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DNA Analysis from Biopsied Specimens for Carcinomas of the Esophagus

Masao Tomita, Shinsuke Hara, Yutaka Tagawa, Katsunobu Kawahara, Hiroyoshi Ayabe, Masayuki Obatake, Terumitsu Sawai, Masaaki Jibiki, Masafumi Morinaga, Toshikazu Matsuo, Satoshi Matsuo, Akira Yoshida, Taizou Furukawa, Ryuichiro Yoshida, Masaaki Eguchi, Takao Takahashi, Tsutomu Sakai, Daikichi Okada and Takao Makiyama

The First Department Surgery, Nagasaki University School of Medicine

Surgical outcome for advanced esophageal cancer patients is not satisfactory in spite of improvements of diagnostic tools, operative techniques, postoperative cares and adjuvant chemotherapy. Postoperative prognosis used to be predicted by the grades of histologic disease progression.

However, it is limited to accurate prediction of its prognosis. Recent studies have been focused on biologic behavior of malignant cells, in particular, nuclear DNA contents which play an important role in cell proliferation and metabolizm.

Clinical use of flow-cytometric measurement of nuclear DNA is now prevalent to assess the grades of malignancy for malignant tumors. In contrast, it is difficult to know nuclear DNA contents preoperatively.

The purpose of this study is to clarify whether or not preoperative biologic behaviors is clinically feasible, from biopsied specimens in comparison with that from the surgical specimens.

Materials and Method

Surgical specimens were subjected to this study and also preoperative biopsied specimens through endoscopy were prepared for this study. Specimens were cut into pieces in 0.4ml of 0.1% Triton X solution and filtrated with 50μm Nylon mesh and added equivalent volume of propidium iodide (100g/ml, RNase 1.0g/ml, finally adjusted to 50g/ml concentration). DNA contents in nuclei were determined by FACScan, and a histogram was constructed.

DNA index (DI) was expressed as a ratio to standard lymphocytes. DI = 1.0 was regarded as diploidy and the other aneuploidy. The cell cycles were analyzed by cell FIT system (Becton Dickinson).

Results

A) Relationship between DNA patterns and sampling taken from the sites of the tumor.

We sometimes encountered in the DNA histogram which was unable to analyse. It is assumed that this fact is based on the reasons that the specimens contained necrotic tissues taken from the center of the tumor. There were more often chances in the specimens taken from infiltrative ulcerations than localized ulcerations in accordance with macroscopic types of ulceration as shown in Table 1.

It showed that surgical specimens should be taken from the periphery of the tumors, in particular, in infiltrative ulceration.

Table 1. Analyzed and unanalyzed DNA pattern according to macroscopic types of ulceration of the tumors

	center of the tumor	periphery of the tumor	
analyzed	95%	100	localizaed ulceration (n = 10)
unanalyzed	5	100	
analyzed	70	100	infiltrative ulceration (n = 8)
unanalyzed	30	100	

B) Relationship between the depth of cancer infiltration and DNA ploidy patterns.

In this series we experienced a case with carcinoma of the esophagus involving the trachea to which cancer infiltration extended across the wall of the esophagus.

The resected specimens were sliced throughout cancer infiltration and each part of cancer infiltration was individually taken to assess the DNA ploidy pattern. Four parts of specimens were taken from the mucosa, the submucosa, the adventitia and invasion to the trachea. The DNA patterns of each part were quite similar, showing DI = 1.0 as shown in

Fig. 1. It was demonstrated that cancer extension arose from the same stem cell line.

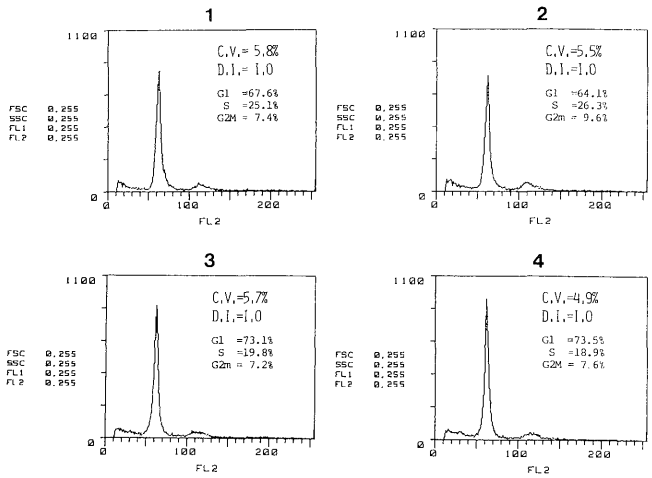

Fig. 1. DNA ploidy patterns in the same specimen according to the depth of cancer infiltation.

C) Heterogeneity in the identical cancer lesion.

Fig. 2 showed heterogeneity of DNA ploidy patterns in the same specimens. The DNA ploidy patterns revealed diploid (DI = 1.0) excent for only one out of the 7 sites. In contrast, an aneuploidy pattern was taken from the periphery of the tumor. Finally, we found 2 cases of heterogeneity out of 10 carcinomas in this series.

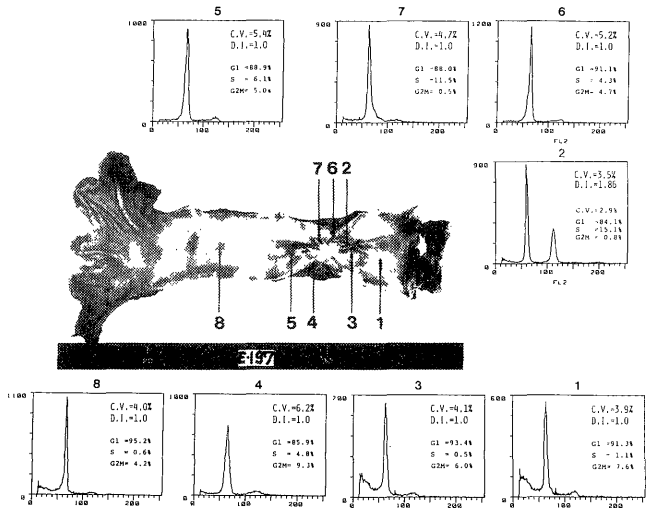

Fig. 2. Heterogeneity of DNA ploidy patterns in the same specimen.

Discussion

In this series, it was ascertained that the procedure of needle aspiration biopsy benefited the assessment of nuclear DNA ploidy pattern. It is of great value to know biologic behaviors of malignant tumor cells preoperatively. It is no doubt that preoperative assessment of DNA ploidy patterns of malignant tumors contributes to determination of therapeutic strategies of choice and also selection of therapeutic steps.

Physicians should be aware of taking samples from the center of the tumor which includes nucrotic mass. This result showed that the sampling from the central zones of the tumors might lead to misunderstanding of DNA ploidy pattern to judge biologic behaviors of malignant tumors.

In addition, it should be emphasized that it is better to take samples from the marginal zones of the tumors as far as possible to avoid misunderstanding. And also three or more biopsied pieces are required for accurate assessment of DNA ploidy including heterogeneity.

Frankfurt[1] reported that the distribution of DNA index in various kinds of tumors varies with variety between primary and metastatic tumors.

Kawamura[2] clarified that the ratio of diploid to aneuploid was 66.7% in the analysis of carinomas of the esophagus. It has been accepted that low ploid aneuploidy pattern is common in esophageal cancer. On the other hand, it has been known that there are the two phasic peaks of low and high ploid aneuploidy patterns in carcinomas of the colon and the kidney and melanomas. These results collaborate that DI distribution represents a special feature on the basis of the tumors and the organs. There was not significant difference in the 5 year survival rates between patients with diploidy and aneuploidy patterns. And also no significant differences in the survival rates were found between a_0 and a_{1+2+3} groups and n (-) and n (+) groups.

In contrast, Sugimachi[3] reported that the outcome of patients with high ploid aneuploid, was apparently poor when compared with those with diploidy and low ploid aneuploidy. It is obvious that DNA ploidy analysis slightly influences on the outcome of carcinomas of the esophagus.

On the other hand, there is a definite difference in the influence on their prognoses between carcinomas of the esophagus and the colon. It is contemplated that DNA ploidy reflects the prognosis more significantly in the advanced cancer stage rather than the early one. The reasons are that early cancers allow complete resection enough to ensure the surgical radicality and achieve complete cure. It is different from the carcinogenesis between carcinomas of the esophagus and colon. In general, carcinomas of the esophagus arise from the squamous epithelium in contrast to grandular tissues in other cancers such as colon and pancreatic cancers.

In the process of cancer progression, an increase in malignant potential does not necessarily accompany DNA abnormality. As a result, DNA ploidy pattern sometimes does not directly imply malignant potential.

However, assessment of DNA ploidy pattern helps judge

biologic behavior of malignant tumor cells and know a presence of heterogeneity on the basis of stem line theory. And also this study collaborates that biopsied specimens are sufficient for assessing DNA ploidy patterns. This procedure made it possible to judge preoperatively or prior to the treatment.

References

1) Frankfurt OS, Slocum HK, Rustum YM et al: Flow cytometric analysis of DNA aneuploidy in primary and metastatic human solid tumors. Cytometry 5:71, 1984.
2) Kawamura Y: Cytometric analysis in DNA ploidy clinical studies in malignant tumors. Akita Med. J. 13:383, 1986.
3) Sugimachi K, Koga Y, Mori M et al: Comparative data on cytophotometric DNA in malignant lesions of the esophagus in the Chinese and Japaneses. Cancer 54:1947, 1987.