Acta Med. Nagasaki 35 : 22—24

Clinical Application of Lung Cancer Cell DNA Analysis

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Received for publication, December 15, 1989

ABSTRACT : Nuclear DNA patterns were evaluated for 115 lung cancers which were surgically resected. Seventeen percent showed diploidy pattern and 83% were aneuploidy. The 3-year survival was 90% in those who showed diploid and 58% in those who revealed aneuploid in analysis of nuclear DNA patterns. In conclusion determination of nuclear DNA pattern for lung cancer cells is of clinical value to assess the surgical outcome besides the histochemical evaluation.

INTRODUCTION

The nuclear deoxyribonucleic acid (DNA) content of tumor cells has been applied for assessment of the prognosis or determination of the treatment of malignant tumors[1)-3)]. It is widely accepted that nuclear DNA contents reflect the biological characteristics of tumors in terms of their malignancy which implies a higher rate of recurrence and metastasis. Histologically stromal reaction is generally regarded as host resistance against tumor infiltratin around healthy tissues and lymphatic or hematogenous metastases. Some investigators confirmed that a prominent lympoid infiltration around tumors was associated with a favorable prognosis in patients with gastric or colonic carcinomas[4)5)].

The purpose of this study is to clarify as to whether the prognosis for patients who undrewent surgical resections correlate with nuclear DNA of lung tumor cells or not and as to whether surgical outcome including the survival time following surgery is accurately anticipated in terms of nuclear DNA analysis on the basis of a result of our experience.

MATERIALS AND METHODS

Nuclear DNA patterns in the surgical specimens resected for the treatment of lung cancer were analyzed from paraffin-embedded preparation by uisug FACS IV. 30μm section, cut from formalin-fixed and paraffin-embedded tissue blocks, were dewaxed (by xylen) rehydrate (100% to 50% ethanol) and then washed by immersing in the running tap water. The sample that was confirmed to be an adequate part by the hematoxylin stained section was incubated overnight in trypsin-citrate buffer and stained using Solution A (trypsin), Soluition B (trypsin inhibitor, RNase A) and Solution C (spermine tetrahydrochloride propidium iodide), and the values of the peak channel numbers of normal and cancer cells were measured by using FACS IV. DNA index was calculated according to a following equation, DNA index=peak channel number of cancer cells/peak channel number of normal cells.

Nuclear DNA patterns were evaluated in 115 patients with resected lung cancers as shown in **Table 1.** Most of them (82.6%) showed a pattern of aneuploidy. It was not characteristic of histologic types.

Table 1. Tumor cell DNA ploidy pattern

histology	PATIENTS (%) diploidy	aneuploidy	Total
ad. ca.	8 (16.3)	41 (83.6)	49 (100)
sq. ca.	12 (18.3)	52 (81.2)	63 (100)
larg. cell. ca.	0 (0)	2 (100)	2 (100)
	20 (17.4)	95 (82.6)	115 (100)

Table 2. Comparison in 3-year survival between types of histology and DNA pattern

histology		3-year survival diploidy	aneuploidy	
ad. larg. ca	N=51	88%	58%	$p<0.05$
sq. ca	N=64	91%	58%	$p<0.05$

The three-year survival between patients with diploidy and aneuploidy of nuclear DNA pattern was compared in this series.

In adenocarcinoma and large cell carcinoma of histologic types, 88% of those who disclosed the DNA pattern of diploidy survived 3 years or more although 58% of those who demostrated aneuploidy survived more than 3 years. In contrast, in squamous cell carcinoma, 91% of those who demonstrated diploidy did well more than 3 years in spite of 58% out of aneuploidy. These were statistically significant difference ($p<0.05$) between the patients with DNA patterns of diploidy and aneuploidy.

On the other hand, a limited operation for small-sized lung cancers of the peripheral type was applied for surgical treatment. However, their prognoses were not uniform, some were satisfactory, some were disappointed.

When nuclear DNA patterns were analyzed, their prognoses were apparently divided into the two groups, better group included diploidy of nuclear DNA pattern and worse one mainly holded aneuploidy of nuclear DNA as shown in **Table 2.**

DISCUSSION

In general, nuclear DNA analysis of tumor cells helps physicians to expect the prognosis and know aggressiveness of tumor cells. It is generally known that aneupliod pattern of tumor cells means high potential of aggressive behavior of the tumor.

The analysis of nuclear DNA content from paraffin-embedded tissue blocks makes it possible to do retrospective analysis of patients who clarify the survival time.

It is accepted that DNA pattern correlates with the prognosis of malignant tumor-bearing or surgical patients.

The prognosis of lung cancer is not yet satisfactory except for early cancer. It is believed that aggressiveness of lung cancer cells is much more increased than that of the other malignant tumor cells. In this study, it is a clarified fact that DNA pattern of aneuploidy in lung cancer cells is a dominanat diploidy pattern in the analysis of nuclear DNA.

On the other hand, most of those in whom aneuplpidy pattern was evidenced failed to survive more than 3 years with statistically significant difference($p<0.05$) regardless of histologic types.

In fact, nuclear DNA pattern clearly indicates as to whether the prognosis and surgical outcome of lung cancer patients would be fair or not. Therefore, it is recommended that aggressive operation and adjuvant therapy are required for patients with aneuploidy pattern in nulcear DNA analysis. It is noteworthy that nuclear DNA analysis is of great benefit as well as great value to assess their prognoses and to determine the necessity or aggressive adjuvant therapy for obtaining a long-term suvival.

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