tyrosine kinase, has been demonstrated in non-small cell lung carcinoma patients with EGF receptor mutations, and so these mutations are a useful marker(s) to find responders to this drug. However recent studies showed that the EGF receptor gene mutation is rare in squamous cell carcinomas of the esophagus and head and neck regions. In the present study we investigated the relationship between BRAK expression and gefitinib efficacy for tumor suppression.
Material and Methods: HNSCC cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum. Nearly confluent cells were cultured overnight in serum-free DMEM. After starvation, they were incubated with or without EGF (10 ng/ml) and/or gefitinib (1 μM). HSC-3 cells were subcutaneously injected into athymic nude mice. Tumor cell-xenografted mice were daily administered gefitinib (50 mg/kg) orally. In some experiments, tumor cells were introduced BRAK ShRNA expressing vector to knockdown BRAK mRNA expression and established stable transformants.
Results: Gefitinib attenuated the effect of EGF, or even stimulated BRAK mRNA expression of HNSCC cells *in vitro*. Oral administration of gefitinib significantly ($P < 0.001$) reduced tumor growth of xenografts in female athymic nude mice accompanied by increased in BRAK expression specifically in tumor tissue. Introduction of BRAK ShRNA vector reduced both the expression of BRAK in the cells and the antitumor efficacy of gefitinib *in vivo*.
Conclusions: Our results indicate that oral administration of gefitinib reduced tumor size, at least in part, through elevation of BRAK expression. Thus, the use of gefitinib for treatment of patients with HNSCC in whom there is an inducing effect of the drug on the BRAK expression in cancer cells in culture may be advantageous. Furthermore, BRAK may be a promising molecule for gene therapy of HNSCC.
This work was performed in collaboration with Drs. Takahide Taguchi, Yukari Imagawa-Ishiguro and Mamoru Tsukuda, Department of Biology and Function in the Head and Neck, Yokohama City University Graduate School of Medicine.

8525 POSTER

Prediction of pathological response of preorerative 5-fluorouracil-based chemotherapy for oral cancer

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Background: The response of chemotherapy is individually different in patients with oral squamous cell carcinoma (OSCC). Prediction of chemotherapy response is very important to select adequate therapy for every patient. We retrospectively investigated the relationship between chemotherapy response and expression of biomarkers in OSCC patients who received 5-fluorouracil (5-FU) based chemotherapy and following radical surgery. The aim of this study is to make the formula which predicts chemotherapy sensitivity using some biomarkers.
Material and Methods: Retrospective analysis of biomarker expressions in biopsy specimen was performed immunohistochemically in 95 OSCC patients. These received 5-FU based chemotherapy such as TS-1®+cisplatin, UFT®+cisplatin, etc. We use 17 biomarkers including dihydropyrimidinedehydrogenase (DPD), thymidylate synthase, EGFR, cyclinD1, Ki-67, c-Met, MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-2, Bax, Bcl-2, CD25, Foxp-3, Caveolin-1. The expression of these biomarkers were evaluated by staining intensity or percentage of positive cells. Pathological effects were evaluated in surgical specimens. We compared these 2 factors and selected significant biomarkers which had association with chemotherapy response. Statistical analysis was performed using Spearmans' correlation coefficient test and logistic regression model.
Results: There was a statistically significant relation between the expression of five biomarkers (DPD, VEGF, MMP-2, Ki-67 and Bcl-2) and the pathological response for 5-FU based chemotherapy. The formula which predicts chemotherapy sensitivity was made by logistic regression model consisted of above five markers. We applied this formula to another 14 OSCC patients for verifying its accuracy. In consequence, the accuracy rate was 85.7%.
Conclusions: The expression of DPD, VEGF, MMP-2, Ki-67 and Bcl-2 were independent predictor for sensitivity of 5-FU based chemotherapy. The combination of these biomarkers was useful for predicting chemotherapy response. The formula was applicable for OSCC patients before chemotherapy and realized high accuracy to predict chemotherapy response.

8526 POSTER

The tumour volume for F-18 fluorodeoxyglucose predicts for response to treatment and progression free survival biomarker in head and neck cancer

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Purpose: To evaluate the prognostic value of metabolic tumor volume measured on 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) imaging and other clinical factors in patients treated for locally advanced head-and-neck cancer (HNC) at a single institution.
Materials and Methods: Between June 2005 and August 2008, fifty nine patients with HNC who underwent pretreatment FDG-PET studies received neoadjuvant chemotherapy and radiation therapy. Metabolically active tumor regions were delineated on pretreatment PET scans by a fixed SUV of 2.5. We evaluated the relationship of 18F-fluorodeoxyglucose-PET maximum standardized uptake value (SUV) and metabolic tumor volume (MTV) with response to treatment, progression-free survival (PFS) and overall survival (OS).
Results: The average SUVmax was 8.9 (range, 1.4−78.0) and the mean MTV was 23.5 cm3 (range, 1.2−170.8) for all patients. Higher MTV was associated with an increased risk of lymph node metastasis at diagnosis ($p = 0.028$) and response to treatment ($p = 0.026$). A Cox proportional-hazards model for progression free survival from head and neck cancer was used to evaluate sex, age, organ, stage, T-stage, lymph node metastasis, MTV, and SUVmax and neoadjuvant chemotherapy type. The results indicated that MTV was the only significant independent factor ($p = 0.021$). A higher MTV of 9.3 cm^3 (median MTV) was significantly associated with an increased hazard of recurrence (2.19-fold, $p = 0.007$). We did not find a significant relationship of maximum SUV, stage, or other clinical factors with response to treatment or PFS or OS.
Conclusions: Metabolic tumor volume is an adverse predictive factor for treatment response and disease progression in HNC. MTV is a direct measure of tumor burden and is a potentially valuable tool for risk stratification and guiding treatment in future studies.

8527 POSTER

A phase II trial of erlotinib after gemcitabine plus platinum-based chemotherapy in patients (pts) with recurrent and/or metastatic nasopharyngeal carcinoma (NPC)

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Background: Although chemotherapy with a platinum compound and gemcitabine (G) is effective in recurrent and/or metastatic NPC, the outcome remains poor with time-to-progression (TTP) <1 year. The epidermal growth factor receptor (EGFR), expressed in 85% NPC, is associated with poor prognosis. We conducted a phase II study to determine the efficacy of the EGFR tyrosine kinase inhibitor erlotinib, given as maintenance therapy, following cisplatin (C) or carboplatin (Ca) + G in pts with recurrent and/or metastatic NPC.
Methods: Pts were treated with up to 6 cycles of chemotherapy (G 1000 mg/m^2 day 1 and 8, C 70 mg/m^2 day 1 or Ca AUC = 5 day 1 if contraindication to C) every 3 week. Pts were switched to erlotinib 150 mg PO daily Q4W after 6 chemotherapy cycles, or before if they progressed on chemotherapy. Primary endpoint was TTP in non progressive disease (PD) pts after 6 chemotherapy cycles and treated with maintenance erlotinib. EBV DNA plasma levels were measured using qRT-PCR.
Results: Of 20 pts, 1 pt never started chemotherapy. Median follow-up was 5.8 months. Pts characteristics were: M:F = 13:7, asian:non-asian = 14:6, median age 56 [range 32−72], PS 0:1 = 10:10, locoregional recurrent:metastasic = 6:14. With G, 8/20 pts (40%) and 12/20 (60%) were treated with C and Ca, respectively. After 96 chemotherapy cycles, the most frequent grade 3/4 adverse events (AE) were neutropenia (63%), thrombocytopenia (47%) and anemia (21%). Of the 19 pts evaluable for response to chemotherapy, 7 pts had a PR (35%), 11 SD (58%) and 1 PD (6%). Of them, 15 pts received 36 cycles of erlotinib (median = 2, range = 0−6). The most frequent grade 3 AE related to erlotinib were lymphopenia (26%), acneiform rash (20%), hand-foot syndrome (13%), neutropenia (13%) and fatigue (7%). No grade 4 toxicity was observed. Of 11 pts evaluable for response to erlotinib, all progressed except 3 pts (27%) with stable disease for 3, 4 and 7 months. Median TTP was 6.3 months for all 17 evaluable pts, and 6.9 months for 13 pts with no PD after 6 chemotherapy cycles. One-year overall survival was 80% for all pts. No correlation between EBV DNA plasma levels or kinetics and clinical outcome was detected.
Conclusions: Maintenance or 2nd line therapy with erlotinib post-chemotherapy is not effective in recurrent and/or metastatic NPC. Historical

comparison with pts treated with the same chemotherapy alone until PD suggests that it may be detrimental to stop chemotherapy after 6 cycles if disease did not progress.

8528 POSTER

Epstein-Barr virus quantification and aberrant host DNA methylation pattern as marker for nasopharyngeal carcinoma in non-invasive nasopharyngeal brushings

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Background: Nasopharyngeal carcinoma (NPC) is the most prevalent head and neck cancer in Indonesia. WHO type III, representing the majority of NPC, is 100% related to Epstein-Barr (EBV) infection. The viral DNA load is elevated in nasopharynx (NP) of most patient at diagnosis. A NP brush was used for in-situ sampling, allowing simpler and less invasive NPC diagnosis. We have shown EBV-DNA load as promising new diagnostic method and use it to screen NPC cases in high risk population. A growing evidence demostrates that aberrant methylation in gene promoter is important in inactivating tumor suppressor gene (TSG) in NPC. This study aimed to quantify EBV-DNA load and determine methylation status of multiple TSGs in NPC, high risk individuals, and healthy EBV-carriers to evaluate wether methylation pattern may have additional value to identify early carcinogenic events.
Methods: NP brushing was taken from NPC, high risk patients presenting chronic problems in head and neck area, and normal EBV-carriers. Paraffin tissue of NPC patient was also included and subjected for DNA isolation in order to verify detection rate of methylation in brushing DNA. EBV-DNA load was measured using a quantitative real time PCR. DNA was modified using bisulfite treatment and amplified by methylation-specific PCR. Seven tumor suppressor genes were included (DAPK, CADM1, p16, RASSF1A, CHFR, RIZ1, and DLC1).
Results: All NPC patients showed elevated EBV-DNA and high frequency of methylated genes (DAPK 69.6%, CADM1 71.4%, p16 68.1%, RASSF1A 73.5%, CHFR 65.9%, RIZ1 41.7%, and DLC1 58.7%). Most of paraffin and brushing DNA revealed a concordance result of methylation status. The high risk individuals, who also demonstrated high EBV-DNA load, showed high frequency of methylated genes of DAPK (76.9%), CADM1 (61.5%), and DLC1 (61.5%), but low or undetected methylated genes of p16, RASSF1A, CHFR, and RIZ1. Healthy individuals showed low DNA load but similar methylation pattern as high risk population.
Conclusion: These result s suggest that EBV infection and promoter hypermethylation might serve as useful markers to screen early NPC. At the time a prospective analysis in high risk group using non-invasive brushing samples to identify early stage NPC is in progress. In Indonesian normals, much abnormal methylation on certain TSGs probably reflects exposure to co-carcinogens in environtment and food.

8529 POSTER

Pattern of locoregional failure after tomotherapy in head and neck cancer

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Background: Helical tomotherapy is a new radiation device delivering a highly conformal dose from a rotational gantry resulting in a more uniform target dose and better avoidance of organs at risk. Treatment failure patterns of head and neck cancer treated with helical tomotherapy and the adequacy of the target volume definitions and delivery techniques currently used were analysed.
Materials and Methods: Between June 2005 and March 2008, 76 consecutive patients with biopsy proven head and neck cancer were treated with helical tomotherapy (Hi-Art TomoTherapy®, Madison, Wisconsin, USA) at the UZ Brussel. For patients with local or regional failure, the volume of failure (Vf) was determined on one or more diagnostic tools as computerized tomography (CT), magnetic resonance imaging or positron emission tomography obtained at the time of failure. The Vf is then contoured with Co-registration of the failure image (Vf) and the initial planning CT was performed. The failures were categorized as local or regional. The dose of radiation received by failure was calculated and analyzed using dose-volume histograms (DVHs) and accordingly it is classified as 1) In-field (InF): in which 95% or more of Vf was within the 95% isodose, 2) Marginal (MF), if 20% to 95% of Vf was within the 95% isodose, or 3) Outfield (OutF) if less than 20% of Vf was inside the 95% isodose. The mean, minimum and maximum doses received by each failure volume were displayed.
Results: Median follow up time was 14.8 months (3.5−38.8). Three-years overall survival, disease free survival and locoregional control were 69%, 47% and 59%, respectively. Twelve patients showed locoregional failure, 5 were local, 6 were regional and one showed both local and regional failure. With DVHs analysis, InF, MF and OutF were 9, 3 and 1, respectively. All MF had a history of surgey before radiotherapy.
Conclusions: Target defintion and coverage were adequate. The majority of locoregional failures were InF i.e. in the high dose regions. Future work on dose escalation to the highest risk regions is recommended. Special consideration for surgically manipulated patients must be taken in volume selections and coverage.

8530 POSTER

Clinical results and prognostic factors in radiotherapy for early glottic squamous cell carcinoma

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Background: The purpose of this study is to determine the prognostic factors for local control in T1a, T1b and T2N0M0 glottic squamous cell carcinomas.
Material and Methods: Data from 249 patients with T1−2N0M0 (T1a: 115, T1b: 48, T2: 86) Stage I-II glottic carcinomas, who were treated with definitive radiotherapy during 1976 to 2002 were analyzed retrospectively. Age, source, total dose, field size, overall treatment time, average fraction size, fractionation regimen, chemotherapy and etc. were set as variables in multivariate analysis.
Results: The 5-year local control rates (LCR) were 92%, 85% and 83% for patients with T1a, T1b and T2 glottic carcinomas, respectively. Only total radiation dose ($p = 0.048$) was a significant prognostic factor for local control in multivariate analysis of T1b glottic carcinoma. Local control in the higher total dose group was better than that in the lower total dose group (5-year LCRs were 100% and 76% for the group of >66 Gy and the group of $\leqslant$66 Gy, respectively. $p = 0.024$, logrank test). None of the treatment parameters were shown to be significant prognostic factors in multivariate analysis of T1a glottic carcinoma. In the analysis of T2 glottic carcinoma, OTT (overall treatment time of radiotherapy) ($P = 0.0003$) and Total dose ($P = 0.0036$) were the significant prognostic factors on local control in multivariate analysis. Higher total dose group ($\geqslant$67 Gy vs. <67 Gy) showed favorable prognosis (5-year LCR: 91% vs. 60%, respectively. $P = 0.0013$: logrank test). And the shorter OTT group ($\leqslant$54 days vs. >54 days) showed favorable prognosis (5-year LCR: 87% vs. 71%, respectively. $P = 0.023$).
Conclusions: Radiotherapy with a total dose of >66 Gy seemed to be required for local control in T1b glottic carcinoma. No significant benefit of total radiation dose >64 Gy was shown in the analysis of T1a glottic carcinoma. Radiotherapy total dose of $\geqslant$67 Gy delivered with shorter period is required for T2 glottic cancer. The fractionation regimens of accelerated hyperfractionation is more effective than conventional fractionation in terms of shortening OTT and delivering high total dose with acceptable toxicity.

8531 POSTER

Prediction of clinical radiation induced toxicity through study of radiation induced apoptosis in peripheral blood lymphocytes (PBLs)

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Background: To analyze radiation induced apoptosis in PBLs of patients with head and neck (H&N) cancer, the application of a semilogarithmic model RID (Radiation Induced cell Death)= $\beta.\ln(Gy)+\alpha$, to assess the association among the defined constants of this model and its utility as a prediction model for toxicity in patients treated with radiation therapy.
Material and Methods: A total of 79 patients with H&N cancer treated with radiation therapy, with or without surgery and chemotherapy were included. PBLs were obtained from peripheral blood samples using density gradient centrifugation (Ficoll Hipaque). Apoptosis was assessed by Anexin V and Propidium Iodide (IP) staining. Triple analysis at doses of 0, 1, 2, and 8 Gy were performed in all patients after 24 hours. Clinical toxicity was assessed by the ROTG classification.
Results: RID was increased by the dose of radiation administered. α(initial value at x axis) and β (apoptosis increase due radiation dose–slope of the curve) constants, defined in the model, were statistically associated. β was associated with radiation induced toxicity, such as grade III or higher xerostomy bivariate ($p = 0.035$) and multivariate analysis ($p = 0.034$; EXP (B) 2,553, 95% CI (1,074−6,070).
Conclusions: Radiation sensitivity of peripheral blood lymphocytes can be estimated using the Anexin IP staining to assess radiation induced apoptosis. The later adjusts to the α/β semilogarithmic model and allows to