Internal environment in cancer patients and proposal that carcinogenesis is adaptive response of glycolysis to overcome adverse internal conditions

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ABSTRACT
In a series of our recent studies, stress was found to induce simultaneously hypothermia and hyperglycemia. These conditions are beneficial to obtain prompt force which depends on the glycolysis pathway and to escape emergency. Since we have noticed that such conditions resemble the internal environment seen in some cancer patients, it was investigated whether such conditions were accompanied with other patients. We selected patients with early and advanced cancer. Body temperature and other parameters including blood gas contents were examined. A difference was seen in body temperature, namely, many patients showed hypothermia, irrespective of cancer stages. Further characterization of other parameters showed that hypothermia and hyperglycemia existed in many patients. They had immunosuppressive state and anemia. Blood gas analysis showed that oxygen contents were low and carbon dioxide contents were high in patients. These results suggest a possibility that the internal environment seen in patients is responsible to induce onset of disease and to maintain their cell growth, because cancer cells have an energy system of predominant glycolysis. Although hypothermia, hypoxia and hyperglycemia are important to activate the glycolysis pathway and to escape from emergency, such responses suppress the mitochondrial pathway for long span and may result in carcinogenesis.

Keywords: Cancer; Hypothermia; Hypoxia; Hyperglycemia; Glycolysis; Mitochondria

1. INTRODUCTION
Many investigators and clinicians have felt that cancer might be a systemic disease although tumor masses are primarily present at local sites. If this is the case, we have to consider specific, common internal environment in cancer patients. In the course of the analysis of many parameters in cancer patients, we noticed that many cancer patients showed simultaneous hypothermia and hyperglycemia.

In light of these findings, we then investigated the internal environment in relation to stress-associated responses [1,2]. Of interest was that both hypothermia and hyperglycemia were induced by stress. Such an internal environment might be beneficial for humans and animals to escape emergencies [3]. Namely, prompt output of force by white muscle fibers depends on the energy production system of glycolysis. Although the efficiency of energy production is low (2 ATP/glucose) in the glycolysis pathway, the ATP synthesis in this system is much quicker (× 100) than that of the mitochondrial system (× 1) [4].

In a short span of time, hypothermia and hyperglycemia are therefore good conditions for escape from stress or emergencies. However, these conditions are not appropriate for energy production of the mitochondrial pathway (i.e., oxidative phosphorylation). Many patients with hypothermia and hyperglycemia suffer from general fatigue, emaciated conditions, diabetic disease, etc.

In the present study, we investigated the internal environment in cancer patients in detail and herein propose on adaptation theory, namely, that the onset of cancer is a phenomenon of a glycolytic adaptation response by living beings to overcome deteriorated internal conditions in the body. Cumulative evidence has shown that
cancer cells have a shift of glucose metabolism from oxidative phosphorylation to glycolysis, eventually resulting in few or defective mitochondria in the cytoplasm [5-7]. Although many investigators have considered that carcinogens such as ultraviolet rays, food additives, air pollution, etc. [8-12], induce multiple mutation steps in proto-oncogenes, there is an alternative possibility that such mutation is a process of glycolytic adaptation by living beings, namely, cancer cells. Hypothermia, hypoxia and hyperglycemia, which are induced by continuous stress (due to the lifestyle in patients), might be important factors which induce the adaptive response.

2. MATERIALS AND METHODS

2.1. Subjects

Patients with early or advanced cancer were first examined as to body temperature (n = 28). They were 54.3 ± 8.0 years old. Age-matched healthy controls (n = 27), 45.8 ± 11.0 years of age, were also examined.

For detailed analysis of many parameters other than body temperature, patients with advanced cancer (n = 13) were then selected (Table 1). Details of the cancer patients are listed in the table, including sex and age (52.1 ± 8.7 years old). At the time of analysis, these patients were receiving neither chemotherapy nor irradiation therapy. Age-matched healthy controls (n = 11), 46.7 ± 10.0 of age, were also examined.

Informed consent was obtained from all subjects.

2.2. Parameters Tested

Blood for the analysis was obtained from a vein. Blood glucose was measured by Precision Xtra TM (Abott Japan Co., Ltd., Chiba, Japan). Venous blood analysis of lactate and of the levels of pH, O₂ and CO₂ was also performed using i-STAT 300F (i-STAT Corporation, NJ, USA).

To analyze the hematological parameters, leukocyte counts of fresh venous blood were determined by hemocytometer and were stained by the Giemsa method. The contents of hemoglobin (Hb) and others in the blood were measured by Sodium Lauryl Sulfate (SLS)-Hb methods and hemocytometer, respectively.

2.3. Statistical Analysis

The difference between the values was determined by Student’s t-test, Mann-Whitney’s U test and Welch’s t-test.

3. RESULTS

3.1. Hypothermia and Hyperglycemia Seen in Cancer Patients

Twenty-eight patients with early or advanced cancer and twenty-seven healthy subjects were examined as to body temperature (Figure 1(a)). It was found that there were many persons with hypothermia (36.1 ± 0.5 °C) among cancer patients in comparison with healthy persons (36.6 ± 0.4 °C), the difference being statistically significant (p < 0.01).

We then analyzed many parameters in patients with advanced cancer (n = 13) and age-matched controls (n = 11) (Figure 1(b)). Hypothermia was confirmed in these patients with advanced cancer (35.9 ± 0.5 °C) in comparison with controls (36.5 ± 0.3 °C). In addition to hypothermia, hyperglycemia was also detected in cancer patients (125.3 ± 28.5 mg/dL) in comparison with controls (106.3 ± 11.1 mg/dL).

Since stress-associated responses simultaneously induce hypothermia and hyperglycemia, other stress-associated parameters were also examined in this experiment (Figure 1(b), bottom). Although there was a tendency that some patients with advanced cancer had a high pulse rate (sympathetic nerve activation) and a high level of lactate, these were not common to all patients (p > 0.05 in both pulse and lactate).

3.2. Immunosuppressive States and Anemia in Cancer Patients

Immunoparameters were examined in cancer patients and...
Figure 1. (a) Comparison of body temperature and other parameters between healthy controls and cancer patients. (a). Body temperature, (b). Further analysis of body temperature and others. In experiment (a), healthy controls (n = 27) and cancer patients (n = 28) were examined. In experiment (b), healthy controls (n = 11) and patients with advanced cancer (n = 13) were examined. In addition to body temperature, the levels of glucose and lactate and the pulse rate were examined in this experiment. Body temperature was measured in the axilla for 3 min. **p < 0.01.

Figure 2(a). The total number of white blood cells (WBC) was lower in patients (4691 ± 1769 /µL) than in controls (6190 ± 1088 /µL) (p < 0.05). When the ratio of WBC (leukocyte) subsets was enumerated, the ratio of granulocytes was found to be high, while that of lymphocytes was low (p < 0.01). The ratio of monocytes was comparable in patients and controls. By calculation, the absolute number of leukocyte subsets was determined. It was found that the most prominent distinction was in lymphocytes, namely, the number of lymphocytes in patients (1334 ± 476 /µL) was extremely low in comparison with the number in controls (2387 ± 538 /µL) (p < 0.01). The decrease in the number of leukocytes seen in patients was found to be due to the decrease in the number of lymphocytes. The ratio and number of monocytes were comparable in controls and cancer patients.

The level of red blood cells (RBC) and related parameters was then examined (Figure 2(b)). In addition to the decrease in the number of WBC, the number of RBC was found to decrease in cancer patients (407 ± 47 × 10^4 /µL) in comparison with controls (446 ± 39 × 10^4 /µL) (p < 0.05). The level of Hb was lower in patients (11.9 ± 1.7 g/dL) than in controls (13.9 ± 1.3 g/dL). The level of hematocrit (Ht) was also lower in patients (37.6 ± 4.1%) than in controls (42.0 ± 3.5%) (p < 0.01). Other parameters of RBC, namely, mean red cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were all comparable between controls and patients (p > 0.05). This was also the case for the number of platelets.

3.3. Blood Gas Analysis in Cancer Patients

It is conceivable that hypothermia and anemia seen in patients with advanced cancer may influence the parameters as shown by blood gas analysis. Therefore, such analysis using venous blood was conducted (Figure 3). It was demonstrated that blood pH was lower in patients (7.36 ± 0.03) than in controls (7.40 ± 0.03) (p < 0.05). The major factors influencing blood pH are known to be the levels of O2 and CO2 contents. Indeed, the levels of PO2 (mmHg) and SO2 (%) were found to be extremely low in patients (p < 0.01, p < 0.05, respectively). On the other hand, the levels TCO2 (mmol/L) and PCO2 (mmHg) tended to be high in patients. BEecf (mmol/L), which shows a base excess in extracellular fluids, was slightly high in cancer patients.

4. DISCUSSION

We herein demonstrated that many cancer patients had hypothermia, hypoxia and hyperglycemia simultaneously. Immunosuppressive states, showing granulocytosis and healthy controls (Figure 2(a)). The total number of white blood cells (WBC) was lower in patients (4691 ± 1769 /µL) than in controls (6190 ± 1088 /µL) (p < 0.05). When the ratio of WBC (leukocyte) subsets was enumerated, the ratio of granulocytes was found to be high, while that of lymphocytes was low (p < 0.01). The ratio of monocytes was comparable in patients and controls. By calculation, the absolute number of leukocyte subsets was determined. It was found that the most prominent distinction was in lymphocytes, namely, the number of lymphocytes in patients (1334 ± 476 /µL) was extremely low in comparison with the number in controls (2387 ± 538 /µL) (p < 0.01). The decrease in the number of leukocytes seen in patients was found to be due to the decrease in the number of lymphocytes. The ratio and number of monocytes were comparable in controls and cancer patients.

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Figure 2. Blood cell analysis. (a) Immunoparameters. (b) Analysis of the number of RBC and the levels of Hb and other parameters. In experiment (a), the absolute number of leukocyte subsets was calculated from the data on the number of WBC and the ratio of leukocyte subsets. In experiment (b), Number of RBC and levels of Hb, Ht, MCV, MCH and MCHC, including the number of platelets, were examined * p < 0.05, ** p < 0.01.
lymphocytopenia, were also present. Although clinicians are empirically aware of the deteriorated conditions such as hypoxia, immunosuppression and anemia in cancer patients [13-20], as shown by a review of the literature, few studies have been done on the simultaneous identification of all these conditions. We propose the possibility that hypothermia, hypoxia and hyperglycemia are beneficial for stress-exposed persons to escape from emergencies inducing stress for short periods of time, but that such internal environment might become cancer-inducing over a longer period of time. This proposal is based on an understanding of the energy production system comprising the glycolysis and mitochondria pathways [3,4].

It has been speculated that the ancestors of eukaryocytes were generated by a connection between living beings with glycolysis and those with mitochondria at approximately 2 billion years ago [21,22]. Under such situation, eukaryocytes had two energy production methods, namely, the glycolysis and mitochondria pathways (Table 2). As shown in this table, the functioning conditions, usage and other characteristics between two pathways are quite different. If we consider the internal environment (i.e., hypoxia and hyperglycemia) seen in cancer patients, these conditions are rather appropriate for the function of glycolysis.

In a recent study, we analyzed in detail the stress-associated conditions in mice exposed to restraint stress [1,2]. Of interest was that such conditions were revealed to be hypothermia and hyperglycemia. In addition, the administration of catecholamines or glycocorticoids also directly induced hypothermia and hyperglycemia [2]. In a short span of time, such internal conditions are beneficial for humans and animals to obtain the prompt force of white muscle fibers via activation of the glycolysis.

**Table 2. Energy production system.**

<table>
<thead>
<tr>
<th></th>
<th>Glycolysis Pathway</th>
<th>Mitochondrial Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>cytoplasm</td>
<td>mitochondria</td>
</tr>
<tr>
<td>Oxygen</td>
<td>– or ±</td>
<td>++</td>
</tr>
<tr>
<td>Source</td>
<td>glucose</td>
<td>pyruvic acid (lactate)</td>
</tr>
<tr>
<td>Temperature</td>
<td>32-36℃</td>
<td>&gt; 37℃</td>
</tr>
<tr>
<td>Usage</td>
<td>cell division</td>
<td>suppression of cell division</td>
</tr>
<tr>
<td>ATP production</td>
<td>quick (× 100)</td>
<td>slow (× 1)</td>
</tr>
<tr>
<td>Efficiency</td>
<td>low (2ATP/glucose)</td>
<td>high (36ATP/glucose)</td>
</tr>
<tr>
<td></td>
<td>sperms</td>
<td>cardiac muscle cells</td>
</tr>
<tr>
<td></td>
<td>cancer cells</td>
<td>neurons</td>
</tr>
<tr>
<td></td>
<td>skin cells</td>
<td>hepatocytes</td>
</tr>
<tr>
<td></td>
<td>bone marrow cells</td>
<td>red muscle cells</td>
</tr>
<tr>
<td></td>
<td>white muscle cells</td>
<td>many other cells</td>
</tr>
</tbody>
</table>

Figure 3. pH and blood gas analysis in healthy controls and cancer patients. Venous blood was used for the analysis. * p < 0.05, ** p < 0.01.
Carcinogens

<table>
<thead>
<tr>
<th>Direct Cause</th>
<th>Secondary Response</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ultraviolet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>food additives</td>
<td>mutation of proto-oncogenes or other genes by carcinogens</td>
<td>Less</td>
</tr>
<tr>
<td>radiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>air pollution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>other carcinogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothermia</td>
<td>mutation of proto-oncogenes or other genes as adaptation to “living beings of glycolysis”</td>
<td>High</td>
</tr>
<tr>
<td>hypoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hyperglycemia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O. Warburg has reported that cancer cells contained a few mitochondria in the cytoplasm and produce energy mainly by the glycolysis pathway [7]. Recent cumulative evidence also supports this earlier observation [30-35]. The functions of oncogenes are eventually related to not only the system of cell-proliferation but also to the system of energy production. The nature of the predominant function of glycolysis in cancer cells is utilized by PET scans [36,37]. The energy produced by glycolysis is used not only to obtain prompt elicitation but also for cell dividing energy (see Table 2 again). In other words, the initial stress-associated response of hypothermia, hypoxia and hyperglycemia is estimated as allostasis (i.e., change of the internal environment to overcome stress). However, continuous stress then turns to allostatic load and induces the carcinogenesis as adaptation responses (i.e., break of “homeostasis” in our body). These concepts on “allostasis” were proposed by B. S. McEwen, F. S. Dhabhar and their colleagues [38-41]. Stressful life events were reported to be related to such allostatic load [42,43].

In our recent study using hyperthermia equipment [44, 45], many cancer patients could live in good conditions without further tumor enlargement when they exposed to mild hyperthermia (i.e., the maximum rectum temperature is 38.0°C for 15-30 min). In some cases, tumor regression resulted from mild hyperthermia [45]. At this time, the values of pH, PO2, PCO2 and other factors improved. Immunosuppression and anemia seen in cancer patients were also alleviated. These results suggest that a slight shift of glucose metabolism from the glycolysis pathway to the mitochondria pathway (i.e., oxidative phosphorylation) might be important to cure malignancy. Local, strong hyperthermia (e.g., 42°C) was not effective and rather acted as severe stress in cancer patients. In other words, systemic improvement of the internal environment is critical to cure malignancies. However, we do not recommend patients to use expensive equipment for hyperthermia. The most important things for spontaneous regression of cancer are as follow: changing harmful lifestyle (e.g., overwork), using hot-water bottle at sleeping time, taking a deep breath several times a day, dietary consideration and control of the fear.

We have herein proposed the possibility that stress-associated conditions are beneficial for humans to escape from emergencies in a short span of time, but that the resulting hypothermia and hyperglycemia act as factors which induce the generation of cancer cells. In other words, the onset of malignancy might be a return to “living beings with glycolysis at 2 billion years ago”. Cancer cells eventually contain only a few mitochondria in the cytoplasm. However, we could not neglect a mitochondrial function in tumor cell growth [46,47].
A hypothesis by J. S. Fang, R. J. Gillies and R. A. Gaten was proposed that evolution of carcinogenesis is an adaptation response to hypoxia and acidosis, showing the interaction of cancer cells and microenvironments in the surrounding tissues [48,49]. At that time, a paracrine signaling between epithelial and stromal cells is known to be important for tumor initiation and progression [50]. We were also able to reveal the systemic, internal environment of hypoxia, acidosis and other conditions in actual cancer patients. However, a further research is required to support our proposal definitely. Such research includes an animal experiment using mice with cancer and a cancer cell culture experiment under conditions of hypothermia, hypoxia and hyperglycemia.

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Lung cancer proliferation correlates with [F-18] fluoro-