日放腫会誌 *J. Jpn. Soc. Ther. Radiol. Oncol.* 3: 219-223, 1991

FLOW CYTOMETRIC ANALYSIS OF NUCLEAR DNA CONTENT AND RADIATION RESPONSE IN ESOPHAGEAL CANCER

Koichi KURISU, Yoshio HISHIKAWA, Midori TANIGUCHI, Norihiko KAMIKONYA and Takashi MIURA

(*Received 13 November 1990, accepted 23 July 1991*)

Abstract Cell nuclear DNA ploidy patterns were determined by flow cytometric analysis in 19 patients with esophageal carcinoma. Aneuploid DNA patterns were observed in 13 patients. Seventeen of the patients were treated by external radiotherapy of 50 Gy or more. Intraluminal brachytherapy was also performed in 15 of these patients and their local response to radiation was estimated by barium esophagograpy one month after completion of the radiotherapy. We investigated relations among the DNA ploidy patterns, clinical chara cteristics, and local radiation response. Four patients with lymph node metastasis were judged to have aneuploid DNA patterns. These patients with aneuploid pattern showed a favorable local response to treatment one month after radiotherapy. It is possible that the ploidy pattern of esophageal carcinoma could be a valuable predictor of the early local response to radiotherapy.

Key words: Esophageal carcinoma, Radiotherapy, Flow cytometry, DNA

INTRODUCTION

Alterations in cell cycle phase distribution and cellular DNA ploidy are considered to be factors that have important influence on the efficacy of radiation. Flow cytometry is a fast precise method for the determination of DNA ploidy and it can also be used to analyze cell cycle phase distribution[1-3]. In this study, we examined the usefulness of the DNA ploidy pattern as an indicator of tumor response to radiation.

PATIENTS AND METHODS

Patients

Nineteen patients with esophageal carcinoma were studied. There were 11 men and eight women ranging in age from 43 to 87 years (mean age: 68.7 years). The tumors were located at the upper, middle, and lower esophagus in 1, 15, and 3 patients, respectively. Specimens for pathological diagnosis and flow cytometric analysis were obtained from all patients by biopsy through esophagoscopy before the initial radiation treatment. Two specimens were obtained from sites as close together as possible. All the tumors were diagnosed as squamous cell carcinoma following the endoscopic biopsy. None of the patients had received chemotherapy before radiation.

DNA analysis

Tumor samples were prepared for flow cytometry in the manner described by Taylor[4]. Briefly, DNA was stained with 50 μg/ml propidium iodide (Sigma, St. Louis) which was made up in a 0.1% solution of nonionic detergent Triton-X-100 and included RNase (Sigma, St. Louis). Immediately before analysis, the samples were filtered through a 40 μm nylon mesh, and the resulting suspension was

栗栖 孝一, 菱川 良夫, 谷口 緑, 上紺屋 憲彦, 三浦 貴士
Department of Radiology, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya-city, Hyogo 663, Japan.

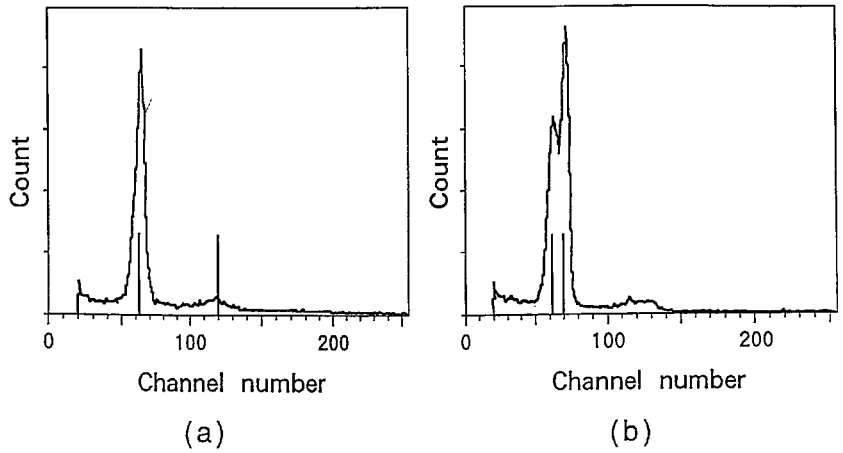

Fig. 1. Represetative histograms. (a) The diploid tumor has one distinctive G0/G1 peak (channel 62). (b) In the aneuploid tumor there is a first normal G0/G1 peak (channel 62) and a second G0/G1 peak (channel 70). The DNA index of this tumor was 1.13.

passed through a flow cytometer (FACS can, Becton Dickinson, Sunnyvale). A 488 nm argon ion laser line run at 600 mW was used for fluorescence. Patterns in the tumor cells on the DNA histograms were divided into diploid and aneuploid types. Fig. 1 shows histograms representative of both types. The diploid type always had a single major peak (Fig. 1a), whereas the aneuploid tumor showed two separate peaks; the second peak being in addition to the normal diploid DNA (Fig. 1b). Histograms of high quality were obtained; the coefficient of variation of diploid peaks ranged from 3.2% to 5.1%. The DNA index was calculated by comparing the channel ratio of G0/G1 populations in normal and tumor cells.

Radiotherapy

Of the 19 patients, 17 were treated by external rediotherapy of 50 Gy or more. The other two patients received dosage of less than 50 Gy due to their poor general condition and preoperative radiotherapy. Intraluminal brachytherapy was combined as a boost therapy in 15 of the 17 patients who received external radiotherapy of 50 Gy or more. The remaining two patients were treated with external radiotherapy alone due to stenosis prohibiting intraluminal brachytherapy and the possibility of fistulas. The dose of intraluminal brachytherapy was 12 Gy, delivered in two fractions in one week, with the exception of one patient who received 6 Gy in one fraction.

External radiotherapy was performed with 10 MV X-rays (LMR 15, Toshiba, Tokyo). All patients received a dose of 2 Gy per day for 5 days a week. Parallel opposed portals were used up to 40 Gy, followed by oblique portals or rotation. The field size was 6 cm wide and 3 cm longer than the tumor size at both the superior and inferior margins of the tumor.

Intraluminal brachytherapy was performed using a high-dose-rate, remote afterloader (RAL-303, Toshiba, Tokyo). Details of the technique and dosimetry of this intraluminal brachytherapy have been reported previously[5].

Evaluation of Radiation Effect

We estimated the local radiation response of the 17 patients who received external radiotherapy of 50 Gy or more.

The local response to radiation was evaluated by barium esophagograpy one month after the completion of radiotherapy. Clinical efficacy was denoted in the following manner[6]: Complete response (CR) denoted 100% regression of disease and partial response (PR) denoted more than 50% reduction in tumor bulk but less than 100% resolution of disease.

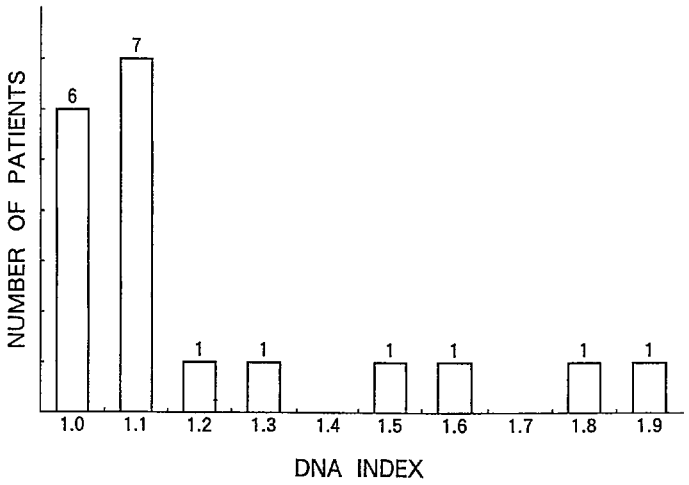

Fig. 2. Distribution of DNA indices in esophageal carcinomas.

RESULTS

DNA Ploidy Pattern

Six of the 19 patients had a diploid DNA pattern; with aneuploid DNA patterns observed in the remaining 13. The DNA indices for aneuploid tumors ranged for 1.11 to 1.93 (mean: 1.35) (Fig. 2).

Correlation Between DNA Ploidy Pattern and Clinical Factors

Table 1 shows relations between the DNA ploidy pattern and a patient's sex, age, tumor length, radiological findings, disease sites, and lymph node metastasis. We found no obvious correlations between DNA ploidy patterns and five of the six above factors; we did find correlation with lymph node metastasis.

Four patients had lymph node metastasis until after the completion of radiotherapy. Three of these had neck lymph node metastasis and one had abdominal lymph node metastasis. The diagnosis of neck lymph node metastasis was based on a physical examination, and the diagnosis of abdominal lymph node metastasis was based on ultrasonic diagnosis and computed tomography. The four patients with lymph node metastasis had an aneuploid DNA patterns.

Correlation Between DNA Ploidy Pattern and Radiation Effects

Table 1. Characteristics of Diploid and Aneuploid tumors

	Diploid	Aneuploid
Total no. of patients	6	13
Sex		
male	4	7
female	2	6
Mean age	72.3	67.1
Tumor length (mean±SD cm)	6.1±3.5	6.1±2.5
Radiological findings		
superficial	1	2
tumorous	1	4
serrated	0	5
spiral	3	1
funnelled	1	1
Site		
upper	1	0
middle	5	10
lower	0	3
Lymph node metastasis		
(+)	0	4
(−)	6	9

Table 2 shows relations between the DNA ploidy patterns and the effects of radiation. Eight of the 12 patients with aneuploid tumor cells showed CR one month after radiotherapy, whereas one of five patients with diploid cells showed CR. Among the 17 patients who received external radiotherapy of 50 Gy or

Table 2. DNA Ploidy Pattern and Local Radiation Effect

	Diploid (n=5)	Aneuploid (n=12)
CR	1	8*
PR	3*	2
MI	1	2

Note. CR=complete response; PR=partial response; MI=minor improvement. *Includes one patient treated by external radiotherapy alone.

more, 9 showed CR and 5 had PR. The remaining three patients had less than 50% reduction in tumor bulk; this was considered to be a minor improvement.

DISCUSSION

It is recognized that the DNA content of tumor cells may differ from that of normal cells as a result of chromosomal alteration. On flow cytometric analysis, an abnormal amount of DNA (aneuploidy) has been demonstrated in tumors of the esophagus[7,8], kidney[9,10], colon[11], and lung[12,13]. In those studies, all tumor specimens were obtained by surgery and correlation between the DNA content of the tumor cells and the patient prognosis was investigated retrospectively. In the present study, however, we obtained specimens by biopsy through esophagoscopy before treatment, and investigated the correlation between the DNA content in esophageal cancer cells and the local radiation response prospectively.

It has been found that most tumor cells are diploid in the early stages of disease, whereas aneuploidy tends to increase in advanced stages[7-13]. In our study of 19 patients with esophageal squamous cell carcinoma, we observed that the patients with aneuploid cell tumors manifested a high frequency of local lymph node metastasis compared to patients with diploid tumors.

Regarding the relations between ploidy pattern and patient prognosis, aneuploid tumors tend to have a high frequency of recurrence and are associated with poor patient prognosis[7-13]. Our results, showed that aneuploid tumors had better local response to radiotherapy than diploid tumors. However, this does not mean that the prognosis for patients with aneuploid tumors treated by radiotherapy is better than that for patients with diploid tumors, since patients with aneuploid tumors tend to have lymph node metastasis and to be in an advanced disease stage. Long-term follow up will be needed to clarify the relations between DNA ploidy patterns and patient prognosis after radiotherapy.

Kaketani et al.[7] analyzed the DNA ploidy patterns of excised malignant esophageal tissue; they reported that the incidence of recurrence within 12 months after surgery was higher in patients with aneuploid tumors (83.3%) than in those with diploid tumors (16.7%). This means that a poor prognosis can be predicted prior to surgery in patients with aneuploid tumors. Therefore, it is important to design more effective therapy for patients with aneuploidy. We suggest that adjuvant therapy should be given to patients with aneuploid tumors that are treated surgically. Judging from our results, radiotherapy should be considered as an effective adjuvant therapy for these patients. If a postoperative prognosis is not improved by adjuvant therapy combined with surgery, we recommend radiotherapy, including a high-dose-rate intraluminal brachytherapy boost, instead of surgery, since a good local response to radiotherapy is expected in patients with aneuploidy, as seen in our study. Of course, the prognosis of patients treated by radiotherapy may not be different from those treated surgically. However, the quality of life obtained for the patient by the two treatment methods is very different. If improvement of dysphagia and food passage can be gained, we recommend that patients with poor prognosis should be treated by moderate methods. Taking these factors into account, we believe that the measurement of DNA content of esophageal cancer cells could be an important factor to be cosidered when making therapeutic decisions.

We conclude that the DNA ploidy pattern is an important indicator of early local response to radiotherapy of esophageal carcinoma.

ACKNOWLEDGEMENT

This study was partially supported by Grants-in-Aid from the Japanese Ministry of Education, Science and Culture (01304039). The authors thank Mrs. Izumi Kurisu for her assistance in the preparation of this manuscript.

REFERENCES

1) Friedlander, M. L., Hedley, D. W., Taylor, I. W.: Clinical and biological significance of aneuploidy in human tumours. *J. Clin. Pathol.* **37**: 961-974, 1984.
2) Frankfurt, O. S., Slocum, H. K., Rustum, Y. M. *et al.*: Flow cytometric analysis of DNA aneuploidy in primary and metastatic human solid tumors. *Cytometry* **5**: 71-80, 1984.
3) Barlogie, B., Raber, M. N., Schuman, J. *et al.*: Flow cytometry in clinical cancer research. *Cancer Res.* **43**: 3982-3997, 1983.
4) Taylor, I. W.: A rapid single step staining technique for DNA analysis by flow microfluorimetry. *J. Histochem. Cytochem.* **28**: 1021-1024, 1980.
5) Hishikawa, Y., Kamikonya, N., Tanaka, S. *et al.*: Radiotherapy of esophageal carcinoma: role of high-dose-rate intracaviatary irradiation. *Radiother. Oncol.* **9**: 13-20, 1987.
6) Kelsen, D. P., Ahuja, R., Hopfan, S. *et al.*: Combined modality therapy of esophageal carcinoma. *Cancer* **48**: 31-37, 1981.
7) Kaketani, K., Saito, T., Kobayashi, M.: Flow cytometric analysis of nuclear DNA content in esophageal cancer: aneuploidy as an index for highly malignant potential. *Cancer* **64**: 887-891, 1989.
8) Jin-Ming, Y., Li-Hua, Y., Qian, G. *et al.*: Flow cytometric analysis DNA content in esophageal carcinoma: correlation with histologic and clinical features. *Cancer* **64**: 80-82, 1989.
9) Baish, H., Otto, U., Kloppel, G.: Malignancy index based on flow cytometry and histology for renal cell carcinoma and its correlation to prognosis. *Cytometry* **7**: 200-204, 1986.
10) Ljungberg, B., Stenling, R., Roos, G.: DNA content and prognosis in renal cell carcinoma. *Cancer* **57**: 2346-2350, 1986.
11) Noguchi, P. D., Gunningham, R. E., Ridge, J. *et al.*: The use of flow cytometry and cell sorting in a human colon carcinoma model. *Cytometry* **6**: 254-259, 1985.
12) Manfred, V., Jurgen, M., Jaroslav, S. *et al.*: DNA distribution in non-small cell lung carcinoma and its relationship to clinical behavior. *Cytometry* **6**: 348-356, 1985.
13) Laura, T., Donatella, T. D., Francesco, M. *et al.*: Non-small cell lung carcinoma: tumor characterization on the basis of flow cytometry detected cellular heterogeneity. *Cytometry* **4**: 174-183, 1983.

要旨：19名の食道癌患者の癌細胞核DNA量測定をフローサイトメトリーを用いて行ない，DNAの異数体(aneuploidy)の有無により2群に分類した．異数体は13名に認められた．50 Gy以上の外照射を行った17名について局所一次効果を治療終了1カ月後の食道造影により判定し，異数体検出の有無と臨床所見および局所一次効果との関係について検討を加えた．17名中15名には高線量率腔内照射を併用した．リンパ節転移の認められた4例は異数体が検出された．放射線治療の局所一次効果は異数体が検出された群が良好であった．フローサイトメトリーによる異数体の有無の判定は放射線感受性予知に際して，有用な指標の一つとなる可能性が示唆された．