Some advanced cancer patients suffer from pungent sulfury malodor. To determine the chemical identity of the odorant, we performed gas chromatography-mass spectrometry-olfactometry analysis of volatiles from fungating cancer wounds. We identified the source of the characteristic smell as dimethyl trisulfide, a compound that is known to be emitted from some vegetables and microorganisms. Controlling the production of dimethyl trisulfide should improve quality of life of patients.

Key words: cancer; odor; GC-MS; olfactometry; dimethyl trisulfide

Some advanced cancer patients are distressed by the unpleasant smell of their fungating wounds.1) Fungating wounds are known as masses or ulcerative lesions, and are defined as the conditions of ulceration and proliferation that occur when malignant tumor cells are infiltrated and erode through the skin. Fungating wounds have been reported to occur in about 5% of patients with cancer.1) These ulcers are usually superinfected with bacteria,2) and therefore the infected area tends to emit malodor. Indeed, antimicrobial preparations have been proposed to reduce the malodor,3) but the odorant(s) causing the malodor associated with fungating wounds has not been revealed. Here we aimed to determine the chemical identity of the cancer wound-derived odor(s).

We examined three female patients with breast cancer (B1, stage IV; B2, stage IIIB; B3, stage IV) and two male patients with head and neck cancer (H1, stage III; H2, stage IV). Informed consent to the experimental protocol was obtained from all the patients. First we evaluated the intensity and quality of the body odors emitted from the fungating wounds of the patients. All of them had a similar pungent sulfury odor (Table 1). In addition to the sulfury odor, B1 and B2 had a cheese-like odor, and B3, H1, and H2 had a rotten fish odor (Table 1).

Next we analyzed the malodor emitted from the wounds of each patient. Sterile gauze pads (Hakujuji, Tokyo) were placed on the fungating wounds of the five cancer patients for 6–12 h. The pads are then sealed in polyvinylidenechloride bags, and the head-space volatiles were extracted onto Carboxen/PDMS (Carboxen760/ Polydimethylsiloxane) SPME (solid phase micro extraction) fibers (75 μm) (SUPELCO, Bellefonte, PA) at room temperature for 2h. Clean gauze pads were utilized as a control. The compounds on the SPME fibers were then analyzed by gas chromatography-mass spectrometry-olfactometry (GC-MS-O), which enabled us to examine the mass spectra and odor qualities of individual GC-separated odorants simultaneously. Shimadzu GCMS-QP2010 (Shimadzu, Kyoto, Japan) (a Stabilwax column of 60 m × 0.32 mm i.d. with a film thickness of 0.5 μm) was combined with a sniffing port equipped with a Sniffer9000 system (Brechbuhler, Houston, TX) in splitless mode (MS and sniffing port at a ratio of 1:1). The column temperature was programmed to rise at 5 °C/min from 50 °C (2 min hold) to 230 °C (30 min hold) (total run time, 68 min). The interface temperature was maintained at 200 °C, and the ion source temperature was maintained at 230 °C. Mass spectra were obtained in full scan mode (range, 29–400) by electron impact using the National Institute of Standards and Technology (NIST) library database. GC-MS-O analysis and evaluation of body odors were performed by three persons.

Table 1. Body Odor Intensities of Cancer Patients

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Odor character**</th>
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<tbody>
<tr>
<td></td>
<td>Sulfury</td>
</tr>
<tr>
<td>B1</td>
<td>Strong</td>
</tr>
<tr>
<td>B2</td>
<td>Strong</td>
</tr>
<tr>
<td>B3</td>
<td>Slight</td>
</tr>
<tr>
<td>H1</td>
<td>Moderate</td>
</tr>
<tr>
<td>H2</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

* B1, B2, B3: breast cancer patients; H1, H2: head-and-neck cancer patients.
** Characteristic body odors of the various patients and the intensities of respective odors are listed.

The intensity of odors is categorized into four groups: not detected (n.d.), slight, moderate, strong.

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Abbreviations: GC-MS-O, gas chromatography-mass spectrometry-olfactometry; TIC, total ion chromatogram; DMTS, dimethyl trisulfide; EIC, extracted ion chromatogram; RT, retention time
odors in patients, B1 and B2 came out at RT = 24.01 min, 25.47 min, and 26.08 min. The rotten fish odor in patients B3, H1, and H2 came out at RT = 3.43 min. The burnt odor was common in all samples at RT = 17.96 min, although this odor was not sensed in the body odors of any of the patients.

The mass spectrum of the peak at about 19.5 min predicted the structure of dimethyl trisulfide (DMTS) (Fig. 2A, arrowhead, and 2B). The mass spectrum and the retention time of authentic DMTS (Wako, Tokyo) were identical to those of the peak compound (Fig. 2A, orange line, and 2C), demonstrating that the sulfury odor at RT = 19.46 min was DMTS. The extracted ion chromatogram (EIC) of the base and molecular ion peak of DMTS (m/z 126) showed the presence of DMTS at a RT of 19.46 min in all the cancer samples (Fig. 2D, red line).

Next we analyzed the odor quality and intensity of various concentrations of DMTS solutions. The threshold concentration (0.001 ppm v/v) was consistent with
Identification of Dimethyl Trisulfide (DMTS) as a Common Malodor from Fungating Cancer Samples.

Fig. 2. Identification of Dimethyl Trisulfide (DMTS) as a Common Malodor from Fungating Cancer Samples.

A, Close-up total ion chromatogram (TIC) of the B1 sample (18–21 min in Fig. 1). The arrowhead indicates the peak corresponding to the sulfury odor. The orange line indicates the TIC of authentic DMTS (1 μl of 0.001% DMTS in ethyl acetate). B, Mass spectrum of the arrowhead peak in Fig. 2A. C, Mass spectrum and the chemical structure of DMTS. D, EICs of the base and molecular ion peak of DMTS (m/z 126) (red line) on the TICs (black line) of the various samples. E, Odor intensities and odor characters of various concentrations of DMTS solution. The intensity of odor was rated by directly sniffing the sample, and was categorized on a 5-point scale; 0 (no odor), 1 (barely perceptible), 2 (slight), 3 (moderate), 4 (strong), 5 (very strong). F, Odor intensities of SPME-absorbed head-space DMTS from a series of DMTS solutions by GC-MS-O analysis (upper panel), and the relationship between the concentration and the peak area of DMTS on EIC (m/z 126) (lower panel). The equation is \( \log Y = 1.14 \log X + 6.76 \). The positions of the five cancer samples based on the peak areas (Fig. 2D) are plotted on the equation curve.

the previously reported value (0.00166 ppm v/v) (Fig. 2E). The DMTS solutions at higher than 0.01 ppm exhibited an uncomfortable sulfury malodor, representative of the cancer patient’s body odor, which was rated at an intensity level of higher than 2 (Fig. 2E). GC-MS-O analysis of SPME-collected head-space DMTS in the dilution series revealed that the patient’s samples showed intensity levels of between 2 and 4 (corresponding to SPME samples from 0.001–0.1 ppm DMTS solutions) (Fig. 2F). However, it is of note that the intensity rating of the SPME-collected GC-MS-O sample cannot be compared directly with that of body odor by direct sniffing. Nonetheless, these data suggest that DMTS was emitted from the cancer wounds at a level high enough to make one feel uncomfortable.

In addition, we were able to identify the structures of the compounds with sour and cheese-like odors (Fig. 1B): acetic acid for the sour odor (RT = 21.17 min), isobutyric acid for the cheese odor (RT = 24.01 min), butyric acid for the cheese and vomit odor (RT = 25.47 min), and isovaleric acid for the cheese and foot odor (RT = 26.08 min). The other odors could not be identified due to low concentration or to overlapping peaks in the TIC.

In conclusion, we identified DMTS as the main odorant that caused severe malodor in some advanced cancer patients. To the best of our knowledge, this is the first to report on the main odorant that represents the smell of fungating cancer wounds. DMTS has been found in volatiles emitted from vegetables, such as...
cooked onion, broccoli, and cabbage, and also sometimes detected in fermented and aged food or drinks such as cheese, milk, whisky, beer, sake, and wine, probably due to contamination by microorganisms, such as *Geotrichum candidum*. DMTS is also produced by aerobes such as *Pseudomonas aeruginosa* that reside in leg ulcers. Thus, although the source of DMTS found in the fungating cancer wounds in this study remains to be determined, DMTS might be a product of infected bacteria in fungating wounds.

To improve the quality of life of patients, development of a way to prevent or reduce the DMTS odor is awaited. Indeed, almost all patients with fungating wounds suffer from this malodor (our unpublished observations). The use of metronidazole, an antibiotic to anaerobes, has been proposed to control malodor. The odors from fatty acid volatiles identified in this study might be reduced, because these are produced by anaerobes. Identification of DMTS as the main source of malodor of fungating cancer wounds should provide new clues to better strategies for the treatment of malodor in cancer patients.

Olfactory diagnosis of cancer has recently been proposed. In fact, well-trained dogs can detect specific odors, in the breath of cancer patients, but identification of the compounds that can be used in such diagnosis has proven difficult, due to very low abundance in breath samples. Our study indicates the power of GC-MS-O to identify very low abundance but yet characteristic odorous compounds from complex cancer-derived volatile samples. The approach undertaken here might be useful in analyzing odorous compounds for cancer diagnosis in the future.

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**References**