Objective: Operative stress of cardiovascular surgery can alter the blood levels of various physiologically active substances (e.g., cytokines, growth factors), and thus potentially affect cancer cell proliferation. How the combination of changes in blood levels of these substances affects cancer cells has not been adequately addressed. We investigated the stimulatory capacity on cancer cells of serum from patients after cardiovascular surgery, using a novel in vitro assay method.

Methods: The subjects were 22 patients undergoing cardiovascular surgery, consisting of 11 off-pump and 11 on-pump procedures. Blood was sampled from each subject immediately before surgery, immediately after surgery, and after transfer to the intensive care unit. Human lung cancer cells were exposed to the serum of each blood sample from each patient, and an MTT assay was conducted to evaluate cell proliferation.

Results: Serum samples of all patients showed an inhibitory effect for lung cancer cell proliferation. This inhibitory effect was lower in postoperative serum compared with serum samples before surgery. As a result, lung cancer cell proliferation was better with postoperative serum samples than preoperative serum samples. The proliferation rate after surgery, when it was compared with preoperative serum, was significantly higher in patients with on-pump procedures than in patients with off-pump procedures.

Conclusion: The results of this study suggest that the operative stress of cardiovascular surgery induces changes in serum to make it less inhibitory for the cancer cell proliferation. This phenomenon is greater in patients with extracorporeal circulation.

Key words: coronary artery bypass grafting, extracorporeal circulation, cancer cell proliferation

Introduction

Most of the patients who undergo adult cardiac surgery are elderly. A survey of the Japanese Association for Coronary Artery Surgery showed that 46.5% of patients who underwent coronary artery bypass grafting (CABG) in Japan in 2005 were aged 70 and over. Patients undergoing cardiovascular surgery sometimes also have malignant diseases.

There are many steps in the progression of cancer, for example, proliferation, migration, and invasion of cells in the primary lesion, invasion to the vessels, adhesion to vascular endothelial cells, and proliferation in the
metastatic sites. Many physiologically active substances, such as cytokines and growth factors, are involved in each process. It is known that serum levels of several growth factors or cytokines are affected by surgical procedures.\textsuperscript{2–5)} Here, we hypothesized that operative stress could alter the blood levels of various physiologically active substances, and thus potentially affect cancer cell proliferation. How the combination of changes in blood levels of these substances affects cancer cells has not been adequately addressed yet.

Especially, cardiovascular surgery is often accompanied with cardiopulmonary bypass (CPB). CPB is usually a more invasive procedure, compared with off-pump procedures, and then it may affect more strongly, the cancer cell proliferation.

In an in vitro study, we previously revealed that lung cancer cell proliferation was better when human serum obtained immediately after a lung resection surgery was added, compared to serum obtained before surgery, using a new in vitro assay method.\textsuperscript{6)} In this paper, we investigated the effect of cardiovascular surgery on cancer cell proliferation using this in vitro assay method.

### Materials and Methods

The subjects were 22 patients undergoing cardiovascular surgery. They consisted of 14 males and 8 females, aged 68 ± 12 (range: 38–80) years. Surgery included 11 off-pump (OPCAB), and 11 on-pump procedures (CPB).

There were no significant differences in age, sex, and operation time between OPCAB and CPB patients. CPB involved a significantly higher number of units of blood transfusion than OPCAB (Table 1).

Blood was sampled from each subject immediately before surgery (PRE), immediately after surgery (POST), and after transfer to the intensive care unit (ICU), which was 2 to 3 hours after the surgery. Serum derived from each blood sample was frozen and stored. Human, non-small cell lung cancer cell lines, EBC-1 and PC-14, were used for the study. EBC-1 was provided by the Japanese Collection of Research Bio-resources Cell Bank (Tokyo, Japan). PC-14 was provided by the Riken Cell Bank (Tsukuba, Japan). EBC-1 was cultured in Dulbecco’s minimum essential medium (DMEM; Life Technologies Oriental, Inc., Japan), supplemented with 10% heat-denatured fetal calf serum (FCS; Life Technologies Oriental, Inc., Japan), 100 U/ml penicillin and 100 µg/ml of streptomycin (Life Technologies Oriental, Inc., Japan). EBC-1 was cultured in Dulbecco’s minimum essential medium (DMEM; Life Technologies Oriental, Inc., Japan), supplemented with 10% heat-denatured fetal calf serum (FCS; Life Technologies Oriental, Inc., Japan), 100 U/ml penicillin and 100 µg/ml of streptomycin (Life Technologies Oriental, Inc., Japan). EBC-1 was cultured in Dulbecco’s minimum essential medium (DMEM; Life Technologies Oriental, Inc., Japan), supplemented with 10% heat-denatured fetal calf serum (FCS; Life Technologies Oriental, Inc., Japan), 100 U/ml penicillin and 100 µg/ml of streptomycin (Life Technologies Oriental, Inc., Japan). EBC-1 was cultured in Dulbecco’s minimum essential medium (DMEM; Life Technologies Oriental, Inc., Japan), supplemented with 10% heat-denatured fetal calf serum (FCS; Life Technologies Oriental, Inc., Japan), 100 U/ml penicillin and 100 µg/ml of streptomycin (Life Technologies Oriental, Inc., Japan). EBC-1 was cultured in Dulbecco’s minimum essential medium (DMEM; Life Technologies Oriental, Inc., Japan), supplemented with 10% heat-denatured fetal calf serum (FCS; Life Technologies Oriental, Inc., Japan), 100 U/ml penicillin and 100 µg/ml of streptomycin. For routine subculturing, cells were detached from the culture flask with 0.05% trypsin and 0.53 mM EDTA (Life Technologies Oriental, Inc., Japan).

The assay was performed using a 96-well microplate. A 100µl of single cell suspension (5 × 10^3 cells/well) of EBC-1 and PC-14 in culture medium containing patient serum was applied to each well. Cells were exposed to

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>CPB</th>
<th>OPCAB</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y.o.)</td>
<td>64 ± 15</td>
<td>73 ± 5</td>
<td>0.056</td>
</tr>
<tr>
<td>Sex</td>
<td>male</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>female</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Surgical Procedures</td>
<td>on-pump CABG</td>
<td>3</td>
<td>off-pump CABG</td>
</tr>
<tr>
<td>TAA graft replacement</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic valve replacement</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral valve replacement</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAD graft replacement</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSD closure</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDA closure</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>op. time (min.)</td>
<td>407 ± 91</td>
<td>347 ± 69</td>
<td>0.097</td>
</tr>
<tr>
<td>bleeding (ml)</td>
<td>N.E.</td>
<td>637 ± 287</td>
<td></td>
</tr>
<tr>
<td>CPB time</td>
<td>215 ± 87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transfusion units</td>
<td>17.8 ± 17.5</td>
<td>3.3 ± 2.9</td>
<td>0.013</td>
</tr>
</tbody>
</table>

CPB, cardiopulmonary bypass; CABG, coronary artery bypass grafting; TAA, thoracic aortic aneurysm; AAD, acute aortic dissection; VSD, ventricular septal defect; PDA, patent ductus arteriosus; N.E., not evaluable
patient serum at a concentration of 10%, 20% or 30% for 48 hours. Triplicate samples were measured in all conditions. An MTT assay was conducted thereafter to evaluate cell proliferation, with an absorbance (optical density, OD) measured at 540 nm (control: 630 nm) using a microplate reader (model 680, Bio-Rad, CA, USA). The rate of decrease in inhibitory capacity of the serum for cancer cell proliferation after surgery compared to PRE was calculated using the following formula: 

\[ \text{Rate of decrease} = \frac{100 \times (\text{OD at POST-OD at PRE})}{\text{OD at PRE}}. \]

This study was approved by our institutional review board, and written informed consent was obtained from the patients.

Statistical analysis

All values were reported as means ± SD. ANOVA was employed to evaluate the significance of differences between the groups. \( p < 0.05 \) indicated statistical significance.

Results

The assay results could be evaluated in all patients. Control (with 0% serum) ODs were 195.6 ± 21.2 in EBC-1 and 822.8 ± 44.5 in PC-14. The patients’ serum at PRE, as well as POST and ICU, significantly \(( p < 0.0001 \) in all data sets) inhibited the growth of lung cancer cells (Fig. 1). Dose dependency in this inhibitory effect was observed in all serum samples \(( p < 0.0001 \)).

The ODs of each condition are summarized in Table 2. ODs after surgery (POST and ICU) were significantly higher than those before surgery, both in the CPB and OPCAB group in each assay condition in EBC-1 cells. In PC-14 cells, the ODs after surgery were also significantly higher than those before surgery in the CPB group. However in the OPCAB group, a significant increase in OD after surgery was observed only in 2 of 6 conditions; 20% at ICU and 30% at PRE.

The rates of decrease in inhibitory capacity of the serum of EBC-1 in a total of 22 patients were calculated as 47.5 ± 43.8% in 10% serum concentration, 48.8 ± 60.0% in 20% serum, and 34.0 ± 35.7% in 30% serum, respectively. Those of PC-14 were 21.2 ± 28.8%, 47.5 ± 73.2%, and 48.3 ± 42.1%, at 10%, 20% and 30% serum concentrations, respectively. The rate of decrease in inhibitory capacity of each group was summarized in

Fig. 1 Effect of patient serum before and after cardiovascular surgery on cancer cell proliferation. Rectangles indicate the data before surgery, closed circles after surgery, and open circles after returning to the intensive care unit. P-values indicate the statistical significance compared to before surgery.
Yamamoto S, et al.

**Table 3** The rate of decrease in inhibitory capacity of the serum for cancer cell proliferation after surgery

<table>
<thead>
<tr>
<th>cell line</th>
<th>serum concentration</th>
<th>CPB</th>
<th>OPCAB</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBC-1</td>
<td>10%</td>
<td>66.4 ± 45.5 %</td>
<td>28.5 ± 34.2 %</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>68.8 ± 71.8 %</td>
<td>28.8 ± 39.1 %</td>
<td>0.062 (n.s.)</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td>37.6 ± 34.8 %</td>
<td>30.4 ± 37.9 %</td>
<td>0.323 (n.s.)</td>
</tr>
<tr>
<td>PC-14</td>
<td>10%</td>
<td>35.8 ± 33.6 %</td>
<td>6.6 ± 12.2 %</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>86.8 ± 85.7 %</td>
<td>8.2 ± 22.4 %</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td>60.8 ± 38.4 %</td>
<td>35.8 ± 43.6 %</td>
<td>0.084 (n.s.)</td>
</tr>
</tbody>
</table>

PRE, immediately before surgery; POST, immediately after surgery; ICU, after transfer to the intensive care unit; CPB, cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass; n.s., not significant

**Discussion**

The majority of patients who undergo cardiovascular surgery are elderly. Therefore, they sometimes also have malignant tumors. In patients with malignant tumors, cardiovascular surgery itself may affect cancer cell progression. However, there was no information available on how cardiovascular surgery might affect malignant tumors.

Mistiaen WP, et al. analyzed 8620 patients referred for cardiac surgery. They observed 205 patients with documented malignant tumors. In their paper, they reported that malignancy before cardiac surgery was a significant factor for a poor prognosis. However, it was not evident whether cardiac surgery itself affected the prognosis of the patients with malignancies. Shimizu H, et al. reported a case of recurrent hepatocellular carcinoma with rapid growth after cardiac valve replacement. They suggested that the extracorporeal circulation in particular triggered the rapid growth of the tumor in this patient. It is well known that serum levels of various cytokines and growth factors are affected by surgical procedures. The alterations of serum levels of cytokines and growth factors have the possibility of stimulating cancer progression. Therefore, we speculate that the surgical procedure itself may promote cancer progression.

Some cytokines and growth factors promote and others inhibit cancer progression. In cardiovascular surgery, serum levels of various cytokines and growth factors change at the same time. Therefore, it seems to be difficult to speculate how cardiovascular surgery affects malignant tumors from the changes in serum levels of individual factors.

We previously reported a novel in vitro assay method to evaluate the promoting effect of patients’ serum on cancer cells. We used serum samples before and after
surgery in 16 lung cancer patients. The result indicated that lung resection surgery promotes cancer cell progression in patients with lung cancer. There was greater promotion of lung cancer cell proliferation in highly invasive surgery patients compared with less invasive surgery patients.

In the present study, we used the same assay procedure to evaluate the effect of cardiovascular surgery on malignant tumors. Our results show that the patient serum strongly inhibits cancer cell proliferation. Lung cancer cell we used for this in vitro assay was not originated from each patient in this study. It appeared to be a reason why the patient serum strongly inhibited cancer cells in our assay method. The inhibitory effect was significantly lower in serum after surgery compared with serum before surgery. As a result, patients’ serum after cardiovascular surgery showed seemingly promotive effect for lung cancer cell proliferation compared with serum before surgery.

Our study was not designed to detect which factors caused this phenomenon. However, the results imply that some inhibitory factors to cancer cells were washed out or consumed by the cardiovascular surgery, especially in patients with CPB.

The inhibitory capacity of the serum for cancer cell proliferation was significantly decreased in the CPB group than in the OPCAB group in this study. The cumulative lifetime risk of cancer incidence is 51% for males and 39% for females in Japan now.9) Cardiovascular surgeons often meet patients with malignancy. It is better to explain to these patients whether cardiovascular surgery affects the malignancies or not. Our results will provide some important information about it. Cardiovascular surgery, especially surgery with cardiopulmonary bypass may reduce the inhibitory capacity of patient serum for cancer cell proliferation. More intense follow-up for malignancy will be needed after cardiovascular surgery.

Folkman already reported in 1997 that serum endostatin levels increase after resection of malignant tumors, and residual metastatic tumors show rapid growth after surgery.10) Endostatin is produced in the primary lesion of malignant tumors, and it inhibits angiogenesis of metastatic lesions. However, our patients did not have malignant tumor, and then endostatin could not affect our results.

Fig. 2  The percent promotion of CPB and OPCAB groups at serum concentrations of 10% in both EBC-1 and PC-14.
Surgical stress in cardiovascular surgery has been investigated mainly in relation to the systemic inflammatory response after extracorporeal circulation. It was reported that serum levels of cytokines such as interleukin (IL)-6, 8, and 10 are affected by extracorporeal circulation. As for growth factors, alterations of serum levels of TNF-alpha, TGF-beta, HGF, VEGF, IGF, and basic-FGF were reported. These are well-known growth factors which play important roles in the progression of malignant tumors.

We are also interested in whether these factors also concern this phenomenon or not. To measure multiple factors requires much patient serum. Unfortunately, we did not preserve a sufficient number of serum samples to measure them. So we are now investigating it from the other aspect; how the mRNA expression of cancer cells is altered with serum after surgery.

Our present study revealed a significant difference only between CPB and OPCAB patients. We also speculate that different procedures, i.e. valve surgery and on-pump CABG, yield different effects on cancer cells. A larger cohort will be needed to investigate it.

**Conclusion**

We speculate that more invasive surgery causes a stronger alteration of serum levels of various factors. The combined effect of these alterations, mainly decrease in serum levels of inhibitory factors, results in the decrease in inhibitory capacity for cancer cell proliferation of the serum. As a result, it appears as the promotion of cancer cell proliferation in cardiovascular surgery, especially in patients with extracorporeal circulation.

**References**

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