Hirosaki Med. J. 51 (Suppl.) : S203—S208, 1999

CLINICAL AND FUNDAMENTAL RESEARCHES OF RADIATION THERAPY

Yoshinao Abe, Yasushi Mariya and Masahiko Aoki

Abstract In this paper, present status and prospects of therapeutic radiology in Department of Radiology are reviewed. The clinical outcomes of head and neck cancers are summarized and radiotherapeutic advantages of other cancers are described. Since three dimensional conformal radiotherapy, that will be introduced in our hospital in the near future, will realize the superior dose distribution clinically, we will be able to prescribe sufficient dose to the tumor target volume with less dose to the surrounding normal tissue. We expect more effective outcomes of radiation therapy from this methods. Biologically, predictive assays that optimize cancer treatment are important and proliferative capacity, survival assay of the cancer cells, capacity of the repair and tumor oxygen status are being studied. Accelerated proliferation during fractions that is observed clinically is one of the factors of radioresistance. Some strategies to overcome accelerated proliferation are postulated ; accelerated fractionation, chemotherapy during radiation therapy and heavy ion therapy. We are also studying accelerated proliferation clinically and experimentally. These works will improve the cancer treatment more efficiently in the future.

Hirosaki Med. J. 51, **Supplement** : S203—S208, 1999

Key words : radiation therapy ; three dimensional conformal radiotherapy ; predictive assay ; accelerated proliferation ; accelerated fractionation.

I. Introduction

Department of Radiology is dedicated to two major radiological fields ; therapeutic and diagnostic radiology. Diagnostic radiology has engaged in multimodal imaging diagnosis and intervention. Therapeutic radiology has focused on cancer treatment. In this paper, recent works in therapeutic radiology are reviewed.

II. Clinical Aspects of Radiation Therapy

In the field of radiation therapy, many clinical works were presented recently and are posted on a home page of our department (http://hippo.med.hirosaki-u.ac.jp/~radio/therapy/). One of the most frequent diseases treated mainly by radiation therapy is the head and neck cancers. Clinical outcome of head and neck cancers are summarized in Table 1[1—5], which are compatible with the results from other hospitals[6,7]. Recently, we are conducting several therapeutic approaches to the head and neck cancers such as accelerated fractionation and combined treatment with chemotherapy. Especially, the former is one of the promising methods

Table 1 Clinical Outcome of the Head and Neck Cancer treated in University Hospital and Affilated Hospitals*

Location of Cancer	TNM or Stage	Number of Patients	Five Year Survival	Five Year Cause Specific Survival	Year of Publications (References Number)
Mesopharyngeal	All Stage	48	55		1994 (1)
	Stage II	7	100		
	Stage III	20	63		
	Stage IV	19	37		
Hypopharyngeal	All Stage	36	31		1992 (4)
Laryngeal	T1, T2	54	83	95	1998 (7)
	T3N0-1	23	55	76	in press
Tongue	T1, T2	47		93	1997 (2)
Maxillary Sinus	All Stage	50		49	1998 (3)

* Aomori Prefectual Hospital ; S. Watanabe, M.D.(chief Radiologist) and Aomori Rosai Hospital ; K. Kattou, M.D. (chief Radiologist)

Department of Radiology, Hirosaki University School of Medicine, Hirosaki 036-8562, Japan
Correspondence : Y. Abe
Received for publication, June 14, 1999
Accepted for publication, September 9, 1999

弘前大学医学部放射線医学講座
別刷請求先：阿部由直
平成11年6月14日受付
平成11年9月9日受理

with biological fundamentals—'do not miss the chance for cure by incidental prolongation of overall treatment time'—and the results will be reported soon. An emphasis should be placed on not only the successful clinical results of head and neck cancers but the more precise analysis of clinical results endorsed by biological assays from the biopsy specimens such as labeling index, flow cytometry data, apoptosis and ploidy. Such biological approaches are our distinctive feature and will help meaningful interpretations to the clinical outcomes.

The treatment results of other cancers are being accumulated. We note here some of the cancers that are characterized by the need of radiation therapy. Recently, breast conservative therapy is widely accepted[8] and demands for radiation therapy are increasing because radiation therapy is the must. In this treatment, we care about not only local control but the cosmesis after treatment. We carefully select the patients dedicated to the boost therapy.

In US and Europe, prostate cancers are treated frequently by radiation therapy[9] but the demands in Japan are not so high. However, epidemically, increasing in number of prostate cancer are reported and will lead to the demands of radiotherapy soon. For this purpose, we are now ready for new radiotherapy technique so called three dimensional conformal radiotherapy (3D-CRT) described below.

Although the numbers of lung cancer patients are increasing for past 20 years, the treatment results are unchanged. Clinical results except early stage have been disastrous. However, combined therapy of chemotherapeutic agents and radiation to the advanced lung cancer is now attempted and the results are promising[10, 11]. Also, small lung cancers that are medically inoperable, such as emphysema, cardiovascular or cerebrovascular diseases, will be also treated using 3D-CRT. Usually these patients are treated by surgery alone but lung cancer having multiple foci, not rare, and concomitant emphysema are problems for surgery.

Intraoperative radiation therapy (IORT)[12] that is mostly dedicated to treat pancreas cancer is now ready to start in our hospital with the collaborations of 2nd Department of Surgery and Department of Anesthesia. With this technique, intractable tumors especially located deeply in the abdomen are expected to be treated.

Evolutionary radiation therapy technique, 3D-CRT[13], are developed and the high voltage machine compatible with 3D-CRT will be introduced in our hospital soon and is highly applicable to the routine clinical use. 3D-CRT is a computer assisted radiation therapy characterized by unique beam distribution consists of multiple small beams from each beam angle, finally reaching 5-7 or more beam angles with multileaf collimator. Many algorithms and methods for beam deliverying systems have been developed and attempted. 3D-CRT has an advantage for focusing dose to the complicated target volume with possibly lowering dose to the surrounding normal tissues. Thus, we will be able to deliver dose to the patients expecting better tumor control and less occurrence of unfavorable complications.

Stereotactic radiation therapy[14] or radiosurgery[15] is a kind of 3D-CRT, only limited to the brain lesions. The problems of these methods such as how to deliver beams, immobilize the patients and prescribe the fraction size and the total dose were discussed. Also the results of brain lesions were reported. Especially the former methods allow us to deliver beams more efficiently to the tumor volume itself using routinely equipped linear accelerator with minor modification.

III. Predictive Assays

III-1. Clinical Results and Predictive Assays

Predictive assays consist of many biological factors of cancer such as repair, repopulation, reoxygenation, survival fractions and the nature of the tumor itself. We dealt with clinical specimen for measuring ploidy that was compared with the clinical outcome.

Ploidy, a kind of tumor's nature, is also related to the prognosis of the cancer[16, 17]. Flow cytometric analysis of DNA ploidy was performed on paraffin embedded biopsy specimen from 129 patients of the head and neck cancer. Aneuploid cancer (64/129 cases) had higher incidence of lymph node metastasis, more advanced clinical stages and more undifferentiated histology of squamous cell carcinoma than did diploid cancer (65/129 cases). Ploidy did not correlate with T-factor and radiation response. Overall survival rate was 31 and 58% with aneuploid and diploid tumors, respectively, and the difference was significant ($p<0.05$). Moreover, even after achieving complete response, the recurrent rate of aneuploid cancer is much higher

than that of diploid one. These results suggest that aneuploid cancer has a potential to wide-spread extension of tumor cells suggesting the possible need of chemotherapy to improve the prognosis[18].

III-2. Fundamental Predictive Assays

For evaluation of intrinsic radiosensitivity, micronucleus assay[19] has been performed. The principle of micronucleus assay is as follows ; one cell cycle with damaged cell induces a micronucleus at the time of mitosis and micronucleus can be easily identified by applying cytochalasin B that does not inhibit the division of nucleus but the division of cytoplasm. Thus the number of micronucleus induced by irradiation is closely related to the cell damage. Using three experimental tumor cell lines from the same origin, clear positive correlation has been obtained between classical colony assays and number of micronucleus formation after irradiation[20]. This study validated the possible use of the micronucleus assay to substitute for classical colony assay. Assays for clinical specimens such as brain tumors have been undertaken and the results have not yet been conclusive.

We have attempted to measure labeling index (LI) of bromo- or iodo-deoxyuridine (Brd- or IdUrd) and potential doubling time (Tpot). Once BrdUrd was available commercially for cancers as a radiosensitizer but can not be used today. Although, in US and European countries, it is permitted to administer these agents intravenously, we could not do it. We attempted in vitro labeling[21] to solve this problem. There still had the problem to apply it for clinical use because of not-easy-handling methods. For animal study, it is easy to administer these agents and to obtain LI and Tpot[22].

Recently we introduce new methods called 'comet assay'[23] and 'near-infra-red light assay' to assess repair capacity and oxygenation status of the cancer[24], respectively. These methods will help us to analyze the clinical results more precisely. Such fundamental researches will lead to much more successful clinical results of the cancer.

IV. Accelerated Proliferation during Multiple Fractions

IV-1. Clinical and experimental proofs

Accelerated proliferation, which is thought to be due to increased number of clonogenic cells during fractionated irradiations, was assessed by either clinical results overall treatment time or animal studies—jejunal colony assay during multiple fractions. This idea was postulated by Withers *et al.* in 1988 analysing a lot of world-wide clinical data obtained from head and neck cancers regarding the total dose to give 50% survival against overall treatment time[25]. They found a bend at around 3-4 weeks before which 50 Gy is enough to control 50% of the tumor and after which increment of 0.6 Gy-a-day is necessary to compensate total dose against prolongation of the overall treatment time. This phenomenon is accounted for accelerated proliferation.

Clinical results of the head and neck cancers showed the worse prognosis is related to the prolonged overall treatment. In our analysis, the main causes of the prolongation are treatment pauses due to severe acute toxicities, national holidays, breakdown of the external beam machines and planning delay between initial therapy and boost therapy[4, 5].

The observation of acute reaction—mucositis—of laryngeal cancer caused by accelerated fractionation—shortening of treatment time employing twice-a-day irradiations—compared with conventional fractionation showed rapid aggravation and rapid resolution in the group of accelerated fractionation[26]. This is the another example of accelerated proliferation found in normal acutely responding tissue.

Experimental studies were performed mostly in jejunal basis because jejunum has the most rapidly cell kinetics in the mammals. Rapid and massive cell production occurs at the jejunal crypt. A newly produced cells are experienced several divisions in the jejunal crypt, then they are moved to the villi, carried to the top gradually and finally fallen out. The numbers of cells in the jejunum are stable because of balance between production and falling out—self-renewal system. Jejunal crypt is good model for proliferation of acutely responding tissues and tumor cells[27].

Experimental studies regarding repopulation during multiple fractions disclosed some novel knowledge in the jejunum that consist of clonogenic number, potential doubling time and cell loss (in preparation). Briefly, in jejunum, immediately after irradiations of 2 Gy once or twice a day induces proliferation. Comparing once a day treatment, twice a day treatment showed more rapid growing

tendency. Tpots obtained from both treatments are shortened rapidly and the extents of both Tpots are the same. The cell loss factor has declined in both cases and showed more declining in twice a day case. It is suggested that the more damage, as far as within the permitted range of self renewal, induce the more accelerated proliferation. Shortening of Tpot and reducing cell loss factor is the possible mechanism of accelerated proliferation during multiple fractions. The mechanism of accelerated proliferation during fractionation is critical for the cancer cure.

IV-2. To find the way to overcome

To overcome accelerated proliferation, our clinical and fundamental approaches are followings : accelerated fractionation, concurrent chemotherapy at the mid-way of radiation therapy and high linear energy transfer (LET) radiation therapy[28] such as heavy ion beams (HI-RT). As described previously, there are some reasons for prolongation of treatment. To overcome such inevitable prolongation, we adopted accelerated fractionation for the treatment of laryngeal cancer and the results revealed excellent outcome[5]. Second, to treat stage III medically inoperable non-small cell lung cancer, we applied concomitant chemotherapy at the mid-way of radiation therapy —it is the time to start accelerated proliferation. The results were compatible with another phase II studies and adverse reactions were tolerable[29]. This approach to applying chemotherapy is promising strategy. Third, to confirm possible inhibition of accelerated proliferation using high LET treatment, we performed experimental researches regarding the effect of multiple fractions of carbon ion beams on proliferative response to the mouse jejunum[30]. The final goal of these experimental works is to clarify the rationale for the use of carbon ion beam to treat the cancer efficiently.

V. Prospects

The goal of the physics and biology of radiation therapy is to give cell kill effect not only totally on the tumor but less on tumor surrounding normal tissues. The physics will help us by the new technology of 3D-CRT and HI-RT ; spatial dose distribution. The biology will help us to clarify the nature of the cancer and to determine how to fractionate the beams ; time-dose distribution. Without such effort, we can not obtain satisfactory outcome of cancer patients. The patient, who can save, must be cured and the patient, who can not save so far, must be cared adequately. Continued efforts to treat such patients will be fulfilled in the future.

References

1) Mariya Y, Tarusawa N, Takekawa S, Yodono H, Mori I, Shinkawa H, Watanabe S, *et al*. Results of radiotherapy and a combined radio- and chemotherapy for hypopharyngeal carcinomas. Jpn J Cancer Clin 1992 ; 38 : 1703-9 (Abstract in English).

2) Mariya Y, Takekawa S, Tarusawa N, Watanabe S, Aoki M, Kattou K. Results of radiotherapy for mesopharyngeal carcinoma. J Jpn Soc Ther Radiol Oncol 1994 ; 6 : 181-92 (Abstract in English).

3) Mariya Y, Watanabe S, Kattou K, Tarusawa N, Fukuda E, Aoki M, Anbai A, *et al*. Results of 226Ra brachytherapy for T1, T2 tongue carcinoma and the clinical implication of ploidy and potential doubling time as prognostic factors. J Jpn Soc Ther Radiol Oncol 1997 ; 9 : 15-24 (Abstract in English).

4) Aoki M, Mariya Y, Matsukura H, Abo M, Watanabe S, Anbai A, Kattou K, *et al*. Combined therapy for maxillary sinus carcinoma—The effect of prolonged treatment time on local control. Jpn J Cancer Clin 1998 ; 44 : 1542-7 (Abstract in English).

5) Mariya Y, Watanabe S, Kattou K, Abe Y, Aoki M, Anbai A, Matsukura H, *et al*. The impact of biologically effective dose corrected for overall treatment time upon tumor control of stage I and II laryngeal cancers. J Jpn Soc Ther Radiol Oncol 1998 ; 10 : 215-22.

6) Inoue T, Mitsuhashi N, Chatani M, Kagami Y, Ii N, Himei K, Nagata Y, *et al*. Treatment results of head and neck cancer in Japan. J Jpn Soc Ther Radiol Oncol 1998 ; 10 Suppl. 2 : 4-10 (Japanese).

7) Chao KSC, Perez CA, Brady LW. Radiation Oncology : Management Decisions. Philadelphia : Lippincott-Raven ; 1998. p. 189-273.

8) National Cancer Institute. PDQ Treatment, Breast Cancer. 1999.

9) National Cancer Institute. PDQ Treatment, Prostate Cancer. 1999.

10) Dillman RO, Seagren SL, Propert KJ, Guerra J, Eaton WL, Perry MG, Carey RW, *et al*. A randomized trial of induction chemotherapy plus high-dose radiation versus radiation alone in stage III non-small-cell lung cancer. N Engl J Med 1990 ; 323 : 940-5.

11) Schaake-Koning C, van den Bogaert W, Dalesio O, Festen J, Hoogenhout J, van Houtte P, Kirkpatrick A, *et al*. Effect of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. N Engl J Med 1992 ; 326 : 524-30.

12) Mariya Y, Aoki M, Anbai A, Matsukura H, Abe Y, Kimura T, Matsuda K, *et al*. Response of unresectable

pancreatic cancer to Intraoperative radiotherapy. Radiat Med 1998 ; 16 : 195-200.

13) Smith AR, Purdy JA. editors. Three Dimensional Photon Treatment Planning. Report of the Collaborative Working Group on the Evaluation of Treatment Planning for External Photon Beam Radiotherapy. Int J Radiat Oncol Biol Phys 1991 ; 21 : 1-266.

14) Aoki M, Watanabe S, Mariya Y, Tarusawa N, Fukuda E, Anbai A, Basaki K, *et al*. Results of fractionated stereotactic radiotherapy with linear accelerator. J Jpn Soc Ther Radiol Oncol 1997 ; 9 : 25-35 (Abstract in English).

15) Uchiyama B, Satoh K, Saijo Y, Joukura E, Boku E, Fujimura S, Abe Y, *et al*. Gamma-knife radiosurgery for metastatic brian tumors from primary lung cancer. Nihon Kokyuuki Gakkai Shi 1998 ; 36 : 503-8 (Abstract in English).

16) Kearsley JH, Bryson G, Battistutta D, Collins RJ. Prognostic importance of cellular DNA content in head-and-neck squamous-cell cancers. A comparison of retrospective and prospective series. Int J Cancer 1991 ; 47 : 31-7.

17) Fu KK, Hammond E, Pajak TF, Clery M, Doggett RL, Byhardt RW, McDonald S, *et al*. Flow cytometric quantification of the proliferation-associated nuclear antigen p105 and DNA content in advanced head and neck cancers : results of RTOG 91-08. Int J Radiat Oncol Biol Phys 1994 ; 29 : 661-71.

18) Aoki M, Mariya Y, Abe Y, Fukuda E, Matsukura H, Abo M, Basaki K, *et al*. Flow cytometric DNA ploidy analysis in head and neck cancer. Jpn J Cancer Clin 1998 ; 44 : 195-6 (Abstract in English).

19) Fenech M, Morley A. Solutions to the kinetic problems in the micronucleus assay. Cytobios 1985 ; 43 : 233-46.

20) Mariya Y, Streffer C, Fuhrmann C, Wojcik A. Correlation of radiation-induced micronucleus frequency with clonogenic survival in cells of one diploid and two tetraploid murine tumor cell lines of the same origin. Radiat Res 1997 ; 147 : 29-34.

21) Mariya Y, Aoki M, Tarusawa N, Takekawa S, Kaimori K, Abe Y. Determination of potential doubling time using *in vitro* continuous labeling method with bromodeoxyuridine : Estimation in head and neck squamous cell carcinoma. Nippon Acta Radiologica 1993 ; 53 : 976-8 (Abstract in English).

22) Abe Y, Takahashi J, Fukuda H, Mariya Y. Potential doubling time assayed in mouse jejunum and a mouse fibrosarcoma, FSa-II : double labeling with bromodeoxyuridine and [3H]-thymidine. J Jpn Soc Ther Radiol Oncol 1995 ; 7 : 195-200.

23) Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in indivisual cells. Exp Cell Res 1988 ; 175 : 184-91.

24) Mariya Y, Steinberg F, Streffer C, Fuhrmann C, Abe Y. Oxygenation status and tumor response during fractionated irradiation in two murine tumor cell lines of same origin but different intrinsic radiosensitivities. Radiat Med 1999 ; 17 : 175-9.

25) Withers HR, Taylor JMG, Maciejewski B. The hazard of accelerated tumor clonogen repopulation during radiotherapy. Acta Oncol 1988 ; 27 : 131-46.

26) Mariya Y, Matsutani H, Abe Y, Nakaji S. Enhanced regeneration response of laryngeal and hypopharyngeal mucosa with accelerated hyperfractionated radiation therapy for glottic cancers. Radiat Med 1998 ; 16 : 469-72.

27) Abe Y, Takahashi J, Fukuda H. G2 arrest following fractionated irradiation in the murine jejunal crypt. Int J Radiat Biol 1996 ; 69 : 757-61.

28) Raju MR, Heavy Particle Radiotherapy. New York : Academic Press ; 1980.

29) Abe Y, Takahashi J, Fukuda H, Ono S, Yoshioka S, Akaizawa T, Kubota K, *et al*. A phase II study of cisplatin, oral administration of etoposide, OK-432 and radiation therapy for inoperable stage III non-small cell lung cancer. Int J Clin Oncol 1998 ; 3 : 365-9.

30) Basaki K, Abe Y, Tatsuzaki H, Akaizawa T, Ando S, Ando K. Relative biological effects of carbon ion beams on mouse intestinal crypts. J Jpn Soc Ther Radiol Oncol 1998 ; 10 : 27-33 (Abstract in English).

放射線治療の臨床と基礎

阿　部　由　直　　真里谷　　靖　　青　木　昌　彦

抄録　この論文では，放射線科で行われている放射線治療の現状と将来への展望についてまとめた．これまで報告した頭頸部腫瘍に対する放射線治療の概略を紹介し，他領域での放射線治療について略記した．数年後に導入予定である三次元原体照射法は優れた線量分布を実現するので臨床的に正常組織の障害を減らしつつ癌に十分な線量を与えることが可能となる．結果として放射線治療の適応拡大が図られていくであろう．生物学的に治療の先行指標を抽出し臨床応用を目的とした研究を進めることがもう一つの課題である．増殖能，癌細胞の放射線による生存率，放射線障害からの回復能力および腫瘍酸素濃度について検討している．分割照射により引き起こされる促進された増殖は重要なテーマであり，増殖を阻止する急速照射，照射中の化学療法ならびに重粒子線照射などが治療戦略として挙げられる．これらの努力は将来の治療成績向上のために必須のものである．

弘前医学 51 補冊：S203—S208, 1999

キーワード：放射線治療；三次元原体照射；治療予測；促進された増殖；急速照射．

弘前大学医学部放射線医学講座