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Enhanced tumor growth in the remaining lung after major lung resection

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ABSTRACT

Background: Pneumonectomy induces active growth of the remaining lung in order to compensate for lost lung tissue. We hypothesized that tumor progression is enhanced in the activated local environment.

Methods: We examined the effects of mechanical strain on the activation of lung growth and tumor progression in mice. The mechanical strain imposed on the right lung after left pneumonectomy was neutralized by filling the empty space that remained after pneumonectomy with a polypropylene prosthesis.

Results: The neutralization of the strain prevented active lung growth. According to an angiogenesis array, stronger monocyte chemoattractant protein-1 (MCP-1) expression was found in the strain-induced growing lung. The neutralization of the strain attenuated the release of MCP-1 from the lung cells. The intravenous injection of Lewis lung cancer cells resulted in the enhanced development of metastatic foci in the strain-induced growing lung, but the enhanced development was canceled by the neutralization of the strain. An immunohistochemical analysis revealed the prominent accumulation of tumor-associated macrophages in tumors arising in the strain-induced growing lung, and that there was a relationship between the accumulation and the MCP-1 expression status.

Conclusions: Our results suggested that mechanical lung strain, induced by pulmonary resection, triggers active lung growth, thereby creating a tumor-friendly environment. The modification of that environment, as well as the minimizing of surgical stress, may be a meaningful strategy to improve the therapeutic outcome after lung cancer surgery.

1. Introduction

Primary lung cancer is a leading cause of death in many developed countries. In addition, the lung is the most frequent site of metastasis from various malignant diseases. Surgical resection remains the only curative option in patients with oncologically resectable primary or metastatic lung cancer. However, the disease frequently recurs in the remaining lung within the first postoperative year regardless of whether a complete resection is achieved. Martini *et al.* reported the rate of postoperative intrapulmonary metastasis and second primary lung cancer after complete resection of stage I lung cancer [1]. In addition, according to our previous study on patients with double lung cancer, the

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surgical resection of one lesion triggered the progression of other lesions, despite the other lesions having been identified as non-increasing, ground-glass nodules based on serial computed tomography [2]. Thus, many investigators have attempted to clarify the relationship between surgical intervention and tumor progression. For instance, we found that tumor resection resulted in the promotion of growth of the remaining tumor because angiostatin, which is released by the tumor itself, and which plays a part in inhibiting angiogenesis in the remaining tumors, is exhausted by the resection of the tumor [3]. We also found that surgical stress caused the recruitment of bone marrow-derived progenitor cells into the tumors, leading to the promotion of tumor progression [4]. Numerous other studies have focused on the contribution of surgical stress to enhanced tumor progression as a result of immunosuppression [5]. Interestingly, Brown *et al.* reported that the progression of pulmonary metastases in the hemilateral lung was enhanced during the early phase after contralateral pneumonectomy in a mouse model [6], while the growth of subcutaneous tumors was not accelerated. Although these results may suggest that the local environment in the remaining lung, as modified by contralateral pneumonectomy, positively contributed to the enhancement of metastatic progression, no causal factors were shown in that study. In addition, the hypothesis that the prevention of lung growth after pneumonectomy resulted in the prevention of enhanced tumor progression remains controversial. Compensatory growth of the lung is induced after contralateral pneumonectomy, primarily to resolve the size mismatch between the original thoracic cage and the remaining lung: Hsia *et al.* reported that filling the postpneumonectomy space with plombage significantly prevented compensatory growth of the contralateral lung in the dog [7]. Accordingly, we hypothesized that after lung resection, the remaining lung represents a tumor-friendly environment, but that the activated environment is neutralized by preventing the growth of the remaining lung. Based on our hypothesis, we sought to identify factors that are associated with enhanced tumor growth in the remaining lung after lung resection.

2. Materials and methods

2.1. Animals

Eight- to ten-week-old male C57BL/6 mice were bought from Japan SLC, Inc. (Shizuoka, Japan). All of the animal procedures were done in accordance with both the institutional and national guidelines.

2.2. The lung metastasis model

A tumor model was established in C57BL/6 mice as described previously [3]. Briefly, the Lewis lung cancer (3LL) cell line was grown by culture in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The cells were used as a single-cell suspension in PBS. The 3×10^5 cells in 0.2-mL PBS were injected in each animal via the tail vein. Three days after the injection, the mice underwent pneumonectomy (described below). Twenty-one days after the operation, the lungs were excised and the metastatic foci on lung surface were identified and counted using a surgical microscope (magnification: × 4). The size and shape of the metastatic foci was not taken into consideration during nodular counting. Basically, the animals do not die of postoperative complications or tumor progression within the study period unless there were procedural errors during operation.

2.3. The surgical procedure

The mice were anesthetized with intraperitoneal injection of 100 mg/kg of pentobarbital. The mice were intubated with a 20-gauge catheter and connected to a rodent ventilator under a respiratory rate of 100 breaths/minute, 10 mL/kg tidal volume, and 21% inspired oxygen. A 20 mm posterolateral skin incision was made, before thoracotomy in the fifth intercostal space. In the sham operation group, the thoracotomy was closed without any additional intervention. In the surgery group (pneumonectomy without plombage), the whole left lung was resected from the pleural cavity. The left main bronchus, with the left pulmonary artery and vein, was ligated at the hilum follwerd by the removal of the lung. The fifth intercostal space was closed with one suture, and the skin and muscle incisions were closed with three sutures. In the other surgery group (pneumonectomy with plombage), we implanted bead-like prostheses, made of polypropylene. The prostheses, each of which weighed 50 mg and which were 3/16 inch in diameter, were implanted into the empty left hemithorax after left pneumonectomy in order to prevent the postoperative growth of the remaining right lung. Five beads were implanted in each mouse because these prostheses corresponded to the physiological volume of the left lung. After the mice were anesthetized at the selected time point by injection of pentobarbital, they were exsanguinated via the inferior vena cava.

2.4. Elisa

In order to measure the level of monocyte chemoattractant protein-1 (MCP-1/CCL2) (a chemokine regulating macrophage) that was released from the lung cells, the right lung was excised 7 days after the operation, minced using a surgical knife, and placed in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum for 12 hours. The medium was collected and the concentrations of MCP-1 were measured using a mouse enzyme linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The values are expressed as the amount of measured MCP-1 per wet lung weight.

2.5. Histological analysis

For the histological analyses, the right lung was inflated via the intratracheal instillation of 10% buffered formalin at a pressure of 15 cm H_2O. The trachea was ligated under the pressure, and the lung was fixed for 24 hours. The fixed right lung volume was measured by volume displacement [8].