□原著論文□

Association between the pH and clinicopathological characteristics of lung cancer tissue

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Abstract

Recent studies have shown that cancer cells surviving in a microenvironment characterized by hypoxia, a low pH, and a low glucose level have the ability to adapt to these adverse conditions. We measured the pH in the central tumor area of primary lung cancer, and evaluated its association with clinicopathological factors. There was a negative correlation between the tumor size and pH; with an increase in the tumor size, the pH decreased. In addition, pH in lung adenocarcinoma was negatively correlated with the expression of tumor markers, although the correlation was not significant. Cancer cells grow at a markedly low pH compared with the physiological environment. There is a possibility that this low pH is a microenvironment that is appropriate rather than adverse for the proliferative ability of cancer cells.

Keywords：hydrogen ion exponent, microenvironment, hypoxia-inducble factor

肺癌組織における pH と臨床病理学的特性との関連性

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抄　録

低酸素・低 pH・低グルコースという微小環境で生存している癌細胞には，その厳しい条件に適応する能力が備わっていることが近年，次第に明らかにされている．今回，原発性肺癌の腫瘍中心部での pH 測定を行い臨床病理学的因子との関係を調べた．腫瘍サイズが大きいほど腫瘍 pH が低い傾向がみられ，負の相関関係が認められた．また肺腺癌の腫瘍 pH と特異的腫瘍マーカーの関係においては，統計学的に有意ではないものの，緩やかな負の関連性がみられた．癌細胞は，生理的環境に比べてきわめて低い pH で生育しているが，その pH はむしろ腫瘍が生育するのに適した微小環境といえるのかもしれない．

キーワード：水素イオン指数，微小環境，低酸素誘導性因子

Ⅰ. Introduction

Cancer cells possess unique abilities to 1) promote their own growth with little external growth signals (self-sufficiency of growth signals), 2) proliferate in the presence of normal cells (insensitivity to anti-growth signals), and 3) continue to multiply without limitation (limitless replicative potential). Cancer cells also adapt to a microenvironment characterized by hypoxia and low pH, and recent studies have focused on understanding the underlying mechanisms that promote cell survival in the tumor microenvironment such as expression of particular genes, metabolic adaptation, and acquisition of treatment resistance. We investigated the relationships between pH in primary lung tumors and several clinicopathological factors in the preceding article[1)]. We added number of cases and reported them as well as between pH in lung adenocarcinoma and the expression of tumor markers in the present study.

受付日：2017 年 4 月 10 日　受理日：2017 年 7 月 27 日

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Ⅱ. Materials and Methods

Patients with primary lung cancer who were scheduled to undergo surgery at our hospital were enrolled in the study. Patients were excluded from the study if they 1) had previously undergone thoracic surgery, or received treatments for other cancers, 2) had obstructive or restrictive ventilatory defects, 3) were receiving medications that could alter the tumor microenvironment, such as antithrombotic therapy and vasodilators, or 4) had similar conditions as in 1−3. Patients were included in the study if they had tumors with a maximum diameter of > 1.0 cm and < 50% ground glass opacity nodules, as seen on chest CT prior to the surgery. All study patients underwent radical lobectomy. Immediately after the surgery, resected lobes were sectioned horizontally along the largest cut surface of the tumors. Tumor pH was measured using pH Spear (Eutech Instruments, Singapore) by inserting the sensor into the center of the tumors. In addition, pH was measured in the normal lung tissue, which was defined by visual inspection in the same cut surface from which the tumor pH was measured. Calibration of the pH sensor and the pH measurement were performed according to the manufacturer's protocol. In addition, arterial blood gas pH was measured at the time of surgery.

For statistical analyses, Pearson's correlation coefficient was calculated if the variables were normally distributed, and Spearman's rank correlation coefficient was calculated if the variables were not normally distributed. All statistical analyses were performed using Statcel 2 (OMS Publishing Inc., Saitama, Japan).

This was a retrospective study using existing data at our institute, and verbal consent was obtained from the study participants. Thus, we did not require the approval of the institutional Research Ethics Board.

Ⅲ. Results

From 2010 to 2013, 20 patients who met the inclusion and exclusion criteria were enrolled in the study (10 men and 10 women; average age: 69.6). According to the histological classification, there were 14 cases of adenocarcinoma, 2 cases of squamous cell carcinoma, 2 cases of small cell carcinoma, 1 case of adenosquamous carcinoma, and 1 case of pleomorphic carcinoma. Tumor pH was measured, and its correlation with tumor size (maximum diameter), pathological stage (pStage), and the location of the tumor (the order of bronchial branching) was examined.

1. Relationship between pH in normal lung tissue and the clinicopathological factors

Tissue pH in normal lungs was measured as a baseline, and varied between 6.45 and 7.88. There was no correlation between normal tissue pH and the clinicopathological factors, including tumor pH and tumor size (Figure 1). Arterial blood gas pH was within the normal range in all patients, and was not correlated with tumor pH.

2. Relationship between tumor pH and tumor size

There was a negative correlation between tumor pH and tumor size, such that the larger tumors had lower pH (Spearman's rank correlation coefficient: $rs = -0.5128$ ($p < 0.05$), regression line: $Y = -0.1595 \times X + 7.1799$) (Figure 2).

3. Relationship between tumor pH and pStage

Although a significant correlation did not exist, tumors of

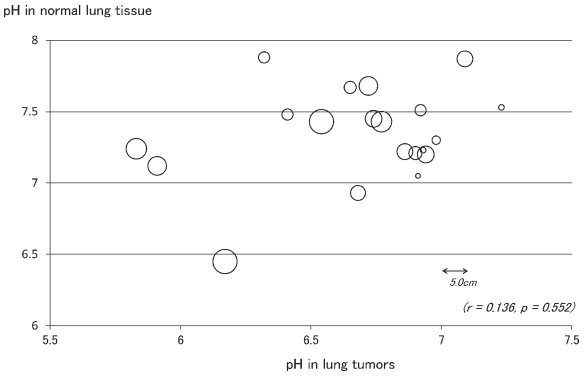

Figure 1　The pH in lung tumors and in normal lung tissue. The maximum diameter of the tumors is represented by the size of the circles.

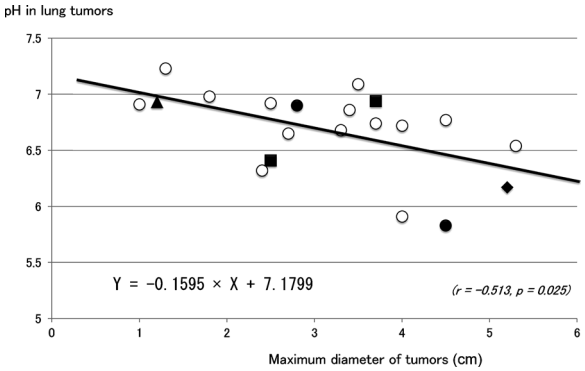

Figure 2　Relationship between tumor pH and tumor size.
Histological classifications of the tumors are represented as follows: ○ adenocarcinoma, ■ squamous cell carcinoma, ● small cell carcinoma, ▲ adenosquamous carcinoma, ◆ pleomorphic carcinoma.

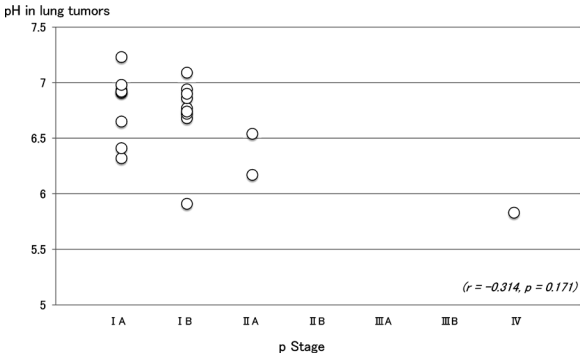

Figure 3　Relationship between tumor pH and pathological stage (pStage).
Tumor pH was plotted against the pathological stages. Tumor pH tended to be lower in advanced stages.

advanced pStage tended to have a lower pH (Figure 3).

4. Relationship between tumor pH and the location of tumors

There was a positive correlation between tumor pH and the location of tumors, such that tumor pH was higher in the distal region of the lobe with increased bronchial branching (Spearman's correlation coefficient: $rs = 0.4496$ ($p < 0.05$), regression line: $Y = 0.1431 \times X + 6.0311$) (Figure 4).

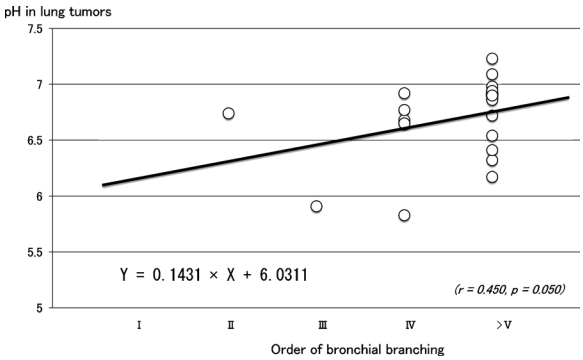

Figure 4　Relationship between tumor pH and the location of tumors in the lung.
Tumor pH was higher in the distal regions of the lobes.

In addition, the expression of tumor markers was measured in patients with lung adenocarcinoma who met the inclusion and exclusion criteria between 2010 and 2015. The measurements were collected prior to, and 1 year after the surgery, in order to examine the relationship between tumor pH in lung adenocarcinoma and the expression of tumor markers. Specifically, carcinoembryonic antigen (CEA) and sialyl SSEA-1 (sialyl LewisX-I antigen, SLX) were selected as tumor markers as they are highly specific to adenocarcinoma. In addition, gastrin-releasing peptide precursor (ProGRP), a marker specific for small cell carcinoma, was selected for comparison. Patients were excluded from the study if they had diseases or conditions that could contribute to false-positive tumor marker expression (Table). Thirteen patients (3 men and 10 women; average age: 69.3) met all the criteria.

5. Relationship between tumor pH in lung adenocarcinoma and the expression of tumor-specific markers

Although a significant correlation did not exist, the level of CEA expression tended to be higher in tumors with a low pH prior to surgery (Figure 5). There was a similar trend between tumor pH and the level of SLX expression (Figure 6). There was no correlation between tumor pH and the level of ProGRP expression (Figure 7). Similar trends persisted 1 year after surgery, such that the high expression levels of CEA and SLX were associated with low pH in tumors, whereas there was no correlation between tumor

Table　Conditions that result in positive or false-positive expression of tumor markers.

Tumor marker	Conditions that result in positive or false-positive findings
CEA	Heavy smoking
	Aging
	Diabetes
	Connective tissue disease
	Chronic lung disease
	Liver dysfunction
	Pancreatitis
	Gastritis, gastric ulcer, ulcerative colitis
	Kidney dysfunction
	Other lung cancers (squamous cell carcinoma, large cell carcinoma, small cell carcinoma)
	Gastrointestinal cancers (esophageal cancer, gastric cancer, colorectal cancer, pancreatic and biliary tract cancer)
	Breast cancer
	Medullary thyroid cancer
	Hypothyroidism
	Gynecological tumors (cervical cancer, ovarian cancer)
	Urinary tract cancer
SLX	Chronic lung disease
	Gastrointestinal cancers (gastric cancer, colorectal cancer, pancreatic and biliary tract cancer)
	Gynecological tumors (ovarian cancer)
ProGRP	Chronic lung disease
	Kidney dysfunction
	Neuroendocrine tumors (carcinoid tumors, large cell neuroendocrine carcinoma)
	Medullary thyroid cancer
	Pancreatic cancer
	Ovarian cancer

Heavy smoking: smoking index > 600, Diabetes: HbA1c (NGSP) > 7.0%, Kidney dysfunction: serum Crea. > 1.3 mg/ml or serum BUN > 24, Cancer cases were only considered if they developed within 5 years of the surgery (excluding early-stage cancers that may be candidates for endoscopic treatment).

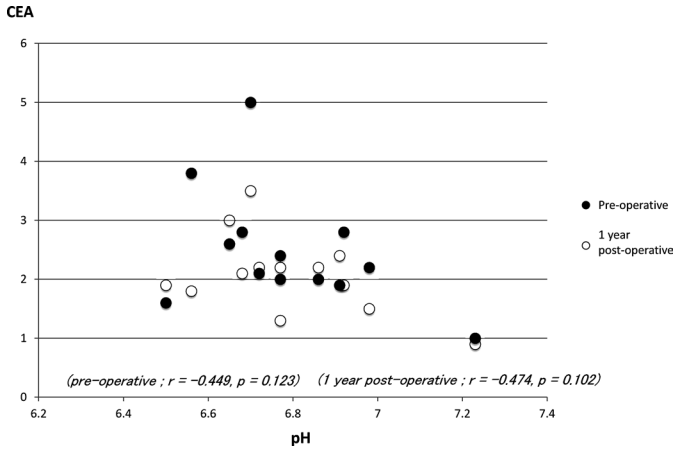

Figure 5　Relationship between tumor pH in lung adenocarcinoma and the expression of CEA.

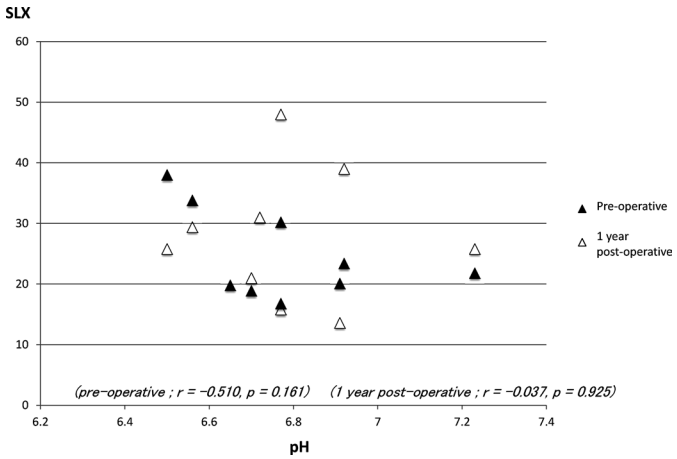

Figure 6　Relationship between tumor pH in lung adenocarcinoma and the expression of SLX.

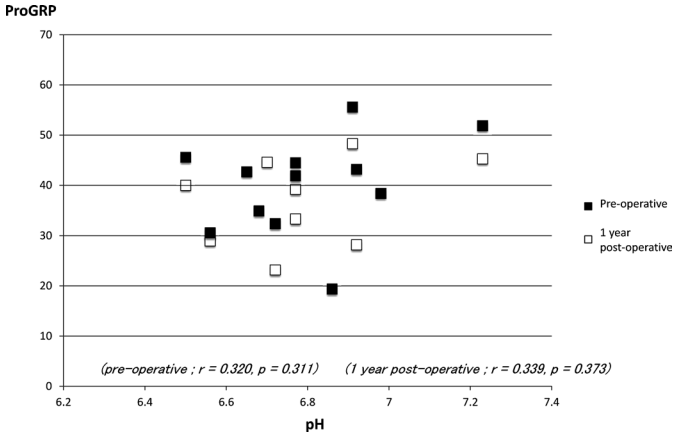

Figure 7　Relationship between tumor pH in lung adenocarcinoma and the expression of ProGRP.

pH and the level of ProGRP expression (Figures 5−7).

Ⅳ. Discussion

Tumor cells proliferate rapidly, outgrowing the vascular supply maintained by angiogenesis. This leads to the insufficient delivery of oxygen and nutrients to tumor cells. Thus, cancer cells survive in a harsh tumor microenvironment characterized by hypoxia, a low pH, and low glucose levels[2−6]. Cell survival in the tumor microenvironment is maintained by hypoxia-inducible factor (HIF)[7,8], which is constitutively activated to regulate the expression of multiple genes involved in adaptation, tumor metastasis, and resistance to treatments[9−13]. Specifically, the oxygen-sensing α-subunit of HIF-1 induces the expression of genes involved in glycolysis, an oxygen-independent metabolic pathway, leading to the suppression of oxidative phosphorylation in mitochondria. This mechanism results in the effective production of ATP by glycolysis under hypoxic conditions. The changes in the cellular pH induced by glycolysis are neutralized in the cytoplasm and plasma membrane, as well as by transmembrane H^+ flux caused by the voltage gradient; however, pH homeostasis is driven more strongly by HIF-1-regulated mechanisms such as the activation of carbonic anhydrase IX, monocarbocylate transporter, and Na^+ / H^+ exchanger. In addition to the pH, HIF-1α regulates metabolic adaptation, erythropoiesis, angiogenesis, and vasoconstriction, as well as cellular growth, survival, and apoptosis. In cancer cells, the overex-

pression of HIF-1α cells to adapt in order to survive in the harsh tumor microenvironment[14]. Thus, the tumor microenvironment, in particular low pH, may in fact be suitable for tumor cells to survive and proliferate.

In the present study, we investigated the relationship between pH and the clinicopathological factors in primary lung tumors. We set the exclusion criteria to minimize the impact of the host to the tumor microenvironment. Specifically, patients were excluded if they 1) had undergone thoracic surgery or had ventilatory defects that could directly affect respiratory functions, 2) received antithrombotic therapy or other cancer therapies that could affect microcirculation via regulation of the blood coagulation system, 3) received vasodilators that could affect gas transport by acting on the microcirculatory system, or 4) had similar conditions as in 1−3.

Next, we sought to determine the appropriate location to measure pH in primary lung tumors. Our results in the normal tissue indicated that pH varies significantly depending on where the pH meter is inserted, suggesting that the presence of normal tissue may interfere with the measurement of pH in tumors. Thus, we selected patients whose tumors had < 50% ground glass opacity nodules, and measured the pH in the center of the tumor, which was composed solely of cancerous tissues as determined by visual inspection.

Our results demonstrated that tumor pH was negatively correlated with the size of tumors, such that the pH measured in the middle of tumors decreased to less than the physiological level with increasing tumor size.

One of the patients in our study exhibited a low pH (pH = 6.45) both in the core of the tumor and in the normal lung tissue. The patient was an 80-year-old male without any notable medical history or comorbidities, and the surgery was performed with curative intent. Histopathological examination led to the diagnosis of stage pT2aN0M0, pStage II A pleomorphic carcinoma. However, metastatic lesions were found in the contralateral lung and pleura soon after the surgery, and the patient died of the metastatic cancer 9 months after the surgery. Thus, the low pH in the normal lung may be an indicator of disease progression that cannot otherwise be detected by clinical observations or data.

There was a similar trend between tumor pH and pStage, which may be related to the relationship between tumor pH and tumor size. Our results also demonstrated that tumor pH increases towards the distal end of the lungs. We believe that bronchial branching has little effect on the partial pressure of CO_2, and therefore on pH, unless there are prominent strictures or airway obstruction. Thus, the observed increase in tumor pH in the distal regions of the lungs may be attributed to the fact that tumors in the distal lung are typically small.

Moreover, we examined the relationship between tumor pH in lung adenocarcinoma and expression of tumor markers that are clinically relevant. Tumor markers are produced by tumor cells, as well as by normal cells in response to the presence of tumor cells. They are indicators of the presence of tumors, as well as the subtypes and the amount of tumor cells present in the body. In particular, as the expression level of these markers reflects the amount of tumor cells present in the body, it may indicate the state of the disease as well as its progression. In the present study, we set additional criteria to exclude patients who had diseases or conditions other than lung adenocarcinoma that could contribute to false-positive tumor marker expression[15–22]. Of note, as these criteria excluded patients with conditions common in men, such as chronic lung disease and heavy smoking, the study patients included a large proportion of women.

We demonstrated that there was a negative correlation between the pH in lung adenocarcinoma and the expression of adenocarcinoma-specific markers, such as CEA and SLX, although the correlation was not significant. On the other hand, there was no correlation between the pH in lung adenocarcinoma and the expression of ProGRP, which is not specific

to adenocarcinoma. Our findings indicate that the measurement of pH in tumors may provide a better understanding of the disease state, leading to improved cancer diagnosis and treatment. For example, direct measurement of the pH in the lymph nodes may result in the detection of metastatic nodes.

Ⅴ. Conclusion

The goal of the present study was to investigate the correlation between pH and clinicopathological factors in lung tumors. Our results supported the notion that the pH inside tumors is lower than the physiological level, and that tumor cells adapt to the harsh tumor microenvironment to survive. Furthermore, our findings indicated that tumor pH may be indicative of disease states in lung cancer.

There is no conflict of interest to disclose.

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