In Vitro Intrinsic Radiosensitivity Of Human Squamous Cell Carcinoma in Primary Culture

Eun Kyung Choi, M.D., Kwang Mo Yang, M.D., Byong Yong Yi, Ph. D.
Hyesook Chang, M.D., Sang-Yoon Kim, M.D.*, Joo-Hyun Nam, M.D.*, Eunsil Yu, M.D.†
and Inchul Lee, M.D.†

Department of Therapeutic Radiology, Otolaryngology, Obstetrics and Gynecology*, Pathology†, Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Korea*

= Abstract =

There are a number of reports suggesting that there may be a correlation between the clinical response to radiotherapy in various tumors and the clonogenic survival of cell lines derived from these tumors following exposure to 2 Gy(SF2). Authors conducted this study to determine SF2 for cells in primary culture from surgical specimens.

The tumor tissues with squamous cell carcinoma of uterine cervix and head and neck were obtained. The tumor tissues were disaggregated to single cells by incubating with collagenase type Ⅳ for 2 hours with constant stirring. Single cell suspensions were inoculated in four 24-well plates precoated with cell adhesive matrix. After 24 hours of incubation at 37℃, rows of four wells were then irradiated, consisting of control set and five other sets each receiving doses of 1, 2, 3, 4, and 6 Gy. After incubation for a total of 13 days, the cultures were stained with crystal violet and survival at each dose was determined by quantitative image analysis system. To determine whether cell growth was of epithelial origin, immunocytochemical staining with a mixture of cytokeratin and epithelial monoclonal antibodies were performed on cell cultures.

During the period of this study, we received 5 squamous cell carcinoma specimens of head and neck and 20 of uterine cervical carcinoma. Of these, 15 yielded enough cells for radiosensitivity testing. This resulted an overall success rate of 60%. The mean SF2 value for 15 tumours was 0.55 ± 0.17 ranging from 0.20 to 0.79.

These results indicate that there is a broad range of sensitivities to radiation in same histologic type. So with a large patient population, we plan to determine whether a different SF2 value is associated with tumours that are controlled with radiotherapy than those that are not.

Key Words : Survival at 2Gy, Primary culture, Squamous cell carcinoma, Head and neck cancer, Cervix cancer

This work is supported by a grant from Asan Institute for Life Sciences.

INTRODUCTION

Radiotherapy outcome for head and neck cancers and uterine cervical carcinomas can be correlated with a number of clinical factors such as clinical performance status, primary site, and T and N stages. Identification of biologic factors that are independent of the known clinical prognostic factors might help better predict tumor response to radiotherapy. The rationale for measuring the *in vitro* sensitivity of cells to radiation is based upon the stem cell model for human tumours, which hypothesizes that tumor stem cells are the target of therapy and that the *in vitro* sensitivity of the stem cells reflects their *in vivo* sensitivity. Fertil and Malaise[1] and Deacon[2] et al suggested that intrinsic radiosensitivity of tumor cells measured by the surviving fraction at 2Gy (SF2) could play a critical role in tumor response to radiation treatment. It was interesting that for a given tumor type the range of survival at 2Gy was relatively large, as is the range of tumor radiosensitivities. Preliminary correlations between the SF2 and local control in patients with head and neck cancers treated with surgery and postoperative radiotherapy were disappointing[3]. On the other hand, correlations between SF2 values and local control in cervical carcinomas treated with radiation alone were extremely encouraging[4]. In this study SF2 has been determined for cells obtained from primary specimens of head and neck cancers and cervical carcinomas taken from patients prior to radiotherapy.

MATERIALS AND METHODS

1. Primary Culture and Survival Assay

Biopsy and surgical specimens were obtained from the department of pathology, where a pathologist dissects normal tissue free from tumor, samples were washed in high antibiotic medium (Dulbecco's modified Eagle's medium supplemented with 20 μg/ml each of gentamicin and amphotericin). And then tumor tissue was minced with scalpel blades and digested to single cells by incubating with collagenase type Ⅳ for 2 hours with constant stirring. The single cell suspension was centrifuged and the pellet resuspended in Hank's balanced salt solution. The yield of trypan blue viable cells was determined by a hemocytometer count of trypan blue negative cell. The cell suspension was diluted with attachment media (culture media plus 0.6% methyl cellulose 4000) to 25000 cells/ml, and 24-well plates precoated with cell adhesive matrix were inoculated with a cell inoculum titration consisting of 25000, 12500, 6250, and 3125 cells per well in the first column of four wells and 25000 cells in each of the 20 remaining wells of each plate. After 24 hours of incubation, the attachment medium was aspirated, and the cultures were washed with 1ml of phosphate buffered saline and fed 1ml of growth medium. A 6cm lead shield was made that permitted a 4 MV X-ray source to irradiate a single column of four wells. Rows of four wells were then irradiated, consisting of a control set and five other sets each receiving doses of 1, 2, 3, 4, and 6Gy. After incubation for a total of 13days with a medium change after 6 days, all plates were rinsed with phosphate buffered saline, fixed in 70 percent ethanol, air dried, and then stained with 0.5 % crystal violet in 20% ethanol. The integrated optical density of the stained cells was measured over the entire surface of each culture well with an image analysis system. The ratio of optical densities of irradiated and control cultures was fitted to the linear-quadratic model, and survival at 2Gy was calculated from the parameters.

2. Immunohistochemistry

To determine whether cell growth was of epithelial origin, immunocytochemical staining with a mixture of cytokeratin and epithelial monoclonal antibodies was performed on cell cultures. Two-week primary cultures, grown on coverslips were stained with the antibody cocktail.

3. Comparison of survival obtained by clonogenic assay and image analysis system

Using fibrosarcoma cell lines(FSa II) we obtained survival curve by both image analysis system and clonogenic assay. We compared SF2 values obtained by both assays.

RESULTS

1. Culture characterization

The culture of squamous cell carcinoma of head and neck and cervix were tested to determine whether cell growth was of epithelial origin. It was common to find heterogeneity of cytokeratin expression within a single culture. This method ruled out 10 cases of significant fibroblast contamination.

2. Survival curves by clonogenic assay and image analysis system

Fig. 1 shows the survival curves of FSa II cell lines obtained by image analysis system and clonogenic assay. The survival fractions by clonogenic assay were 0.61±0.11, 0.37±0.05,

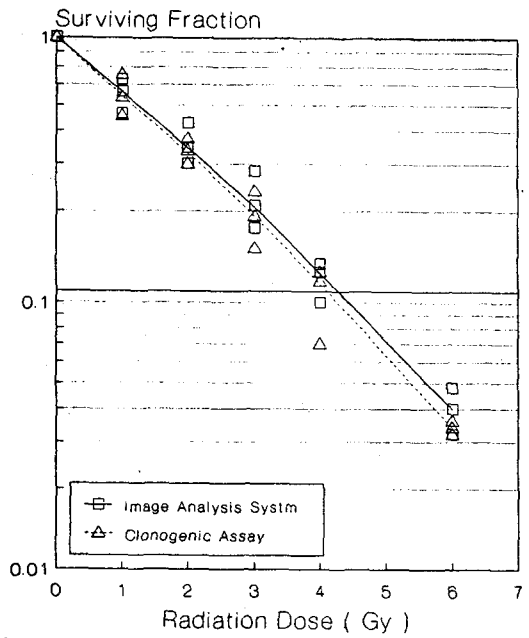

Fig. 1. Cell survival curve of FSa II obtained by clonogenic assay and image analysis system

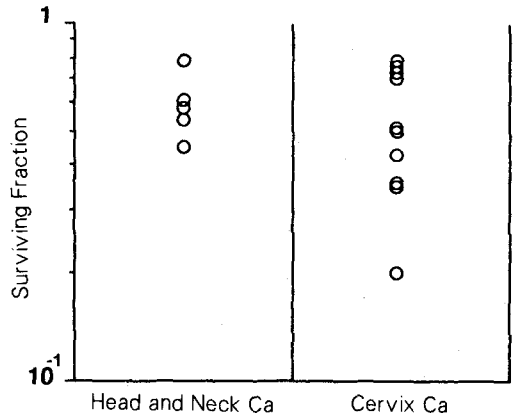

Fig. 2. Surviving fraction at 2Gy for cells obtained from tumor specimens

0.10±0.03, and 0.034±0.002 at 1, 2, 3, 4, and 6Gy of radiation. The end point of this assay correlated well with clonogenic survival as tested so far.

3. Variation in SF2 measurements

Of 5 squamous cell carcinoma specimens with head and neck, 5 yield an evaluable results but of 20 with cervical carcinomas only 10yielded an evaluable results. The surviving fraction at 2Gy (SF2) was determined in 60%(15/25) of the cases when the cell yield was high enough to allow cell cultures to be set up. The mean SF2 was 0.55±0.17 with a range of 0.20 to 0.79. The distribution of the SF2 values was similar in head and neck cancers and cervical carcinomas with a mean value of 0.59 and 0.53 respectively. (Fig. 2)

DISCUSSION

A group of solid human tumor primary cultures was set up from a variety of tumor types for the purpose of measuring survival after increasing doses of radiation. The group of histology types included melanoma, squamous cell carcinoma of the lung and head and neck, ovarian, cervical, and breast, although other types were also represented. The percentage of cultures successfully grew from these tumor cell suspensions on

the cell adhesive matrix continues to be about 80%, as reported by Baker et al[5]. The failure of a culture to grow is usually due to one of the following reasons: (1) too few cells in the initial suspension; (2) contamination of the specimen by microorganisms; (3) the culture does not grow; or (4) the culture overgrows. Our overall success rate of primary culture was 60% and the reason of failure was the fibroblast cells overgrow instead of squamous cell carcinoma cells. In this study success rate of primary culture was higher in head and neck cancers than in uterine cervical carcinomas. Cell growth condition of head and neck cancers might be optimized ideally. The end point of this assay correlated well with clonogenic survival as tested so far, but further studies must be performed to fully characterize exactly what is being measured in this assay. Reported mean SF2 values of other types of tumors were 0.46 for ovarian cancer[6], 0.49 for colon cancer[7], 0.43 for melanoma[8], 0.44 for the squamous cell carcinoma of the head and neck, and 0.47 for cervical cancer [9]. In our study, the mean SF2 value for cervical carcinomas was higher than that obtained by K. West with the Courtenay assay[4]. The mean SF2 value for head and neck cancers in our study was slightly higher than that found by Brock[3]. A possible explanations for these discrepancies might be due to the different cell culture methods and the small specimen numbers of our study.

These results indicate that there is a broad range of sensitivities to radiation in same histologic type. The preliminary evidence reported here suggests that it is possible to establish primary human tumor cell cultures with high efficacy and measure their radiation sensitivity. With a large patient population it should be possible to determine whether a different SF2 value is associated with tumors that are controlled with radiotherapy than those that are not.

REFERENCES

1. **Fertil B, Malaise EP**: Inherent cellular radiosensitivity as a basic concept for human tumor radiotherapy. Int J Radiat Oncol Biol Phys 7:621–629, 1981
2. **Deacon J, Peckham MJ, Steel GG**: The radioresponsiveness of human tumors and the initial slope of the cell survival curve. Radiother Oncol: 317–323, 1984
3. **Brock WA, Baker FL, Wike JL, et al**: Cellular radiosensitivity of primary head and neck squamous cell carcinomas and local tumor control. Int J Radiat Oncol Biol Phys 18:1283–1286, 1990
4. **West CML, Davidson SE, Hunter RD**: Evaluation of surviving fraction at 2Gy as a potential prognostic factor for the radiotherapy of carcinoma of the cervix. Int J Radiat Biol 56:761–765, 1989
5. **Weichselbaum RR, Epstein AJ, Little AJ, et al**: Inherent cellular radiosensitivity of human tumors of varying clinical curability. Am J Roentgenol 123: 1027–1032
6. **Weichselbaum RR, Rotmensch J, Ahmed SS, et al**: Radiobiological characterization of 53 human tumor cell lines. Int J Radiat Biol 56:553–560, 1989
7. **Leith J, Faulkner L, Papa G, et al**: In vitro radiation survival parameters of human colon tumor cells. Int J Radiat Oncol Biol Phys 20:203–206, 1991
8. **Rofstad E**: Radiation biology of malignant melanoma. A review article. Acta Radiol 25:1–10, 1986
9. **Davidson S, West C, Roberts S, et al**: Radiosensitivity testing of primary cervical carcinoma: evaluation of intra-and intertumour hetero-geneity. Radiother Oncol 18:349–356, 1990

국문초록=

인체 상피 세포암의 일차 배양을 이용한 방사선 민감도 측정

울산대학교 의과대학 서울중앙병원 치료방사선과학교실 이비인후과학교실* 산부인과학교실** 병리학교실+

최은경 • 양광모 • 이병용 • 장혜숙 • 김상윤* • 남주현** • 유은실+ • 이인철+

종양의 조직학적 형태에 따라 또 같은 조직의 종양에서도 각 환자에 따라 방사선 치료에 대한 반응 정도에는 많은 차이가 관찰된다. 이러한 방사선 감수성을 예측하는 한 방법으로 각 환자에서 떼어낸 종양 조직을 일차 배양하여 방사선 조사에 의한 세포 생존 곡선을 구한뒤 2Gy에서의 생존(SF2)을 얻었다.

방사선 치료가 계획된 두경부 종양과 자궁경부암 환자의 종양 조직을 얻어 기계적인 방법으로 미세절편으로 만든 후 collagenase type Ⅳ와 2시간 배양하여 단일 종양세포 혼탁액을 얻었다. Cell adhesive matix로 전처리된 24 well plate에 각 well당 일정수의 세포를 넣어 24시간 배양한뒤 각 열에 0, 1, 2, 3, 4, 6Gy의 방사선을 조사하였다. 13일간 배양후 crystal violet으로 염색한뒤 image analysis system을 이용하여 각 well의 광학밀도를 측정하여 세포 생존을 구한다. Linear quadratic model에 의한 생존 곡선을 얻은 뒤 2Gy에서의 생존율을 구하였다. 배양된 세포가 편평상피암세포임을 확인하기 위하여 cytokeratin과 epithelial monoclonal 항체를 이용한 immunocytochemical 염색을 하여 형광 현미경으로 관찰하였다.

5명의 두경부종양 환자와 20명의 자궁경부암 환자의 종양조직을 얻어 실험하여 15명(60%) 종양의 2Gy 생존을 얻는데 성공하였다. 10명의 일차 배양 실패의 원인은 단일 종양세포 혼탁액에 종양세포가 너무 적었거나 세포 이식후 배양이 잘 자라지 않은 것으로 판정되었다. 15편평 상피암 세포의 SF2의 평균은 0.55±0.17이었으며 범위는 0.20에서 0.79까지로 같은 편평상피암이라도 각 환자에 따라 SF2 값에 큰 차이를 보이는 것을 알 수 있었다.

이상에서 같은 부위에 생긴 같은 조직 유형의 종양이라도 각 환자마다 SF2 값의 차이가 큰 것으로 보아 방사선 치료의 효과를 예측할 수 있는 한 인자로 SF2 값을 이용할 수 있을 것으로 생각된다.