma cells was observed but elevation of the induced photon emission intensity on excitation with white light was reported. Bustamante et al. [6] presented a difference in emission intensities between the phase of proliferation from malignant melanoma cell culture, however, their measurements were done with harvested cell suspension and not in physiological conditions. Shimizu et al. [7] and Amano et al. [8] measured the surface of carcinoma lesions by employing animal implantation models. In these reports, Amano et al. [8] presented a two-dimensional biophoton image of a growing tumor with suggestions for clinical applications of biophoton imaging, for example, in cancer diagnosis.

Cell cultures have been routinely examined as an attractive model in many clinical applications. These models have advantages for strict and variable control of the environment compared to animal implantation models and are not restricted to cell lines which do not grow in animals. Despite these advantages, very few studies on ultraweak biophoton emission from malignant cell cultures have been reported due to the extremely weak intensity of light emission [4–6]. Also, as continuous measurement of biophoton emission with cell culture is expected to detect temporal alteration of physiological conditions like cell proliferation or cell damage caused by drugs, studies on changes in emission intensities related to cancer cell proliferation or spectral analysis may enable us to extract specific information on cancer progression and response to drugs.

In the present study, we report the biophoton emission characteristics from an esophageal cancer cell line, TE9. Esophageal cancer belongs to the highest mortality group in malignancies and has a relatively high incidence in Asian countries. Surgery, chemotherapy, radiotherapy or a combination of these are employed for the treatment of esophageal cancer. Pathologic diagnosis of esophageal cancer is one of the most desirable choices for therapeutic tactics. Measurement of biophoton emission could therefore be applied to detect the behavior of growth which is one of the chief factors that define malignancy and used in experiments on the sensitivity or resistance of cancer cells to anticancer drugs that would improve prognosis.

The temporal changes in emission intensities and the cell population along with the spectral analysis of an esophageal cancer cell strain are reported using a highly sensitive measurement apparatus coupled with a flow culture system.

2. Materials and methods

2.1. Chemicals

Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) without Phenol Red. RPMI 1640 medium was purchased from Life Technologies (Grand Island, NY) and FBS was purchased from ICN (Costa Mesa, CA). Ethylene diamine tetraacetic acid (EDTA) and trypsin were used to detach cells from the bottom of sample cuvettes and culture flasks. EDTA was purchased from Life Technologies (Grand Island, NY) and trypsin was purchased from ICN (Costa Mesa, CA). All chemicals were culture grade.

2.2. Cell line

The cell line employed for this study was an esophageal carcinoma cell line (TE9) originating from pleural effusion of a patient and was established in the Second Department of Surgery, Tohoku University, School of Medicine [9].

2.3. Instrumentation

2.3.1. Flow culture system

A flow culture system was used to maintain stable nutritional conditions for cell proliferation throughout the measurement period (93 h). This system was comprised of a quartz flow culture cell, a gas perfusion system and a medium perfusion system (Fig. 1). The medium was supplied at a constant velocity of 2 ml/h

by a syringe pump (TOP-5200, TOP, Tokyo, Japan) and drained by a micro tube pump (EYELA MP-3, Tokyo Rikakikai, Tokyo, Japan). A 5% CO_2/air flow was maintained by a gas regulator (SEC-400 mass flow system, STEC, Kyoto, Japan) at a constant velocity of 20 ml/min.

2.3.2. Detection of ultraweak biophoton emission

Biophoton emission measurements were made with a low noise and highly sensitive photon-counting system that is capable of spectral analyses [10,11]. The system consists of a temperature-controlled light-tight sample chamber, a photomultiplier tube (PMT; R1333, Hamamatsu Photonics, Hamamatsu, Japan) cooled to −40°C, a rotating wheel with a set of short wavelength sharp cut-off glass filters and a computer-controlled photon counter (Fig. 1). The PMT is sensitive in the range of 300–900 nm with a maximum spectral sensitivity around 650 nm. The filter wheel has an open port for the measurement of the total emission intensity and a closed port for dark current measurement. The spectral resolution of the system varies between 10 and 40 nm in the wavelength range of 250–850 nm. Recorded spectral data were corrected for the wavelength response of the PMT and transmittance of the cut-off filters. The useful wavelength range of the system is approximately 400–850 nm with the combination of the PMT and the cut-off filters.

2.4. Experimental details

2.4.1. Measurement of biophoton emission

Cells were cultured as a monolayer at 37°C on the bottom of a quartz cuvette of the flow culture system. Before the measurement of biophoton emission an equal number of cells was disseminated in two quartz cuvettes, i.e. a sample and a reference cuvette. Then the cells were incubated for 6 h in a CO_2 incubator and allowed to adhere to the bottom of the cuvettes. After incubation, the cell population in the reference cuvette was counted with a hematocytometer to estimate the initial cell population, as 10–20% of the disseminated cells do not stick to the cuvette during 6 h of incubation. The sample cuvette was then placed in the measurement system. After 93 h of measurement, the final cell population in the sample cuvette was counted.

Biophoton emission intensities were calculated by

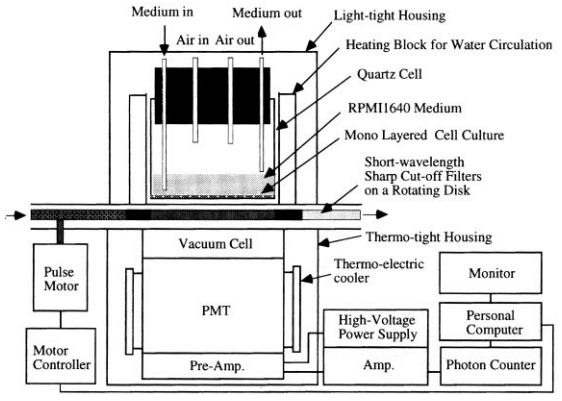

Fig. 1. Schematic representation of the measurement apparatus with the flow culture system.

subtracting the background counts (dark current with the closed port of the filter wheel) from the total emission intensities (through the open port of the filter wheel). The gating time for each portion of the disk was 20 s and it took 14 min for one cycle. The sampling period for each experiment was 400 cycles (93 h).

2.4.2. Growth curve of TE9 in the flow culture system

The growth curve of TE9 in the flow culture system was independently obtained by counting the cell population at 12, 23, 47, 70 and 93 h of culture for comparison with the changes in emission intensity. The average initial cell population for each experiment was 5×10^5 cells and the data displayed in Fig. 2 are an average of four to six measurements.

2.4.3. Statistical analysis

The statistical significance of the differences in the emission intensities between TE9 culture and the medium were estimated by the two-tailed Student's *t*-test.

3. Results

The growth curve of the TE9 cells in the flow culture system (Fig. 2) displays a lag phase, a logarithmic growth phase and a stationary phase similar to that of static cultures. The increases in the cell population were 1.0, 1.2, 3.5, 5.4 and 4.9 times the initial population at 12, 23, 47, 70 and 93 h, respectively. The

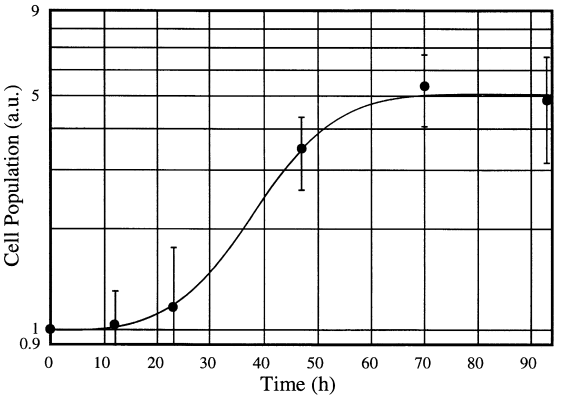

Fig. 2. Growth curve of a cancer cell line (TE9) in the flow culture system. Changes in the cell population of a cancer cell line in the flow culture system were estimated at 12, 23, 47, 70 and 93 h. An increase in the cell population is indicated in relative numbers.

doubling time of TE9 in the flow culture system was calculated as 15.2 h. Cells at 70 and 93 h were in the subconfluent–confluent state on microscopic observation.

Changes in the emission intensities obtained on averaging five different experiments from TE9 culture and medium are displayed in Fig. 3. A drastic decrease in light emission during the initial 18 h was observed both in the cell culture and in the medium. This decay is attributed to the delayed fluorescence from the medium on exposure to weak room light before the measurements. After this decrease, the emission intensity from the TE9 culture followed a

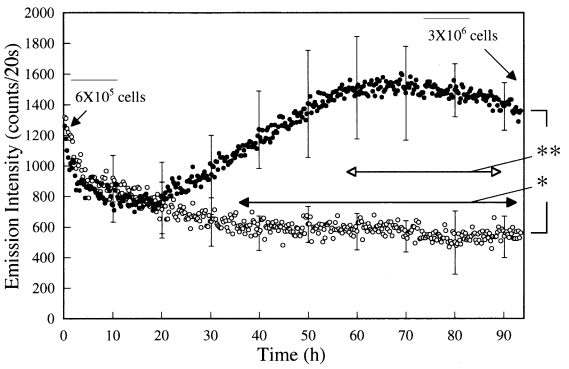

Fig. 3. Changes in the emission intensity from carcinoma cells (TE9) and the medium (RPMI 1640). Temporal changes in the emission intensity from TE9 cells (●) and RPMI 1640 medium (○) are shown. Each dot represents the mean ± SD of five different experiments. *Statistically significant at $P < 0.01$ (34–93 h); **statistically significant at $P < 0.001$ (57–90 h).

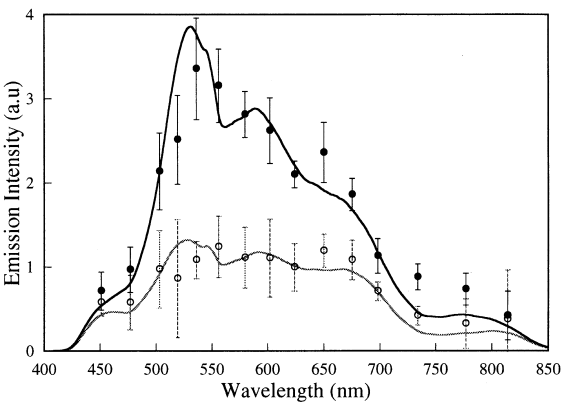

Fig. 4. Spectral distribution of biophoton emission of a carcinoma cell culture (TE9) and the medium (RPMI 1640). The emission spectra of TE9 with medium (●) and RPMI 1640 medium (○) in the flow culture system are shown. Each spectrum was obtained from five different experiments. Spectra of TE9 and the RPMI 1640 medium culture were obtained from each period of 67–93 h.

similar course to that of the growth curve. This increase in emission intensity following the lag phase continued until 60 h to the level of the second plateau.

The emission intensity at 14 h in the first plateau obtained on averaging ten sequential cycles (140 min) was 748 counts/20 s. The emission intensity reached a maximum level of 1533 counts/20 s (at 70 h) in the second plateau and then decreased to 1369 counts/20 s (at 93 h). The emission intensities of TE9 culture were significantly higher than that of RPMI 1640 medium in the period of 34–93 h ($P < 0.01$) and 57–90 h ($P < 0.001$). In contrast, the emission intensity of the medium continuously decreased to the level of 574 counts/20 s at 93 h.

The spectral distribution of biophoton emission from TE9 culture and RPMI 1640 medium was obtained by averaging each of five different experiments. Both are shown in Fig. 4 after subtraction of background data (dark current). The emission intensity from the TE9 culture was more intense than that from the medium in the range of 400–850 nm. The spectrum from TE9 culture showed peaks around 530, 590 and 770 nm and shoulders around 450, 540 and 660 nm. The spectrum from RPMI 1640 medium showed peaks around 460, 530, 540, 590, 670 and 800 nm. Although TE9 culture and the medium exhibited almost the same position of peaks and shoulders, the ratio of the spectral peaks to that of the shoulders

was considerably different. The emission intensity of the peaks is significantly higher, especially in the range of 500–700 nm ($P < 0.001$ at 536, 556, 580, 602, 624, 650, 675 and 734 nm; $P < 0.005$ at 519 and 698 nm; $P < 0.01$ at 503 nm; $P < 0.05$ at 777 nm). The spectral pattern observed in the cell culture in the lag phase was similar to that of the medium. In addition, the emission spectrum of the cell culture in the logarithmic growth phase was almost the same as that in the stationary phase.

4. Discussion

Comparisons of the cell population of the cancer cell line TE9 with emission intensities from cell culture after 10 h show that they both followed almost the same course. The emission intensity exhibited a first plateau phase from 10 to 20 h, followed by an increase in the emission intensity from 20 to 60 h and a second plateau phase after 60 h. These data correspond to the lag phase, the logarithmic growth phase and the stationary phase in the growth curve, respectively. This comparison indicates that there is a close relationship between the biophoton emission intensity and the cell population. Thus, continuous measurement of biophoton emission can be a novel method to assess cell proliferation non-invasively. Furthermore, there is a possibility that a comparison of emission intensity kinetics between different cell lines could be useful for identifying cell types. Monitoring the changes in biophoton emission intensity could also provide additional information on the changes in growth behavior during repeated reculture of a cell line.

Earlier, Quickenden and Que Hee [12] reported two emission peaks during the logarithmic growth phase and another two peaks in the late stationary phase of *saccharomyces cerevisiae*. They attributed prominent third and fourth peaks observed in the late stationary phase to changes of glucose-based metabolism to ethanol-based metabolism. Bustamante et al. [6] reported a difference in the emission intensity between the logarithmic growth phase and the stationary phase using human malignant melanoma cells. They attributed it to the difference in the activity of catalase in cells. The contribution of these physiological conditions to biophoton emission is not clear at present. In our studies, the emission intensity from the cells was low at 0 h and slightly decreased during the late stationary phase. As the emission intensity is reflective of the underlying metabolic reactions in the cells, emission intensities at 0 h and in the late stationary phase could also carry useful physiological information. Further studies are in progress to clarify the relationship between emission intensity and physiological condition, especially during various phases of the cell cycle.

Spectra of ultraweak light emission from biological systems are reported to provide information on emitters and molecular mechanisms of the physiological process. Studies on the mechanisms of biophoton emission from living cells have been extensively carried out on subcellular organs. In these studies, active oxygen species generated from mitochondria, peroxisomes or microsomes are proposed to be one of the contributors [1,13,14]. Active oxygen species are subsequently involved in oxidative reactions of membrane lipids leading to the generation of triplet carbonyls and singlet oxygen [1,10], which are emitting species. Energy transfer from active oxygen species or radicals to other fluorescent molecules is also suggested as a possible light emission mechanism [1]. In our study, the cancer cell line TE9 exhibited specific spectral peaks and shoulders in the range of 500–700 nm compared to that of the medium. A light emission shoulder at around 450 nm can be attributed to triplet carbonyls [1,15] and emission at around 580, 634 and 703 nm can be attributed to singlet oxygen. Peaks at around 530 and 590 nm may be attributed to tyrosine and tryptophan, respectively [16]. Similarities in the spectral positions of peaks or shoulders observed in the cell culture and the medium may be due to the similarity of molecular components in both. The difference in the ratio of the peaks to shoulders of emission intensity between the TE9 cell culture and the medium can be explained by biased quantitative distribution of molecular components. Furthermore, spectral signatures of individual cell types can be used as a marker to extract information from complex biological systems as in the case of an in vivo system. The medium must also be considered as a possible field of light emission in the case of cell culture [17], as hydrogen peroxide generated in cells can penetrate the cell membrane. Leakage of hydrogen peroxide may be the cause of subsequent oxidization of medium components with generation of light emis-

sion. As spectra of biophoton emission contain information on metabolic activities related to molecular mechanisms in cells, spectral difference can be used to detect differences in properties between cell lines for clinical diagnoses.

In conclusion, we report temporal changes of ultraweak biophoton emission intensities along with cancer cell proliferation and spectral distribution of ultraweak biophoton emission from the TE9 cancer cell line. To the best of our knowledge, ultraweak light emission from a continuous cell culture using a flow culture system has not been reported before. Our results suggest that biophoton measurement could be a novel method for assessing growth activity and physiological or pathologic conditions of cancer cells and it could contribute to clinical applications for malignancy in future.

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