Acta Med. Nagasaki 32 : 92－97

Assessment of Surgical Outcome for Lung Cancer in Relation to Cellular DNA and RNA Contnt Ànalysis

Masao TOMITA, Norio YAMAOKA, Yutaka TAGAWA
Hiroyoshi AYABE, Katsunobe KAWAHARA, Hideki TANIGUCHI
Nobuko KUROSAKI, Tomonori NAKASONE, Hiroshi HASHIYADA
Naoki KAWAZOE

First Department of Surgery
Nagasaki University School of Medicine

Received for publication, June 3, 1987

To improve the surgical outcome for the treatment of lung cancer, as well as to avoid early recurrence following surgery, the application of multidisciplinary treatments after surgery is inevitable.

The indication for postoperative disciplinary treatment was assessed by means of flowcytometric DNA and RNA analysis on the basis of clinical experience with early recurrence following curative operation.

In conclusion, high levels of RNA contents of the tumor cells were mostly indicative of postoperative meticulous cares including multidisciplinary treatments.

INTRODUCTION

The selection for modes of multidisciplinary treatments for lung cancer was dubious until recently.

In the treatment of lung cancer, permanent cure is not necessarily warranted on accout of early recurrence sometimes seen following curative operation. Needless to say, multidisciplinary treatment is required for prevention from recurrence and for improvement of its prognosis. However, it is difficult to decide as to which modality is most beneficial for each case. The authors, therefore, attempted to find a better way to achieve multidisciplinary treatment by means of analyzing cellular DNA and RNA contents on the basis of a clinical experience with early recurrence.

富田　正雄，山岡　憲夫，綾部　公懿，川原　克信，谷口　英樹，黒崎　伸子，仲宗根朝紀
橋谷田　博，川副　直樹

METHODS

The cellular DNA and RNA contents were measured by flowcytometry using Acridine Orange staining method by DARZYNKIWICZ.[1] Fig. 1 shows the cytogram and histogram drawn by PC-1 cells derived from squamous cancer cells of the lung. DNA was shown on the longitudinal axis and RNA on the transverse axis of the scattergram. Analysis of G_{1S} and G_{2M} phase was satisfactorily feasible in combination with the measurement of the cellular DNA and RNA contents.

And also the cellular volume showed a constant value in relationship of cell growth curve of PC-1 to cell cycle analysis although the cellular RNA content become increased at cell-proliferation stage and decrease at confluent stage, proportionating to cell proliferation and reflecting an indicative of cell activity.

Surgical specimens used in this study were 51 lung cancers (24 adenocarcinomas, 26 squamous cell carcinomas, 1 large cell carcinoma) in whom all were operated upon at the first Department of Surgery, Nagasaki University Hospital during a period from October 1984 to March 1986 and the cellular DNA and RNA contents per 2×10 cancer cells were measured by means of flowcytometry.

DNA and RNA indices were also compared with calculated DNA and RNA amounts of cancer cells to lymphocyte counts. The DNA index of diploidy in cancer cells was presented as being 1. The other remainder were to be aneuploidy.

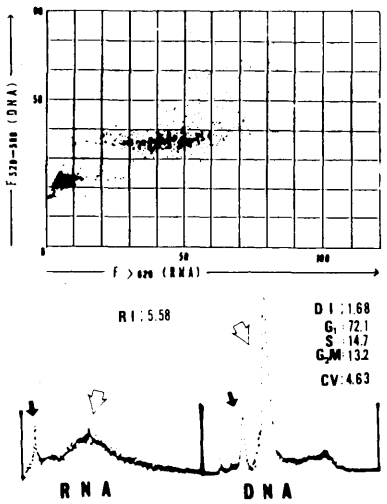

Fig. 1. cytogram and histogram drawn by PC-1 cells derived from squamous cancer cells of the lung.

RESELTS

The DNA and RNA indices were compared with the disease stages as shown in Fig. 2. The DNA indices were increased according to advances in the desease stages of lung cancer except in Stage Ⅳ. These, however, were not statistically significant. Stage Ⅳ cases were only 3, not sufficient for evaluating and drawing some considerations. In contrast, the RNA index was raised according to progression of the disease stage. There were statistically significant differences in RNA indices between Stage Ⅰ and Ⅱ ($P<0.02$) and between Stage Ⅰ and Stage Ⅲ ($P<0.01$). The cellular RNA content in advanced cancers increased (much more). Fig. 3 shows the analysis of RNA and DNA indices evaluated

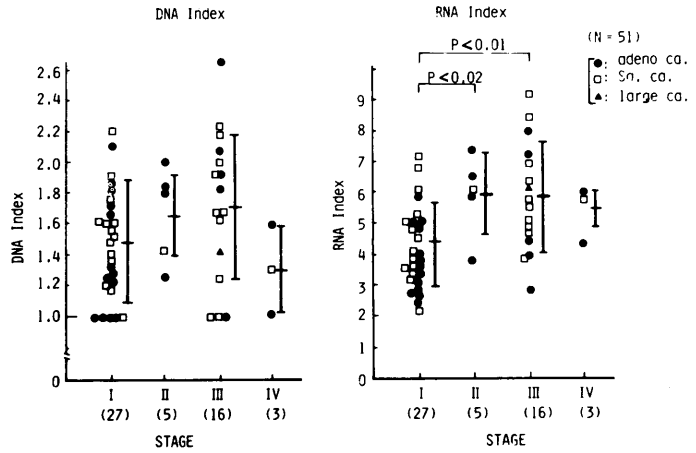

Fig. 2. Relationship between desease stages of lung cancers, and DNA and RNA indices

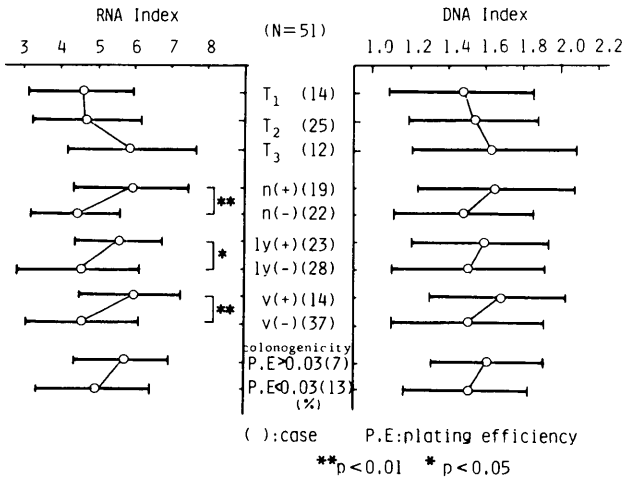

Fig. 3. Relationship between various factors of the disease, and RNA and DNA indices

according to various factors such as T, n, ly, v factors and colonogeniety presented by planting efficiency (PE).

There was no statistically significant difference among T1, T2 and T3 factors. The DNA levels in n(+) were higher than those in n(−) without statistical significance. However, RNA contents differed from the DNA one. The RNA contents in n(+) was much more significantly increased rather than those in n(−) as was similar to those seen in ly and v factors. According to the tumor size, the relationship between node metastasis and cellular RNA content was evaluated. Node metastasis tended to occur in case of increasing the cellular RNA content regardless of the tumor size and correlated closely with the cellular RNA volume as shown in Fig. 4.

According to the disease stages, the RNA and DNA indices were compared with mean value + standard error of each stage as indicated in Fig. 5. The DNA an RNA contents in Stage II and/or III diseases were raised more than those in Stage I one, in particular much more significant in the RNA volume. Twelve out of 28 patients subjected to this study suffered from early recurrence within 1 year following surgery. The DNA and RNA indices in those who had early recurrence were kept higher rather than who did not have early recurrence.

In the patients with an early recurrence as shown in Fig. 5, rise in RNA contenst was statistically significant, although that in DNA was not significant. It was clearly shown

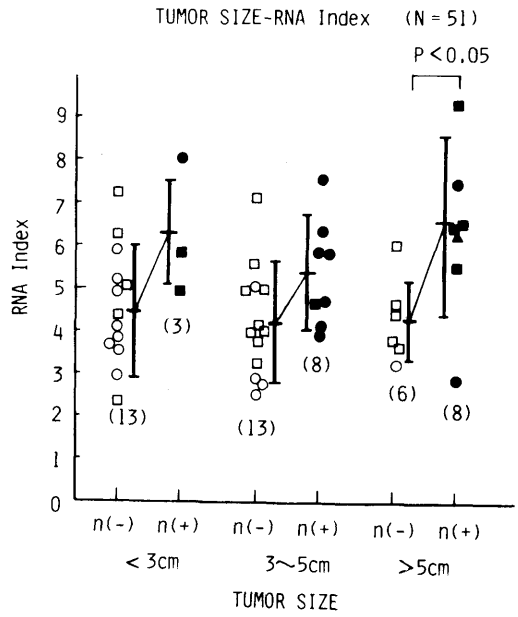

Fig. 4. Relationship between nodal involvement and RNA index according to the tumor sizes

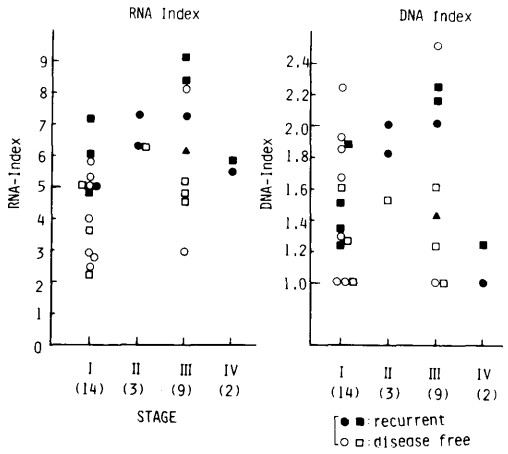

Fig. 5. Relationship between early postoperative recurrences according to disease stages, and RNA and DNA indices

that an increase in RNA coincided well with early recurreence.

In Stage Ⅲ disease, most of whom underwent non-curative operation on account of far advanced cancer, the DNA and RNA values showed much higher. In particular, a rise of the RNA volume of the tumor cells at sugery straight forwardly indicated to be more liable to predispose to early recurrence of cancer rather than that of the DNA volume.

DISCUSSION

The outcome of surgical treatment for lung cancer is referable to various factors related to background.

Flowcytometry in clinical cancer research is of wide use to quantitiate the cellular DNA and RNA volumes in each cancer cell.[2)]

The measurements of cellular DNA and RNA contents are of value of grading the malingnat cell proliferation, and predicting the progression of malignant disease[3)] and the survival rate.

In this series, the cellular DNA and RNA contents were compared with the extension of nodal involvement, the disease stage according to TNM classification, surgical outcome and likelihood of early recurrence following surgical intervention.

Positive nodal involvement well correlated with high level of RNA content with statistically significant difference.

Such was almost similar to those of positive histologic ly and v factors. On the basis of a result of cell cycle observation, in the S phase of tumor cells nodal involvement was predominantly seen, indicating that tumor cells themselves increasing proliferative activity.[4)]

In analysing our surgical experiences with early recurrences within 1 year postoperatively, a rise in cellular RNA and DNA contents was commonly seen, in particular high level of cellular RNA volume was mostly suggestive of appearance of early postoperative recurrence.[5)-8)] It is of interest to draw an conclusion that one of the clinical advantages toward clinical use of flowcytometric study is to assess the level of cellular RNA to predict the clinical outcome for lung cancer patients following surgery.

SUMMARY

The measurements of the cellular DNA and RNA contents of lung cancer cells were beneficial in grading malignant potential and predicting an appearance of early cancer recurrence. It is of interest to emphasize that high RNA value is mostly suggestive of occurrence of early cancer recurrence following surgery even in curative operation performed.

REFERENCE

1) DARZYNKIEWICZ, Z., TRAGANOS, E., SHARPLESS, T and MELAMED M. R.: Conformation of RNA in situ as studied by acridine orange and automated cytofluorometry. *Exp. Cell. Res.*, **95**: 143−153, 1975.

2) AMDREFF, M., DARZYNKIEWICZ, Z., SHARPLESS, T., CLARKSON, B and MELAMED M. R.: Discrimination of human leukemia subtypes by flow cytometric analysis of cellular DNA and RNA. *Blood* **55**: 282−293, 1980.

3) BARLOGIE, B., GOHDE, W., JOHNSTON, D.A., SMALLWOOD, U., SCHUMANN, J and DREWINKO B.: Discrimination of ploidy and proliferative characteristics of human solid tumors by pulse cytophotometry. *Cancer Res.*, **38**: 3333−3339, 1978.

4) BUCHNER, T. H., BARLOGIE, B., ASSEBURG, V., HIDDENMAN, W., KAMANABROO, D and GOHDE W.: Accumulation of S phase cells in the bone marrow of patients with acut leukemia by cytosine arabinoside. *Blut.* **28**: 299−300, 1979.

5) CRISSMAN, H. A. and TOBEY, R. A.: Cell cycle analysis in 20 minytes. *Science* **184**: 1297−1298, 1974.

6) BARLOGIE, B., HITTELMAN, W., SPILZER, G., HART, JS., TRUJILLO, J. M., SMALLWOOD, U., J and DREWINKO B.: Correlation of DNA distribution abnormalities with cytogenetic findings in human adult leukemia and lymphoma. *Cancer Res.*, **37**: 4400−4407, 1977.

7) BICHEL, P., FREDERIKSEN, P., KJAER, T., THOMMESEN, P and VINDELOP LL.: Flow microfluorometry and transrectal fine-needle biopsy in the classification of human prostatic carcinoma. *Cancer* **40**: 1206−1211, 1977.

8) TRIBUKAIT, B., ESPOSTI, P and RONSTROM, L.: Tumor ploidy for characterization of prostatic carcinoma: flowcytofluorometric DNA studies using aspiration biopsy material. *Scand. J. Urol. Nephrol. Suppl.* **55**: 59−64, 1980.