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Ultraweak biophoton emission imaging of transplanted bladder cancer

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Abstract Biophoton emission or spontaneous ultraweak light emission has been observed from almost all living organisms, with intensities ranging from 10^{-19} to 10^{-16} W/cm^2. The measurement of biophoton emission offers the attractive possibility of noninvasive monitoring of the underlying physiological function of a living system. In the present study, ultraweak biophoton emission from mice with transplanted bladder cancer was detected by a two-dimensional photon-counting system. Photon counts were observed to be 1.51–4.73 times higher from the regions of untreated tumor than from normal regions. Our study suggests that this novel technique may be applicable to the diagnosis of superficial tumors.

Key words Ultraweak light · Biophoton emission · Two-dimensional imaging · Bladder cancer

Ultraweak light emission, often referred to as biophoton emission, has been observed from most living organisms, including animals, plants and microbes [1, 4–6, 8–10, 12]. The intensity of this ultraweak emission is in the order of 10^{-19}–10^{-16} W/cm^2 [6, 9].The biophoton emission described here is a naturally occurring emission associated with metabolic processes. This is different from the bioluminescence of, for example, fireflies, and the delayed fluorescence of chlorophyll and other pigments following exposure to light [8, 9].

The measurement and characterization of this weak light has led to interesting applications in the fields of biology and medicine, such as the study of the fertilization process in sea urchin eggs [10] and analysis of smokers' blood [16] and human breath [7].

In the present study, we present the first report of the two-dimensional imaging and photon counting of ultraweak light emission from bladder cancer transplanted into the feet of nude mice.

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Materials and methods

Tumor model

Female nude mice (BALB/c nu/nu, Nihon SLC, Hamamatsu, Japan) with a body weight of about 25 g were used in this study. The KK-47 cells were established from grade I superficial bladder cancer [11]. A saline suspension of 1×10^7 disaggregated KK-47 bladder cancer cells was injected s.c. into the left feet of the mice [2]. The biophoton emission measurements were performed 2 weeks after implantation.

Two-dimensional imaging and counting of biophoton emission

The measuring system included a video-intensified system (Argus-100/VIM, Hamamatsu Photonics, Hamamatsu, Japan) that was modified in our laboratories. A block diagram of our measurement system is given in Fig. 1. Briefly, the biophotons emitted from the specimen were imaged onto the detector assembly by a lens. The detector assembly consisted of a sensitive photocathode and a microchannel plate. The active surface of the photocathode was 15 mm in diameter and the spectral response of the photocathode was between 370 nm and 700 nm. The photocathode was cooled to $-20\,^{\circ}$C to reduce the thermal noise, which was approximately 4 counts/s on cooling. The focal length and the aperture of the lens were infinity and 0.82, respectively. The surface area under investigation was approximately 1 cm^2.

The biophoton imaged on the photocathode by the lens generated photoelectrons which were multiplied by the microchannel plate and focused onto the imaging tube. The two-dimensional positions of the

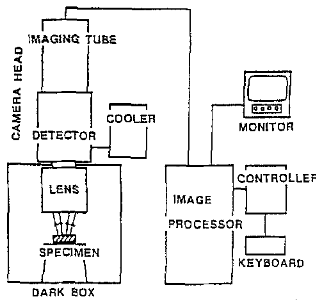

Fig. 1 Schematic diagram of the two-dimensional photon-counting and imaging system

incident biophotons were computed by an image processor and displayed on a monitor in a 512 × 512 pixel (picture element) format. The photon count per unit area was calculated in the tumor and normal regions after subtraction of the background counts [9].

When the tumors reached approximately 250 mm^3 in volume (usually within 2 weeks of inoculation), the mice were anesthetized with pentobarbital sodium (Nembutal, Abbott Laboratories, Ill., USA, 20 mg/kg i.p.) and ketamine hydrochloride (Ketalar, Sankyo, Tokyo, Japan, 10 mg/kg i.m.). The position of the mice was then fixed with a nonfluorescent tape on a small stage covered with an aluminum foil. Photon counting was performed for 2 h in each mouse, and once for each mouse. The data from the tumor and normal area were expressed as photon number/pixel per hour after subtraction of the background count. Seven mice were examined in this study. The statistical significance of the results was assessed by the Mann-Whitney test.

Histological studies of transplanted bladder cancer were performed on a hematoxylin and eosin preparation.

Results

Figure 2 shows the images obtained from the monitor of our measurement system. The image in Fig. 2a was obtained on illuminating the measurement region with dim white light. This was done for the purpose of focusing and positioning the specimen. The mouse was then adapted to the dark for about 20 min, during which period delayed fluorescence due to illumination with light subsided. The tumor was located in the left foot of the mouse. The biophoton image is shown in Fig. 2b. The bright area in the image corresponds to the tumor region. The biophoton emission pattern from the normal areas such as the right foot, abdomen and tail were not clearly discernible. The background counts were studied for one to three areas in each examination.

The photon counts for individual mice (normal and tumor-bearing legs) are shown in Table 1. The photon count rate after subtraction of background count from normal mouse legs was $(10.0 \pm 5.4) \times 10^{-3}$ photons/pixel per hour and $(27.4 \pm 20.7) \times 10^{-3}$ photons/pixel per hour for the tumor-bearing mouse legs. The count from the tumor-bearing legs was significantly higher (approximately 1.51–4.73 times) than those from normal legs ($P = 0.0367$, Fig. 3).

Figure 4 shows the results of histological studies of the transplanted bladder cancer. It can be noted that the cells are actively mitosing with no evidence of hemorrhage or necrosis.

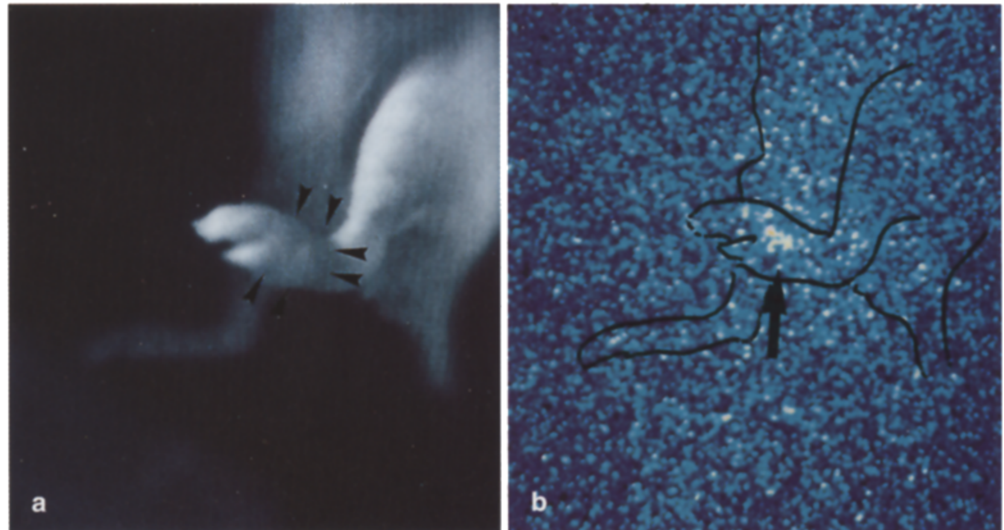

Fig. 2 a Image obtained on illumination with dim white light. The tumor can be observed in the left leg (*arrowheads*). **b** Biophoton emission pattern from tumor-bearing nude mouse. The *bright area* corresponds to the tumor region (*arrow*)

Table 1 Photon counts for individual mice ($\times 10^{-3}$/pixels·h)

Mouse No.	1	2	3	4	5	6	7	Mean ± SD
Normal	15.8	6.7	8.7	5.5	14.3	3.0	16.2	10.0 ± 5.4
Tumor	68.0	20.5	13.1	9.5	40.9	14.2	25.4	27.4 ± 20.7

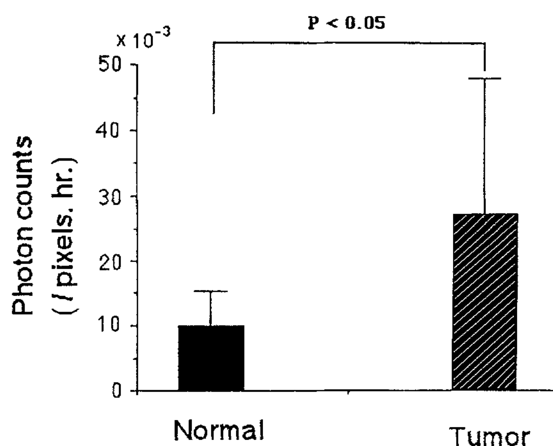

Fig. 3 Photon count from normal and tumor-bearing regions. The photon counts from tumor legs were significantly higher than those from normal legs ($P = 0.0367$)

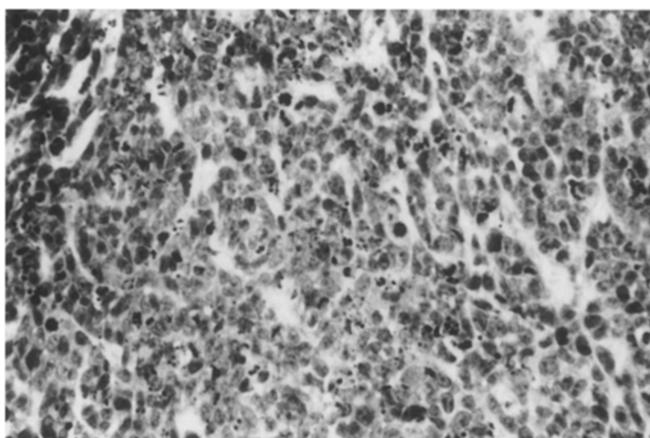

Fig. 4 Histological section of the region of transplanted KK-47 bladder cancer. H&E, reduced from × 100

Discussion

The KK-47 cells enter the log phase of growth 1–6 weeks after implantation [2], and during this period necrosis, hemorrhage, leukocyte infiltration or crusta formation are not observed (Fig. 4). However, during the low growth rate phase 6–7 weeks after implantation, necrosis, hemorrhage and crusta formation were observed. Although the biophoton emission intensity is reported to increase on artificial injury and crusta formation in mouse [12], the present experimental results suggest that biophoton emission is also observed in the implanted tumor region containing actively proliferating log phase cancer cells. Our earlier reports on the biophoton image from germinating plant seedlings also suggest that the emission is high at injured sites and regions actively involved in cell multiplication [8, 9].

In a related study it was suggested that the emission intensity from cells after illumination (induced photon emission) is dependent on the cell type and degree of differentiation [13, 14]. Therefore, the characteristics of biophoton emission from other tumor and non-tumor cell lines also need to be investigated.

One of the possible mechanisms which would explain the biophoton emission phenomena is attributed to the endogenous generation of electronically excited states during oxidative metabolic reactions [6, 15]. It has been reported that the biophoton emission from rat liver nuclei is dependent on oxygen with the possible involvement of singlet oxygen [5]. The active oxygen species including singlet oxygen, superoxide radicals, hydrogen peroxide and hydroxyl radicals have also been implicated in carcinogenesis and tumor promotion [3], suggesting a relation between biophoton emission phenomena and carcinogenesis studies. The relationship between tumor, active oxygen species and biophoton emission must be further investigated to give an understanding of the exact mechanism of the biophoton emission.

The extreme weakness of this phenomenon makes it difficult to apply this technique to the study of light emission from deeply located tumors. Although this novel technique can only be adapted to the detection of photons from superficial tumors at present, it should be pointed out that no pre-examination preparation is required. The biophoton emission measurements are simple and noninvasive with immense potential applications in cancer prognosis/diagnosis. Further refinements and advancement in detection technology in conjunction with a better understanding of emission mechanisms could lead to the detection of bladder tumors including carcinoma in situ noninvasively.

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