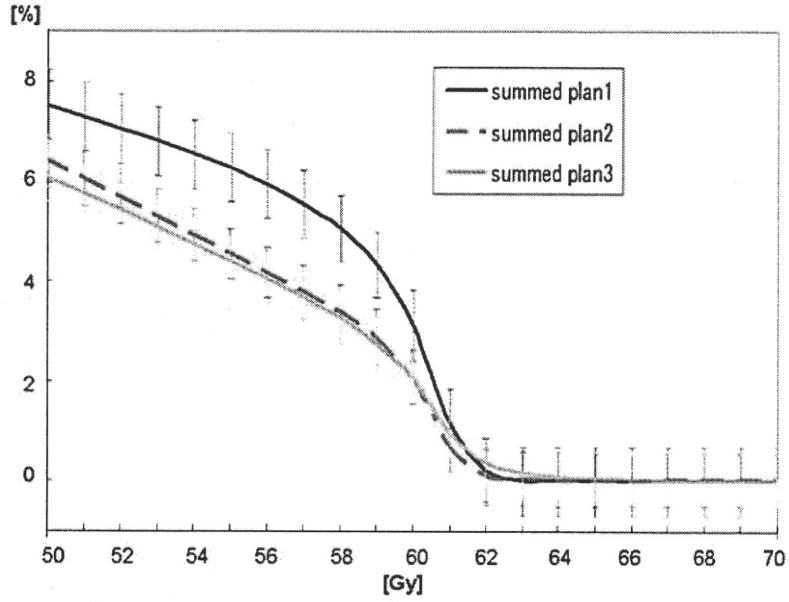

Figure 5 Mean DVH (focused on high dose area (50 to 70 Gy)) of small bowel PRV in each summed plan.

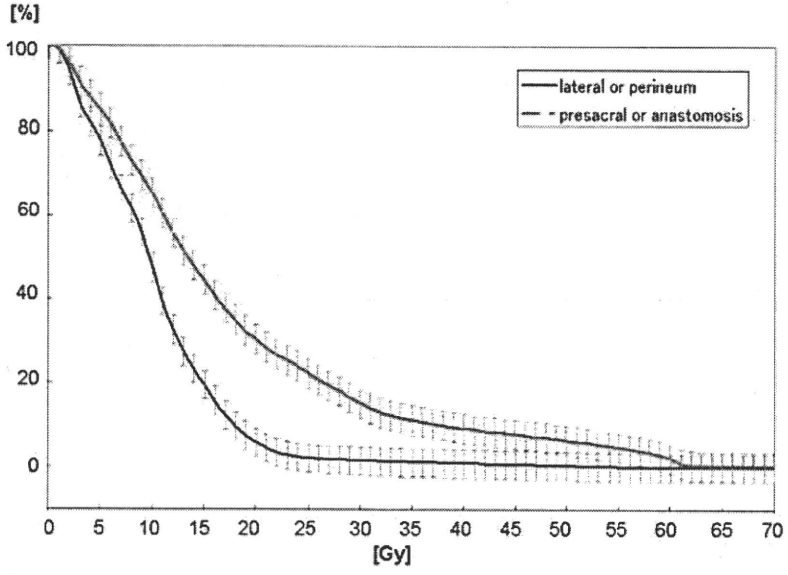

Figure 6 Mean DVH of small bowel PRV of *summed plan 3* in patients with lateral pelvic lymph node metastasis or perineum recurrence and that in patients with presacral or anastomostic recurrence. In patients with lateral pelvic lymph node metastasis or perineum recurrence, the mean DVH of small bowel PRV of *summed plan 3* was significantly lower than that in patients with presacral or anastomostic recurrence.

FDG. There have been many reports on contouring target volume according to 40~50% of maximal SUV value, source-background ratio, and arbitrary SUV value in some malignant tumors [21-25]; however, it remains inconclusive. Bayne et al. pointed out that SUV value had problems with accuracy and reproducibility [26]. In the present study, although we used an arbitrary SUV of 2.0, we consider that it is not a clear border between malignancy and non-malignancy but a region with relatively high malignant potency and with resistance to radiation at 40 Gy including a subclinical margin like CTV margin. Although SUV of 2.5~3.0 was used as a threshold value between malignancy and non-malignancy in many previous studies, we used SUV of 2.0 as

the threshold value for BTV based on the fact that patients in the present study had already been irradiated with 40 Gy and based on the fact that Haberkorn et al. reported the mean SUV of recurrent rectal cancer after radiotherapy with 40 Gy to be 1.8 [27].

Furthermore, in the present study, since normal tissues around the GTV were also irradiated with 40 Gy, the possibility that radiation-induced inflammation masked a residual malignant tumor must also be considered. For other tumors such as head and neck cancer and lymphoma [28,29], chemoradiation-induced inflammatory response causes sufficient numbers of false-positive results limiting PET being performed less than 2 months after chemoradiation. It may be inappropriate to use FDG-PET for radiation planning during radiation therapy. Recently, chemotherapy consisting of 5-FU or Capecitabine with or without the addition of Oxaliplatinum has commonly been performed for recurrent rectal cancer. Also, in the present study, all patients underwent concomitant and/or previous chemotherapy with radiation therapy. Findlay et al. mentioned the so-called flare phenomenon that occurs at 1~2 weeks after the initiation of chemotherapy and that can be observed as a marked increase in FDG metabolism in lesions that show response later [30]. We may also have to investigate the appropriate thresholds of FDG accumulation for BTV for each type of chemotherapy. However, in rectal cancer, many investigators revealed that the positive predictive value of FDG-PET assessment of therapy response during or soon after chemoradiation was very high and was not significantly limited by post-chemoradiation changes [31]. The timing of FDG-PET after chemoradiation for the most accurate assessment of tumor response in rectal cancer is controversial. Further larger prospective surveys of the time courses of tumor FDG uptake during and after chemoradiation in rectal cancer are required.

There are other major problems regarding the use of PET/CT for radiation therapy planning: misalignment of the fusion of PET and CT images due to body movement, bowel peristalsis and difference in volume of urine between the transmission scan and emission scan as well as artifacts due to FDG in urine, so-called "hot urine". These problems can be resolved to a large extent by overnight fasting before PET/CT and by starting the emission scan from the position of the pelvis. Moreover, in the present study, a 5-mm circular margin was attached to each target volume and OAR; however, it might not be sufficient to cover such misalignment. It is necessary to investigate such misalignment using on-line imaging (e.g., cone-beam CT) before clinical application.

In the present study, since V_{30}, V_{40}, V_{60}, D_{mean} and NTCP of small bowel PRV were not increased and V_{50} of small bowel PRV could be reduced due to the differences between GTV2 and BTV, focal dose escalation by 6 Gy to regions with SUV above 2.0 using IMRT with dose-painting boost for postoperative local recurrent rectal cancer is considered to be safe. FDG-PET-guided IMRT has the possibility of improving local control of postoperative local recurrent rectal cancer without increasing the risk of radiation injury of small bowel PRV. However, although NTCP which reflects account all the DVH data was not increased, D_{max} of small bowel PRV in the summed plan using focal dose escalation was significantly higher than that in other summed plans. While the differences in mean D_{max} of small bowel PRV between *summed plan 3* and the other plans were only about 2.5 Gy in the present study, D_{max} of small bowel PRV in *summed plan 3* was more than 65.0 Gy in 4 of the 8 patients with anastomotic or presacral recurrence, and NTCP in *summed plan 3* in 2 of the 4 patients was more than 10%. Since it is known that the small bowel is a "serial organ" and that the dose at which probability of obstruction or perforation is 50% within 5 years after treatment (TD50/5) of the small bowel is 55 Gy [32], although NTCP shows that focal dose escalation is acceptable, dose escalation by only 6 Gy from 60 Gy even using PET-guided IMRT is relatively risky. Therefore, if the region of high FDG accumulation is near the OARs, it might be necessary to reduce the degree of dose escalation and/or reduce the volume to increase irradiation dose (e.g., lesion with SUV > 2.5). Alternatively, using IMRT from the beginning of radiotherapy, using a belly board, and inserting a spacer between the recurrent tumor and OARs may further facilitate dose escalation without increasing the risk of radiation injury. When PTV-PET overlaps PRV, we may have to further modify the irradiation dose setting of the overlapping part.

Rectal cancer is known to have many hypoxic fractions [11]. Some studies have provided evidence that hypoxia has a negative impact on tumor response to radiation and other methods of therapy [33-36]. Although we used FDG for radiotherapy planning in this study to determine the region with high tumor cell density, it may be more important for improving the effect of radiotherapy for rectal cancer to determine the hypoxic regions. There are some tracers for detecting a hypoxic region (e.g., [^{18}F]Fluoromisonidazole-3-fluoro-1-(2'-nitro-1'-imidazolyl)-2-propanol ([^{18}F]FMISO), Cu-diacetyl-bis(N4-methylthiosemicarbazone (Cu-ATSM) and 1-(2-fluoro-1-[hydroxymethyl] ethoxy)methyl-2-nitroimidazole ([^{18}F]FRP170)) [37-39]. Although Lin et al. have already reported the effectiveness in head and neck cancer [40], increasing the irradiation dose with IMRT to the hypoxic region may also be effective for treating postoperative recurrent rectal cancer.

Conclusions

Our findings suggest that FDG-PET/CT-guided IMRT can facilitate focal dose escalation to regions with SUV above 2.0 while providing normal tissue protection in patients with postoperative local recurrent rectal cancer. However, we do not recommend routine clinical use of focal dose escalation using FDG-PET/CT-guided IMRT. In cases in which the region of high FDG accumulation is near the OARs, careful radiotherapy planning is necessary. Based on the results of this planning study, we will start a clinical phase I/II study of focal dose escalation using PET-guided IMRT for patients with postoperative local recurrent rectal cancer in our institution.

Additional file 1: Supplementary tables.

Acknowledgements
This study was partially supported by funding from the Japan Radiological Society.

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Authors' contributions
KJ, ST, YO, KN, HA and SY participated in the design of the study. KJ and YT performed the statistical analysis. KJ, TK, TM and LK conceived of the study and participated in its design and coordination. RU and YO helped to draft the manuscript. HA, KT, KN, KF and MK acquired data. MM and NM verified and calculated DVH and NTCP. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 4 December 2009 Accepted: 7 April 2010
Published: 7 April 2010

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Pre-publication history

The pre-publication history for this paper can be accessed here: http://www.biomedcentral.com/1471-2407/10/127/prepub

doi:10.1186/1471-2407-10-127
Cite this article as: Jingu *et al.*: **Focal dose escalation using FDG-PET-guided intensity-modulated radiation therapy boost for postoperative local recurrent rectal cancer: a planning study with comparison of DVH and NTCP.** *BMC Cancer* 2010 **10**:127.

Int J Clin Oncol (2010) 15:184–190
DOI 10.1007/s10147-010-0044-y

ORIGINAL ARTICLE

^{18}F-fluorodeoxyglucose positron emission tomography immediately after chemoradiotherapy predicts prognosis in patients with locoregional postoperative recurrent esophageal cancer

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Received: 6 August 2009 / Accepted: 16 November 2009 / Published online: 10 March 2010

Abstract
Objectives The objectives of this study were to reveal the utility of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) within 7 days after chemoradiotherapy to predict prognosis in patients with postoperative recurrent esophageal cancer.
Materials and methods Patients scheduled to undergo concurrent chemoradiotherapy for postoperative locoregional recurrence of esophageal cancer were recruited. Selection criteria were: (1) locoregional recurrence, (2) no previous radiation therapy, (3) planning treatment with concurrent chemoradiotherapy, (4) FDG-PET performed <2 weeks before chemoradiotherapy, and (5) no serious diabetes. FDG-PET was performed <7 days after chemoradiotherapy. No more treatment after chemoradiotherapy was given until disease progression was diagnosed according to the Response Evaluation Criteria in Solid Tumors (RECIST). Correlations of FDG-PET findings with cause-specific survival and local control rates were investigated prospectively.
Results Twenty patients were enrolled. Median observation period of patients who survived was 45.0 months. Median maximum standardized uptake value (SUV_{max}) after chemoradiotherapy was 2.4, and median SUV_{max} before chemoradiotherapy was 8.4. Cause-specific survival and local control rates were significantly better for patients with $SUV_{max} \leq 2.4$ after chemoradiotherapy (log-rank test, $P = 0.033$ and 0.010, respectively). SUV_{max} before chemoradiotherapy tended to be correlated only with cause-specific survival rate (log-rank test, $P = 0.076$). Change in metabolic activity of FDG was significantly correlated with local control rate (log-rank test, $P = 0.042$).
Conclusions FDG-PET performed even <7 days after chemoradiotherapy predicts prognosis in patients with postoperative recurrent esophageal cancer.

Keywords FDG-PET · Recurrent esophageal cancer · Prognosis · Radiotherapy · SUV_{max}

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Introduction

The usefulness of positron emission tomography using ^{18}F-fluorodeoxyglucose (FDG-PET) in patients with esophageal cancer, particularly for staging and detecting recurrence, has been reported [1–3]. There are also reports of the usefulness of pre- or posttreatment FDG-PET for predicting prognosis of primary esophageal cancer [3–9]. However, there is no report on the usefulness of FDG-PET for predicting prognosis of postoperative recurrent esophageal cancer. Recently, chemoradiotherapy (CRT) has been shown to improve the prognosis of patients with postoperative recurrent esophageal cancer [10–16]. At our institution, CRT including prospective study is actively performed for postoperative locoregional recurrent esophageal cancer.

CRT generally causes local inflammatory reactions in normal tissue. FDG uptake in inflammatory lesions is a

well-known phenomenon [17]. Increased FDG uptake caused by radiation-induced inflammation may limit the use of FDG-PET for metabolic measurement in esophageal cancer soon after CRT. Therefore, it has been recommended that FDG-PET be performed several weeks or even months after completion of radiotherapy to assess tumor response [18, 19]. However, we often experienced significant reduction or loss of FDG accumulation even <1 month after irradiation in patients with squamous cell carcinoma. In this study, we prospectively investigated the potential of FDG-PET performed <7 days after CRT completion to predict prognosis and local control of postoperative recurrent esophageal cancer.

Materials and methods

We recruited patients scheduled for CRT in our institution for locoregional recurrence (including para-aortic lymph node metastasis) of esophageal cancer without distant metastasis after no residual tumor (R0) resection that involved extended radical esophagectomy with two- or three-field lymph-node dissection. Although squamous cell carcinoma was histologically proven in all patients before the operation, recurrences were diagnosed comprehensively by upper gastrointestinal endoscopy, ultrasonography, computed tomography (CT), FDG-PET, physical findings, and/or cytology.

Study design

Patient selection criteria were: (1) locoregional recurrence (including para-aortic lymph node metastasis) without distant metastasis after no residual tumor (R0) resection and extended radical esophagectomy with two- or three-field lymph node dissection, (2) no previous radiation therapy, (3) planning for concurrent CRT, (4) FDG-PET performed <2 weeks before CRT, and (5) no serious diabetes. Patients underwent FDG-PET <7 days after CRT completion. They had no more treatment after CRT until progressive disease (PD) was diagnosed according to the Response Evaluation Criteria in Solid Tumors (RECIST). There were no particular rules for treating patients with recurring relapse.

Follow-up

Follow-up evaluations were performed every 1–3 months for the first 2 years and every 6 or 12 months thereafter by endoscopy and enhanced CT. Cause-specific survival and local control rates were calculated from the first day of radiotherapy. We defined PD according to RECIST as failure (recurring relapse) and PD in the irradiated area as local failure.

Endpoints

The primary endpoint of the study was to reveal correlations between maximum standardized uptake value (SUV_{max}) in FDG-PET after CRT and cause-specific survival rate or local control rate. The secondary endpoints were to reveal correlations between SUV_{max} before CRT or changes in metabolic activity of FDG and cause-specific survival rate or local control rate.

Analysis

When patients were divided into two groups, each median value was used as a threshold value. Survival estimates were calculated using the Kaplan–Meier method, and differences were evaluated by the log-rank test. A paired sample *t* test or Kruskal–Wallis test was then performed to calculate significance of differences. Statistical significance was defined as $P < 0.05$. SPSS software for Windows version 11.0 was used for all calculations.

Ethics

Written informed consent was obtained from all patients, and the study was performed according to the principles of the Declaration of Helsinki and approved by the local ethics committee.

FDG-PET

PET scans were performed 1 h after FDG administration at 3.1 MBq/kg with either a Biograph PET/CT scanner or an ECAT EXACT HR^+ PET scanner (Siemens, Hoffman Estates, IL, USA) after >4 h of fasting. PET was performed in each patient using the same scanner. A transmission scan was performed for attenuation correction before emission scans (using a CT scan with the Biograph PET/CT scanner or ^{68}Ge rod sources with the ECAT EXACT HR^+ PET scanner). Seven bed positions were used for emission scans, with an acquisition time of 2 min per position. All scans were reconstructed using an attenuation-weighted, ordered subset expectation maximization algorithm (OZEM). For semiquantitative analysis of increased FDG uptake lesions, SUV_{max} based on body weight (g) was calculated and converted into a value based on lean body mass:

$$SUV = \text{tissue activity concentration (Bq/ml)} / [\text{administered activity (Bq)}/\text{weight (g)}].$$

In cases with multiple recurrence sites, the highest SUV_{max} of all lesions was used for analysis. The percentage changes in FDG metabolic activity ($SUV_{\Delta\%}$) between SUV_{max}

before CRT (pre-SUV) and SUV_{max} after CRT (post-SUV) were calculated using the following formula: $SUV_{\Delta\%}$ = [(pre-SUV − post-SUV)/pre-SUV] × 100 (%).

Treatment

All patients underwent the following concurrent CRT without interruption.

Radiotherapy

A linear accelerator (4 or 10 MV) was used as the X-ray source. The target volume was localized for radiotherapy in all patients in CT planning. The daily fractional radiotherapy dose was 2.0 Gy, administered 5 days a week, and the total dose was 60.0 Gy. For patients who had lymph node metastasis in some regions or metastasis of many lymph nodes in one region, a T-shaped field (including the bilateral supraclavicular, mediastinal, and abdominal regions) was used. For the other patients, local fields with a margin of 1–2 cm from the macroscopic tumor were used. After a total dose of 40.0 Gy, the field was changed for all patients to avoid the spinal cord, and only macroscopic lesions were irradiated, with a margin of 1–1.5 cm.

Chemotherapy

A platinum-based combination regimen, at the very least, was used concurrently with radiotherapy for all patients. Most patients underwent chemotherapy consisting of two cycles of nedaplatin (70 mg/m^2/2 h) and 5-fluorouracil (5-FU) (500 mg/m^2/24 h for 5 days) according to our institutional protocol.

Results

From January 2002 to December 2007, we enrolled 20 patients. Patient characteristics are shown in Table 1. The last observation date was 30 September 2009. At that date, 14 of the 20 patients had relapsed: 9 had recurrence inside the irradiation field, 2 had lymph node metastasis out of the irradiation field, and 5 had distant organ metastasis (2 patients with both lymph node and distant organ metastasis). Fourteen of the 20 patients died: 11 patients died due to progression of disease, 2 due to intercurrent diseases, and one due to an iatrogenic cause. No patient was lost to follow-up. The median observation period was 23.8 (range 4.0–75.0) months for all patients and 45.0 (range 24.0–75.0) months for patients who survived. CRT was completed in all patients, and FDG-PET was performed <7 days after CRT. Blood glucose levels were <150 mg/dl in all patients before FDG administration.

Table 1 Patient characteristics

Characteristics	Number
Gender	
Male	16
Female	4
Age (years)	
Median (range)	63 (54–77)
ECOG PS	
Median (range)	1 (0–1)
Site of recurrence (number of patients)	
Anastomosis site	2
Supraclavicular lymph node	4
Mediastinal lymph node	15
Abdominal lymph node	5
Baseline sum longest diameter (cm)	
Median (range)	4.5 (2.0–11.5)
SUV_{max} before CRT	
Median (range)	8.4 (3.0–20.0)
SUV max after CRT	
Median (range)	2.4 (1.2–5.2)
Chemotherapy (number of patients)	
CDGP + 5-FU	16
CDGP + DOC	3
CDDP + 5-FU + DOC	1
Tumor response (RECIST) (number of patients)	
Complete regression (CR)	7
Partial regression (PR)	10
Stable disease (SD)	3
Progressive disease (PD)	0

ECOG PS Eastern Cooperative Oncology Group Performance Status, *CRT* chemoradiotherapy, *CDGP* nedaplatin, *5-FU* 5-fluorouracil, *DOC* docetaxel, *CDDP* cisplatin, *RECIST* Response Evaluation Criteria in Solid Tumors

Median SUV_{max} in the 20 patients after CRT was 2.4 (range 1.2–5.2), and median SUV_{max} before CRT was 8.4 (range 3.0–20.0). CRT significantly decreased SUV_{max} of in all 20 patients (paired sample t test, $P < 0.001$). The best overall response rate, including complete responses (CR) in 7 patients and partial responses (PR) in 10 was 85.0%. The 1-year and 3-year cause-specific survival rates in the 20 patients were 80.0% [95% confidence interval (CI), 62.5–97.5%] and 48.0% (95%CI 25.6–70.4%), respectively, with a median cause-specific survival period of 24.0 months (95% CI 3.0–45.0). The 1-year and 3-year local control rates in the 20 patients were 69.1% (95% CI 48.4–89.7%) and 51.8% (95% CI 28.9–74.7%), respectively. The median local control period could not be calculated. There was a significant difference between cause-specific survival rates in patients with SUV_{max} >2.4 (n = 10) and patients with SUV_{max} ≤2.4 (n = 10) after CRT (3 years, 20% vs.

77.8%; $P = 0.033$, Fig. 1), and there was also a significant difference between local control rates in patients with SUV_{max} >2.4 ($n = 10$) and patients with SUV_{max} ≤2.4 ($n = 10$) after CRT (3 years, 23.3% vs. 78.8%; $P = 0.01$, Fig. 2). Furthermore, there tended to be a significant difference between cause-specific survival rates in patients with SUV_{max} <8.4 ($n = 10$) and patients with SUV_{max} ≥8.4 ($n = 10$) before CRT (3 years, 67.5% vs. 30.0%; $P = 0.076$, Fig. 3), but there was no significant difference between local control rates in patients with SUV_{max} <8.4 ($n = 10$) and patients with SUV_{max} ≥8.4 ($n = 10$) before CRT (3 years, 46.7% vs. 58.3%; $P = 0.98$).

The median $SUV_{\Delta\%}$ was 68.5% (range 40.2–92.9%). The local control rate in patients with decreases of >68.5% ($n = 10$) was significantly higher than in patients with decreases ≤68.5% ($n = 10$) (3 years, 77.1% vs. 30.0%; $P = 0.042$, Fig. 4). However, there was no significant difference in cause-specific survival rate (3 years, 57.1% vs. 40.0%; $P = 0.89$).

Table 2 shows median (range) SUV_{max} before and after CRT and $SUV_{\Delta\%}$ in patients who showed tumor response. There were no differences among patients who showed tumor response.

Other clinical prognostic factors [Eastern Cooperative Oncology Gropup Performance Status (ECOG PS) and age], as reported previously [16], and baseline sum longest diameter were not correlated with cause-specific survival, local control, or overall survival. Results of univariate

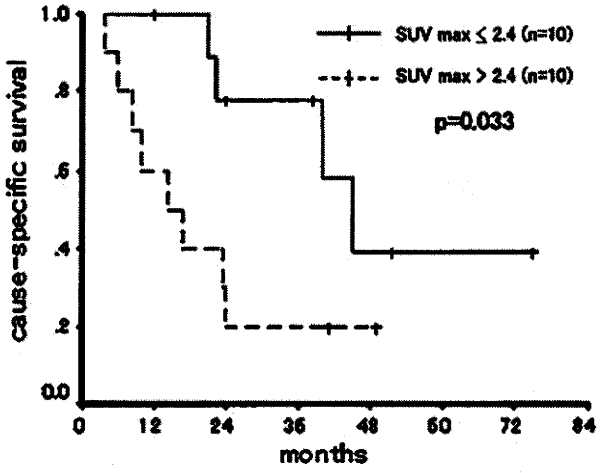

Fig. 1 Cause-specific survival curve for patients with low SUV_{max} after chemoradiotherapy and that for patients with high SUV_{max} after chemoradiotherapy

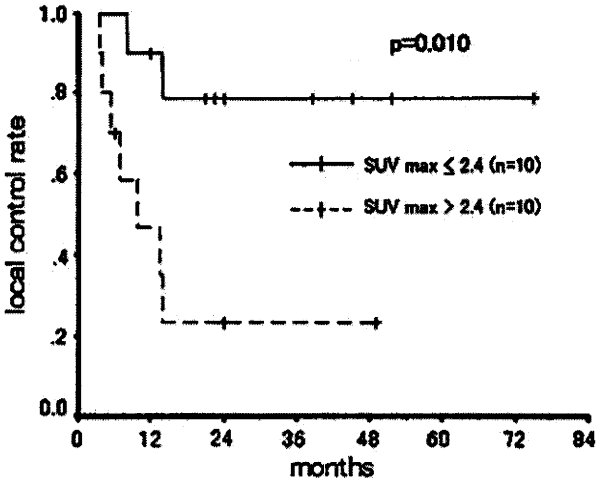

Fig. 2 Local control survival curve for patients with low SUV_{max} after chemoradiotherapy and that for patients with high SUV_{max} after chemoradiotherapy

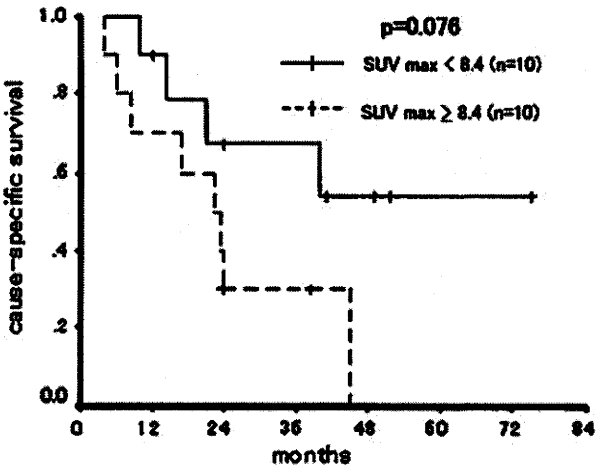

Fig. 3 Cause-specific survival curve for patients with low SUV_{max} before chemoradiotherapy and that for patients with high SUV_{max} before chemoradiotherapy

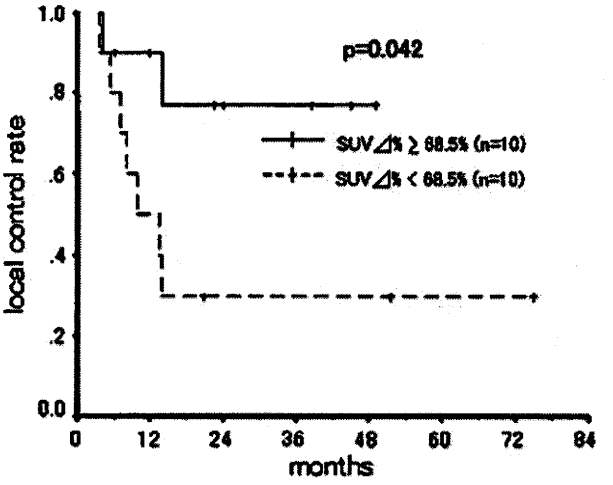

Fig. 4 Local control survival curve for responder patients and that for non-responder patients

Table 2 Median (range) maximum standardized uptake value (SUV_{max}) before and after chemoradiotherapy (CRT) and $SUV_{\Delta\%}$ of each tumor response

	Tumor response (RECIST)			Kruskal–Wallis test
	CR ($n = 7$)	PR ($n = 10$)	SD ($n = 3$)	*P* value
SUV_{max} before CRT	9.5 (3.0–20.0)	8.25 (3.8–16.1)	8.7 (8.3–10.0)	0.62
SUV_{max} after CRT	1.2 (1.2–3.5)	2.6 (1.5–4.0)	3.2 (1.9–5.2)	0.13
$SUV_{\Delta\%}$	84.8 (42.5–92.9)	65.75 (42.1–82.0)	68.0 (40.2–36.9)	0.34

RECIST Response Evaluation Criteria in Solid Tumors, *CR* complete regression, *PR* partial regression, $SUV_{\Delta\%}$ percentage changes in ^{18}F-fluorodeoxyglucose positron emission tomography (FDG) metabolic activity

Table 3 Univariate analysis of predictive factors for cause-specific survival and local control

	Cause-specific survival (months)			Local control (months)		
Predictive factor	Median	95% CI	Log-rank test	Median	95% CI	Log-rank test
FDG-PET after CRT						
$SUV_{max} \leq 2.4$	45.0	34.6–55.4	0.033	NA	NA	0.010
$SUV_{max} > 2.4$	14.5	3.7–25.3		10.0	0.9–19.1	
FDG-PET before CRT						
$SUV_{max} \leq 8.4$	NA	NA	0.076	14.0	NA	0.98
$SUV_{max} > 8.4$	22.5	12.4–32.6		NA	NA	
$SUV_{\Delta\%}$						
<68.5%	21.0	10.9–31.1	0.89	10.0	1.5–18.5	0.042
≥68.5%	40	8.7–71.3		NA	NA	
Age						
<63	40.0	0–82.7	0.76	NA	NA	0.97
≥63	24.0	19.5–28.5		NA	NA	
ECOG PS						
0	45.0	0.9–89.1	0.40	NA	NA	0.22
1	24.0	12.5–35.5		14.0	8.2–19.8	
Baseline sum longest diameter						
<4.5 cm	40.0	8.3–71.7	0.66	NA	NA	0.60
≥4.5 cm	23.5	13.9–33.1		13.5	NA	

ECOG PS Eastern Cooperative Oncology Group Performance Status, *CRT* chemoradiotherapy, $SUV_{\Delta\%}$ percentage changes in ^{18}F-fluorodeoxyglucose positron emission tomography (FDG) metabolic activity, *CI* confidence interval, *NA* not available

analysis of the above predictive factors for cause-specific survival and local control are summarized in Table 3.

The 1- and 3-year overall survival rates in the 20 patients were 75.0% (95% CI 56.0–94.0%) and 40.0% (95% CI 18.5–61.5%), respectively, with a median overall survival period of 23.5 months (95% CI 21.3–25.7). SUV_{max} before and after CRT and $SUV_{\Delta\%}$ had no significant correlation with overall survival ($P = 0.236$, 0.11, and 0.858, respectively).

Discussion

Although there have been some reports on the possibility of FDG-PET predicting prognosis in patients with primary esophageal cancer [3–9], to our knowledge, this is the first report on FDG-PET in patients with postoperative recurrent esophageal cancer. The reason we used cause-specific rather than overall survival as the endpoint was that some patients were expected to die due to intercurrent diseases or treatment complications [16]. In fact, three patients died from causes other than esophageal cancer. We found that SUV_{max} even <7 days after CRT was correlated with local control and cause-specific survival. This may mean that low FDG uptake after CRT indicates not only loss of activity at the lesion but also metastatic ability of malignant cells. This result also agrees with results obtained by Swisher et al. [7], showing that post-CRT SUV_{max} of primary esophageal cancer was correlated with percentage of viable cells in pathologic specimens at the time of esophagectomy.

In patients with primary esophageal cancer or other malignant tumors, a high false-positive rate due to radiation-induced inflammatory changes has been reported. Therefore, so far, there has been no consensus concerning the timing of FDG-PET after CRT for the most accurate assessment of tumor response. Early determination of residual disease would enable the next salvage therapy (e.g., adjuvant chemotherapy, molecular-targeted therapy, additional irradiation) to be started earlier, but many investigators have reported that FDG-PET should be performed 4–6 weeks after CRT. The best timing for FDG-PET after CRT for primary esophageal cancer is unknown; however, there was, at least, a low false-positive rate for postoperative recurrent esophageal cancer in this study, despite the fact that FDG-PET was performed <7 days after CRT. There were no patients with SUV >3.0 during the first 7 days after CRT among 11 patients who were controlled in the irradiated field at the last observation date. Most patients had recurrences in lymph nodes, not in the anastomotic region. The reason findings of FDG-PET performed even <7 days after CRT might be accurate could be because performing FDG-PET so early avoids radiation-induced mucositis, which is known to result in a relatively high and prolonged FDG accumulation. However, SUV_{max} after CRT in two anastomotic recurrent lesions were 2.2 and 2.0, respectively (SUV_{max} before CRT: 5.4 and 7.7, respectively). FDG accumulation in squamous cell carcinoma is known to be decreased by CRT more than that in adenocarcinoma [20]. All patients in our study had squamous cell carcinoma, which might also have had some effect on the results.

According to RECIST, accurate confirmation of response requires a waiting period of at least 4 weeks after response criteria are first met. In fact, although 7 patients in our study showed CR as the best response record, the recurrent tumor vanished on CT immediately after CRT in only one patienty. From our results, it appears that the FDG-PET modality might provide the earliest possible assessment of treatment response and survival prognosis after CRT in patients with locoregional postoperative recurrent esophageal cancer. Furthermore, the results could be used commonly in squamous cell carcinoma lymph node metastasis from other primary sites (e.g., head and neck, lung, uterus).

Although some studies have shown that a comparison of FDG uptake in the early phase and that in the delayed phase is useful for distinguishing malignancy from inflammation [18, 21–26], from results of our study, a one-phase scan might be sufficient to predict cause-specific survival and local control rates in patients who receive CRT for postoperative locoregional recurrent esophageal cancer. Furthermore, we showed that SUV_{max} in FDG-PET before CRT tended to be correlated with cause-specific survival but not with local control rate. This might mean that FDG uptake before therapy closely reflects the overall malignancy of recurrent tumors, including metastatic ability. However, it might not reflect tumor radiation sensitivity. Thus, high SUV levels before treatment may be indicative of the need for more aggressive systematic therapy. We found no significant correlations between SUV_{max} before CRT and other pretreatment prognostic factors (performance status, age, recurrent pattern), as reported previously [16]. FDG-PET has the possibility of being used as an independent prognostic survival estimation tool before CRT in patients with locoregional postoperative recurrent esophageal cancer.

Several studies have shown that changes in metabolic activity were correlated with tumor response and patient survival [27–29]. In our study, there was a significant correlation between $SUV_{\Delta\%}$ and local control rate, but greater $SUV_{\Delta\%}$ did not prolong survival. Similar observations have been reported previously [7, 20, 27–30]. The observed metabolic change in our study is similar to the time course of tumor FDG uptake during CRT shown by Wieder et al. [30]. However, they showed that the metabolic change was correlated significantly not only with tumor response but also with survival. In our study, we could not determine why $SUV_{\Delta\%}$ was not correlated with survival. In most similar studies, patients underwent esophagectomy with lymph node dissection after CRT, and FDG-PET was performed >4 weeks after CRT. These differences in the procedure may have caused the different results. As described above, FDG accumulation before CRT is considered to reflect overall tumor malignancy, not radiation sensitivity. It is therefore reasonable that $SUV_{\Delta\%}$ is not correlated with survival.

Our study results suggest that a single FDG-PET within 7 days of CRT may be sufficient to predict tumor response and survival prognosis. This issue is controversial. It is thus necessary to perform a multicenter study with multivariate analysis and a much larger number of patients to determine which factor, including SUV_{max} before and after CRT and/or changes in metabolic activity, is the most important for patients with locoregional postoperative recurrent esophageal cancer.

Conclusions

This prospective study showed that FDG-PET after CRT predicts survival prognosis in patients with locoregional postoperative recurrent esophageal cancer. We particularly emphasize that FDG-PET performed even <7 days after CRT enables prognosis prediction. FDG-PET could be the earliest diagnostic modality for local control and survival

prognosis in patients with locoregional postoperative recurrent esophageal cancer.

Conflicts of interest statement There is no conflict of interest in relation to this study.

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放射線生物研究 45(1), 2010
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＜速　報＞

ヒト大腸癌細胞株(DLD/1)においてCapecitabine (Xeloda)と2-methoxyestradiol(2-ME2, Panzem)を用いた化学放射線療法が及ぼす抗腫瘍効果についての基礎的検討

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1　始めに

5-フルオロウラシル(5-FU) はウラシルの代謝拮抗剤であり(1)、DNA合成阻害(2)(3)、RNA機能障害(4)の両方に作用するとされており、消化器癌を主とする各種固形癌で幅広く用いられている代表的な抗癌剤である。その中のひとつCapecitabine (N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine, Xeloda®)は酵素活性型5-FU誘導体であり結腸直腸癌や乳癌等で良好な成績が得られている。Capecitabineは経口吸収後、腫瘍組織内で5-FUに変換し抗腫瘍効果を発揮する(5)。一方Estrogen誘導体である2-ME2 (2-Methoxyestradiol, Panzem®)は強力な血管内皮細胞の増殖および遊走の阻害物質であり(6)、HIF-1αの発現を転写後に抑制する(7)、経口で投与する低分子化合物の血管形成阻害剤である (8, 9)。現在多発性骨髄腫、前立腺癌などさまざまな腫瘍に対しての臨床試験が行われている (10, 11)。チューブリンの重合を阻害するため、微小管・紡錘糸の形成以上を起こし、分裂中期で細胞分裂が停止するとされている (12)。マウスにおける基礎実験では乳癌、前立腺癌などでの抗腫瘍効果が確認されている (13)。このようにどちらも非常に優秀な抗癌剤としての性質を持つが、副作用の点が問題になってくる。Capecitabineでは骨髄抑制、手足症候群等が、2-ME2では貧血、下痢、吐き気、倦怠感等が主な副作用とされている (10)。最小限度の副作用で最大限の抗腫瘍効果を得るために、今回我々はCapecitabineと2-ME2を用いた二剤併用化学放射線療法の可能性をマウスを用いた基礎実験により検証した。

目的：ヒト大腸癌細胞株(DLD-1)において、Capecitabine(N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine, Xeloda®)と2-ME2(2-Methoxyestradiol, Panzem®)でのCombination Chemor-

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キーワード：Capecitabine、 2-methoxyestradiol、 ヒト大腸癌細胞株、化学放射線療法

adiotherapyが及ぼす抗腫瘍効果を検証する。

2　材料及び方法

2-1. マウス及び細胞

本研究で用いたマウスは、BALB/C-nu/nuヌードマウス4～5週齢♀（CLEA JAPAN）を用いた。マウスは1ケージあたり4～5匹とし、実験、飼育にあたっては東北大学動物倫理委員会規則を遵守した。細胞株は、ヒト大腸癌細胞株（DLD-1；大鵬薬品工業株式会社福島先生より譲渡いただいた）を用いた。培養条件はRPMI1640(GIBCO)に10 %FBS（MP Biomedicals)、1 %ペニシリン・ストレプトマイシン（GIBCO）を添加した培地で37 ℃、5 %CO_2の条件下で培養した。Source tumorはヌードマウス背部皮下に1×10^7個の培養細胞を移植して作成した。大きさが200-300 mm^3に成長した時点で、腫瘍を摘出し、2 mm角の小片に刻み、マウスの右足大腿部皮下にトローカー針を用いて移植し実験に用いた。腫瘍が80-100 mm^3になった時点で治療を開始した。

2-2. 抗癌剤

本研究ではCapecitabine(Xeloda; Cyugai/Rosh)、2-ME2(2-Methoxyestradiol, Panzem®)の二種類の抗癌剤を用いた。どちらもマウスには経口投与とした。Capecitabine、2-ME2はそれぞれ100 mg/kg、50 mg/kgの投与量でday=1～day=21まで一日一回経口投与を行った。Vehicleは5%カルボキシメチルセルロース（CMC)とし、抗癌剤非投与群ではvehicleを経口投与した。

2-3. 放射線照射

本研究ではSHIMAZU社製の小動物用X線照射装置を用いてマウスを専用のジグに固定し0.72 Gy/minの線量率でday=1で5 Gyの単回照射を行った。

2-4. Tumor Growth Delay Curveの作成

マウスを8グループに分け（vehicle control、5 Gy単回照射、Capecitabine、2-ME2、Capecitabine +2-ME2、5 Gy+ Capecitabine、5 Gy+2-ME2、5 Gy+ Capecitabine +2-ME2)、マウスの右足大腿部に皮下移植した腫瘍が80-100 mm^3になった時点で治療を開始した。Capecitabine、2-ME2はそれぞれ100 mg/kg、50 mg/kgの投与量でday=1～day=21まで一日一回経口投与を行った。放射線と組み合わせた治療の場合、照射一時間前に抗癌剤を投与した。治療開始直後から2日、あるいは3日毎に腫瘍体積を計測し、Tumor Growth Delay Curveを作成した。各グループあたりのマウスの数は6匹とした。腫瘍体積が1000 mm^3に成長した時点でマウスを安楽死させた。抗腫瘍効果の判定としてEnhancement Factor(EF)を用いた。そのためにまず、Absolute

tumor growth delay、Normalized tumor growth delay を求めた。Absolute tumor growth delay は、Capecitabine，2-ME2,放射線の単独治療、またはcombinationでの治療群におけるTumorが4倍の体積に成長する日数から、無治療群（コントロール）でのTumorが4倍の体積に成長する日数を引いたものである。Normalized tumor growth delay はCapecitabine，2-ME2，放射線のcombinationでの治療群のTumorが4倍の体積に成長する日数からCapecitabineあるいは2-ME2での治療単独群でのTumorが4倍の体積に成長する日数を引いたものである。EFは、Capecitabineあるいは放射線あるいは2-ME2を組み合わせたNormalized tumor growth delayを放射線単独でのabsolute growth delayで割ったものである。Capecitabine，2-ME2，放射線が全てcombinationされた治療群の場合は、Normalized tumor growth delayをCapecitabine＋放射線、あるいは2-ME2＋放射線でのabsolute growth delayで割ったものである。

3　結果及び考察

3-1. Tumor Growth Delay

図1にTumor Growth Delay Curveを示す。ここではX線との併用効果を判定するため、Capecitabine，2-ME2が同程度の抗腫瘍効果を示すよう、かつX線単独での効果よりも若干抗腫瘍効果が低めになるよう容量を設定した。Control群に比べ、いずれの群も抗腫瘍効果が現れているが、X線と併用する事でCapecitabineと2-MEの効果が単独治療よりも僅かに高いことがわかった。特

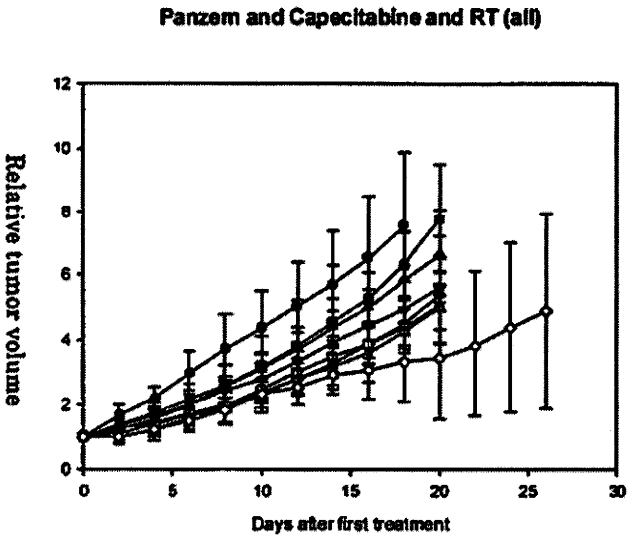

図1　Tumor Growth Delay Curve
横軸は治療後の日数、縦軸は治療開始の腫瘍体積を1とした場合の相対値。
●：control、■：Capecitabine、▲：2-ME2、▼：XRT5Gy、◆：Capecitabine＋2-ME2、
□：Capecitabine＋XRT、△：2-ME2＋XRT、◇：Capecitabine＋2-ME2＋XRT

にCapacitabine、2-ME2、X線の二剤併用化学放射線療法を行った場合、最も抗腫瘍効果が高くなったことがわかった。

図2に腫瘍が4倍に成長するに要する日数を示す。この日数を見るとControlが10日前後であるのに比して、一番右のCapecitabine＋2-ME2＋XRTでは約20日前後と約2倍ほどの腫瘍成長遅延があることがわかった。

表1は腫瘍が4倍に成長するに要する日数、absolute growth delay、normalized growth delay、enhancement factor(EF)の値をまとめたものである。

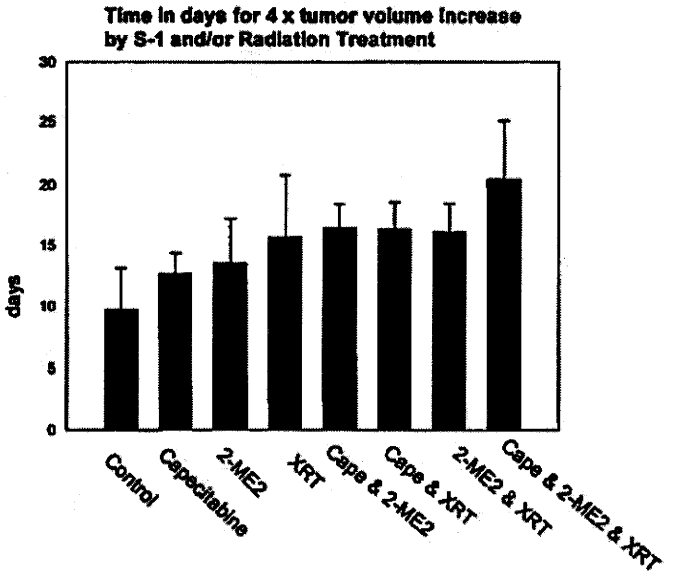

図2　腫瘍の体積が4倍になるまでの日数
横軸は治療別のグループ、縦軸は日数を示す。

表1　各治療群でのabsolute growth delay, normalized growth delay, enhancement factor(EF)の値のまとめ

Summary: DLD-1, Capecitabine, 2-ME2 and XRT

	days of 1-4 folds	se	absolute growth delay	normalized growth delay	EF
control	9.73	3.40			
capecitabine100mg/kg	12.70	1.70	2.97		
2-ME2 50mg/kg	13.55	3.71	3.82		
RT 5Gy	15.74	5.05	6.01		
cape& 2-ME2	16.54	1.98	6.81	3.84	1.01
cape&RT	16.38	2.24	6.65	3.68	0.61
2-ME2 &RT	16.18	2.34	6.45	2.63	0.44
Cape& 2-ME2 &RT	20.51	4.60	10.776	7.81(Cape)	1.21 (Cape)
				6.95(2-ME2)	1.04 (2-ME2)

Capecitabine EFの値は1.21であることから、Capecitabineが2−ME２と放射線の効果を僅かではあるがadditive以上にはenhanceしているという事がいえた。しかし、Capecitabine、2-ME単剤ではX線の効果はさほどenhanceしていなかった。これを、in vitroの面からも検証するため、現在上述の二種類の薬剤が放射線増感効果があるか否かをclonogenic cell survival assayで調べているところである。マウスの毒性であるが、2-ME2を経口投与していたグループではマウスの衰弱が激しく体重の減少が大きかった。Capecitabine、2-ME2共に放射線に対する増感作用がin vivoにおいて報告されている(14、15)。今回我々の実験でも増感作用はわずかながら見られたが、そのEFは非常に小さいものであった。これは二剤併用の化学放射線療法を検証するため、各抗癌剤の投与量を極力低く抑えたためであるのも一因していると考えられる。

Tumor Growth Delayにおいて、Capecitabineあるいは2-ME2と放射線の併用、さらには2剤と放射線を併用した場合の効果が単剤治療よりも大きい事がわかったが、そのメカニズムについて考えてみる。過去の文献においては2-ME2がHIF-1α発現を減少させるという報告がある（16、17、18)。さらに5-FUのprodrugであるTS-1が放射線と併用した際、HIF-1α活性を抑制させることも報告されている（19)。実際、我々ののところでも、*in vitro*での Western Immuno Blotting AssayによりHIF-1αの発現を調べてみた結果、2-ME2、CapecitabineはHIF-1α発現を減少させ、さらにCombinationさせたグループにおいてはその減少が顕著であるデータが最近得られた（未発表)。現在、このHIF-1αの上流域、下流域のシグナリングがどう変化しているのかを調べているところである。もしこの2剤を併用する事で、癌の低酸素部分に関わる因子が減少するのであれば、in vivoで見られた2剤併用での放射線の増強効果は癌の低酸素領域部分の改善によるものが一因となっていると言えるかもしれない。癌の低酸素状態は、癌細胞の低酸素応答を惹起し、HIFを介した関連遺伝子の誘導により、腫瘍血管新生、腫瘍増殖を惹起し、ひいては再発や転移を促進することによって腫瘍の悪性度を増加させることが知られている（20)。臨床的にも子宮頸癌の局所の浸潤性やリンパ節への転移性、また、軟部肉腫では術後の転移性が、腫瘍の高度な低酸素状態で増すことが報告されており、低酸素細胞の存在そのものが腫瘍の悪性度、浸潤性の指標になることが判ってきている。また、腫瘍内低酸素細胞は手術、放射線治療、化学療法などすべてのがん治療に対する大きな抵抗性因子となっていることが判っている(21)。従って、低酸素細胞の克服することが、有効で、効率の高い癌治療を実現させるためには極めて重要となっている。Capecitabine、2-ME2は投与量如何では副作用の懸念がある。もし低容量のCapecitabineと2-ME2とを併用させる事で低酸素領域を克服できるのであれば、臨床的にも極めて重要な意義を持つものとなるかもしれない。今後はCapecitabine、2-ME2での低酸素応答に及ぼす効果をより詳しく調べていきたいと思っている。

4　まとめ

今回の研究で、ヒト大腸癌細胞株（DLD-1）において、Capecitabine、2-ME2を併用させた化学放射線療法は、すぐれた抗腫瘍効果を示す事がわかった。そのメカニズムとしてはHIF-1 αの減少によるものであろうということが推察されるが今後の研究成果を期待したいところである。

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2664 Three-dimensional Non-coplanar Conformal Radiotherapy for the Treatment of Stage I Non-small Cell Lung Cancer: Is it Equally Safe and Effective for So-called Central Tumors?

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Purpose/Objective(s): Three-dimensional non-coplanar conformal radiotherapy (3-DNCCRT) has recently been considered promising for the treatment of stage I non-small cell lung cancer (NSCLC). Usually, it is performed in the form of SBRT using 10 - 20Gy fraction dose. However, this method is considered to be contraindicated for so-called central tumors because of the toxicity of serial organs, such as bronchus, great vessels, etc. We have been treating these tumors with relatively small fraction dose (usually 3Gy) keeping BED10 at the similar level to that of SBRT. In this study, we compared the results of central tumors and peripheral tumors to see if the results of the central tumors are comparable with those of peripheral tumors.

Materials/Methods: Eligibility criteria were as follows: maximum tumor diameter not greater than 5cm, PS between 0 and 2, and no limitation regarding age and pulmonary function. Radiotherapy was given with 6MV photon beam by fixed 10 non-coplanar conformal beams to a total dose of 75Gy in 25 fractions in 5 weeks. Irradiation was aiming at the internal target volume with proper margins. No elective nodal irradiation was given. Between January 2002 and November 2008, 80 eligible cases were treated. Age ranged from 53 to 91 (median 77). The male/female ratio was 59/21. There were 48 T1 tumors and 32 T2. Fifteen tumors were SQCCA, 56 adenoca, 2 large cell ca and 7 NSCLC NOS. There were 66 inoperable cases (83%), among them poor pulmonary function was in 48 (60%), and 14 operable cases, who refused operation. The average tumor size was 3.0 cm (range: 1.0 to 5.0 cm). There were 75 PS 1 and 5 PS 2 cases. Among the entire cases, 37 cases were central tumors and the other 43 were peripheral tumors. There were no differences in the patient characteristics between central and peripheral tumors. Median follow-up period was 37 months.

Results: Three-year local control rate, overall survival rate, cause-specific survival rate, and relapse-free survival rate for overall cases were, 86%, 66%, 78%, and 59%, respectively. For central tumors, they were, 85%, 68%, 79%, and 55%, respectively, and for peripheral tumors, they were, 86%, 64%, 77%, and 61%, respectively. There were no differences between the two groups. There were 7 Grade 3 pulmonary toxicities (9%, 3 in central, 4 in peripheral, n.s.), but there were no severe adverse effects concerning serial organs in either group.

Conclusions: The 3-DNCCRT for stage I non-small cell lung cancer has been safe and effective both for central and peripheral tumors, and might become an alternative treatment for central tumors, which SBRT might cause serious toxicities. Further follow-up with a larger number of cases were necessary.

Author Disclosure: K. Karasawa, None; N. Okano, None; N. Kubo, None; M. Fukayama, None; Y. Tagawa, None; N. Mitsui, None; G. Kuga, None; T. Chang, None; N. Hanyu, None.

2665 Concurrent Chemoradiotherapy Increases the Risk of Radiation-Induced Pericardial Effusion in Patients with Locally Advanced Non-small Cell Lung Cancer

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Purpose/Objective(s): To evaluate radiation-induced pericardial effusion in non-small cell lung cancer (NSCLC) patients treated with 3-dimension conformal radiotherapy (3DCRT) with or without concurrent chemotherapy. The influence of treatment parameters on the incidence of pericardial effusion was also analyzed. The hypothesis of this study was that the addition of chemotherapy increases the risk of radiation-induced pericardial effusion and that pericardial dosimetric factors are associated with subsequent pericardial effusion.

Materials/Methods: From March 2004 to February 2008, 41 patients with stage I-III inoperable/unresectable NSCLC were enrolled in prospective studies at the University of Michigan and Ann Arbor Veterans Hospitals. Patients were treated with radiation alone (n = 12) or concurrent chemoradiation (n = 29). Pericardium was contoured on axial CT scans. The primary endpoint was pericardial effusion, which was assessed on follow-up CT scans. Student's t-test was used to determine the effect of pericardial dose and chemotherapy on the development of pericardial effusion.

Results: Minimum follow-up was 2 years. Pericardial effusion was seen in 19 of 41 (46%) patients. Chemotherapy and dosimetric factors were significantly associated with radiation-induced pericardial effusion. Nineteen of 29 (60%) patients treated with concurrent chemoradiotherapy versus 0/12 (0%) patients with radiotherapy alone developed pericardial effusion ($p<0.01$). Dosimetric analysis showed that V10, V20, V50, V60 of heart dose were associated with a greater risk of pericardial effusion ($p<0.05$).

Conclusions: Treatment with concurrent chemoradiotherapy may increase the risk of radiation-induced pericardial effusion over that of radiation alone. Heart dosimetric parameters are also significant factors for development of radiation-induced pericardial effusion. Every effort should be made to minimize incidental irradiation of the heart while maintaining adequate coverage of target volumes.

Author Disclosure: J. Liu, None; L. Xu, None; R. Ten Haken, None; J. Hayman, None; N. Ramnath, None; D. Tatro, None; K. Cease, None; G. Kalemkerian, None; F. Kong, None.

2666 Pemetrexed (PEM) and Cisplatin (CIS) in Concurrent Combination with High Dose of Thoracic Radiation (RT), after Induction Chemotherapy (CT), in Patients (pts) with Locally Advanced Non-small Cell Lung Cancer (NSCLC): A Phase I Study

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