1 normal FDG-activity (pr recurrence) occurred. In 4 patient we found a hypometabolism in the region of dose deposition.

Conclusion: These preliminary results with 5/11 recurrences at the field edge seem to justify a further examination of the usefulness of pretreatment image fusion with PET to delineate an improved treatment volume.

430 POSTER

Establishment of a nitrosourea-resistant in vivo brain-tumor model: For evaluation of different appraoches to conquer the resistance

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Purpose: Chloroethyl-nitrosourea (ACNU in Japan) is one of the most potent chemotherapeutic agents for brain tumors. However, acquired resistance to this drug has become a serious problem for treatment of patients with these tumors. The main mechanism of resistance is a recruitment of the O6-methylguanine-DNA methyltransferase (MGMT) in the tumor cells. Many approaches including decoy, antisense, and ribozyme, have been reported to overcome the resistance. To evaluate these strategies properly, we designed a syngenic rat brain-tumor model resistant to ACNU.

Methods: The 9L rat gliosarcoma cells were retrovirally transfected with MGMT cDNA. After selected with geneticin, cells expressing MGMT (9L-MGMT) were isolated and compared to the parental (9L-WT) or control cells (9L-Neo). Next, we stereotactically injected these cells into the brain parenchyma of syngenic rats and scored the survivals. Using this model, we also evaluated an RNA antisense approach.

Results: The 9L-MGMT cells, were significantly resistant to ACNU (IC50 = 330 ug/ml, vs. 9L-WT = 48 or 9L-Neo = 40). Further transduction of MGMT gene under CMV promoter did not confer the additional resistance. When cells were implanted into the cerebrum, all rats died within 15 days without treatment (median survival = 13 days). When treated with ACNU, rats with 9L-MGMT died earlier than the other control groups (median survival = 15 days vs. both controls = 23 days, $p < 0.0001$). Transduction of an RNA antisense did not alter the sensitivity to the drug.

Conclusions: Because of the limited intracranial spaces, animals presented a dependable survival curve in this model. Since these survivals were highly reproducible, our system may have a great roll for evaluation of the treatments of drug-resistant brain-tumors.

431 POSTER

Hypermethylation of the hMLH1 promoter region in high-grade gliomas

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Purpose: To analyse the methylation status of the 5'CpG islands of the mismatch-repair (MMR) gene hMLH1 promoter in 26 patients with high-grade gliomas (22 glioblastoma multiforme and 4 anaplastic astrocytomas). We evaluated the relation of hypermethylation at hMLH1 promoter and survival and prognosis.

Methods: MSP (methylation-specific PCR) was performed, a technique that permits distinguishing methylated and unmethylated alleles based on sequence changes produced by treatment of DNA with sodium bisulfite, which converts unmethylated cytosines into uracils, whereas methylated cytosines remain unmodified.

Results: The rate of hypermethylation at hMLH1 promoter gene detected in our series was 4/26 (15.38%). All tumors with hypermethylation at MLH1 promoter gene showed relapse of neoplastic disease. In this way, 2/19 (10.5%) high-grade gliomas with histology of glioblastoma multiforme and harboring normal methylation status remained without evidence of disease after 19 and 24 months from the end of therapy. However, overall survival revealed no significant difference between patients with hypermethylation and normal methylation status.

Conclusion: Hypermethylation at hMLH1 promoter is not a frequent event in advanced gliomas. All patients with hypermethylation showed worse outcome within a period shorter than 24 months.

432 POSTER

Linear accelerator based stereotactic radiosurgery (SRS) as an initial treatment for brain metastases: We can control the tumors 2 cm or less with SRS alone

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Purpose: To evaluate the local effects of linear accelerator based SRS for brain metastases.

Methods and Materials: From April 1993 to March 1998, we treated 146 brain metastases in 67 patients with SRS. Of them, 57 tumors in 38 patients treated with SRS alone as an initial treatment were analyzed. All 38 patients in this study were followed for a minimum of 12 months or to death. The median survival time of these patients was 9.3 months and the follow-up periods with magnetic resonance or computed tomographic imaging were 0.8–61.5 (median 5.2) months. The major primary sites were lung (19 patients) and breast (8 patients). The volume and equivalent spherical diameter of 57 tumors was 0.1–18.3 (median 1.67) ml and 6.0–34.6 (median 17.2) mm respectively. In SRS, all tumors were enclosed by the 31.0–80.0 (median 54.0) % isodose line with 1–3 isocenters and prescribed 15.0–30.0 (median 25.0) Gy on the margin, 18.8–80.6 (median 45.0) Gy at the hot spot. We defined the condition without tumor regrowth nor radiation necrosis after SRS as 'locally controlled'.

Results: The overall local control rate was 84.2% (48/57 lesions). The actuarial local control rate using the Kaplan-Meier method was 86.9% at 6 months and 68.4% at 12 and 24 months for all tumors. Tumor size (<20 mm vs. >20 mm), marginal dose (<20 Gy vs. >20 Gy), maximum dose (<45 Gy vs. >45 Gy) and ratio of marginal/maximum dose (<60% vs. >60%) were evaluated as factors which might influence the local effects. Although univariate analysis revealed tumor size (<20 mm, $p < 0.01$) and marginal dose (>20 Gy, $p < 0.03$) as significantly favorable factors for local control, tumor size is an only significant factor in multivariate analysis ($p < 0.05$). For the small tumors (<20 mm), the actuarial local control rate was 97.1% at 6 months and 84.9% at 12 and 24 months.

Conclusion: Linear accelerator based SRS is an effective treatment modality for brain metastases. Especially for small tumors (<20 mm), excellent local control can be achieved with SRS alone.

433 POSTER

Increased expression of heterodisperse Alu-containing transcripts in glioblastoma multiforme

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Purpose: Characterization of genes which are activated or repressed in astrocytomas should provide new data for understanding of the mechanisms of their arising and progression, may have potential implication for the prognosis and therapy.

Methods: Differential hybridization of high density cDNA filter arrays of human fetal brain cDNA library was used to identify genes with expression significantly changed in tumor cells as compared to normal brain.

Results: Among clones which displayed increased hybridization signals with glioblastoma multiforme cDNA, four clones contained Alu-repetitive sequences. Using these tumor-enhanced cDNAs as individual probes in Northern analysis, we detected no discrete bands in RNA from either normal human brain or astrocytic tumors; instead, hybridization to RNAs of all sizes (from 0.3 to 7.0 kb) was detected. The hybridization signals to glioblastoma multiforme RNA were much more intensive as compared to RNAs of anaplastic astrocytoma, normal adult and fetal brain, as well as some other fetal tissues. No changes in expression level of corresponding Alu-containing genes were revealed, when non-repeated adjacent sequences of cDNAs were used as hybridization probes. Further investigations showed the polymorphism of Alu-containing gene expression. If some samples of glioblastoma multiforme RNA included an abundant amounts of repetitive sequences, other ones did not display the increase of their contents.

Conclusion: The accumulation of high levels of heterodisperse RNAs with Alu-repetitive elements may suggest the abnormality of synthesis, degradation or processing of RNA in tumor cells. The deregulation of these processes may contribute to the progression of astrocytomas to glioblastoma multiforme.