

# Neuro-Oncology

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**Basic and Translational  
Investigations**

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**Clinical Investigations**

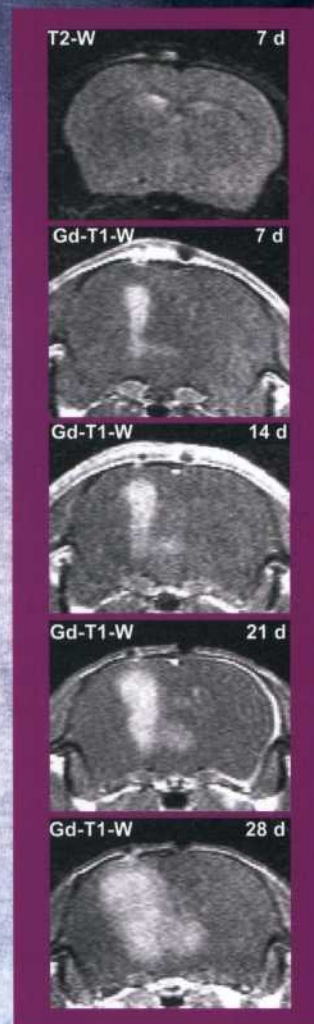
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**Review**

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**Abstracts from the World  
Federation of Neuro-Oncology  
Second Quadrennial Meeting  
and the Sixth Meeting of  
the European Association for  
Neuro-Oncology**

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Official Journal of the Society for Neuro-Oncology since 1997

Japan Society for Neuro-Oncology since 2000



European Association for Neuro-Oncology since 2002



World Federation of Neuro-Oncology Societies since 2002



NO with O<sub>2</sub> or with superoxide radicals (O<sub>2</sub><sup>-</sup>). The first was tested by measuring levels of guanyl cyclase and of cGMP, and the second by assay of nitrotyrosine formation, in NO-donor-stimulated cells of each grade. A clear correlation was shown between iNOS expression and the degree of tumor malignancy, with lowest expression seen in benign tumors and highest seen in those of anaplastic grade. In malignant meningioma cells both membrane-bound and soluble guanylyl cyclase (sGC) were un-regulatable, and the cGMP levels were very low despite significantly high levels of iNOS expression. In contrast, in benign meningioma cells, sGC responded to NO normally, and a marked increase of cGMP was observed upon NO donor stimulation. Thus, the downstream pathways of NO signaling in malignant meningioma appear to be shut down. In additional experiments, a significant increase in nitrotyrosine formation (a biomarker for cGMP-independent NO action) was detected in malignant cells. We conclude that iNOS expression correlates with degree of malignancy in meningiomas. However, in anaplastic meningiomas, cGMP-dependent signaling pathways are inactive, but cGMP-independent pathways are not. These data suggest that in malignant meningioma cells, iNOS overexpression may contribute to a survival advantage mediated by protein tyrosine nitration.

**66. MOLECULAR GENETIC STUDY FOR HEMANGIOBLASTOMAS AND FUNCTIONS OF VHL GENE**  
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Molecular genetic analysis for germline or somatic mutation of von Hippel-Lindau (VHL) gene in central nervous system (CNS) hemangioblastomas (HBs) with and without VHL disease contributes to surgical treatment and follow-up. Forty-nine patients bearing HBs (sporadic 33, VHL 16) underwent surgery and genetic diagnosis for germline and somatic VHL mutations. Locations of the tumors were cerebellum, 39; brain stem, 3; and spinal cord, 10. Fourteen of 16 VHL cases had multiple tumors while 3 of 32 sporadic cases had one tumor. Twelve sporadic HBs showed somatic mutations (missense 6, truncation-type 6) but not germline mutations. In addition, 24 sporadic HBs showed loss of heterozygosity (LOH) on 3p, in which the VHL gene is located. These results suggested that the inactivation of VHL genes on both alleles was a cause of genesis in the majority of sporadic hemangioblastomas and that the VHL gene functioned as a tumor suppressor gene. Eleven of 15 VHL cases showed VHL germline mutations (missense 8, truncation-type 4). Patients with truncation-type VHL germline mutation were more frequently associated with renal cell cancer (RCC). Causes of death were postoperative complications in 2 sporadic patients and tumor development in 2 VHL patients. Clinically ambiguous cases, whether sporadic or VHL, should be analyzed for VHL germline mutation. It might be recommended that HBs with VHL should be surgically treated if symptomatic, while asymptomatic small ones should be observed or treated with radiosurgery. Functions of the VHL gene include not only tumor suppression in HB as the above but also neuronal differentiation, which we demonstrated. Herein we show neuronal regeneration with donor of VHL-gene or peptide transferred stem cells (neural stem cell, bone marrow stromal cell, skin stem cell, ES cell). Transplantation with VHL-gene transferred stem cells dramatically improved symptoms of neuronal disease model rats (Parkinson, cerebral infarction, spinal injury), and they functioned as neurons in the brain. It was suggested that neuronal differentiation by VHL protein was related to ubiquitination and resolution of Notch under normoxia but not under hypoxia. In addition, synthetic VHL oligopeptide (elongin-binding site at a-domain) showed induction potential for neuronal differentiation equal to transduction with viral vector. In the future, neuronal regeneration with VHL gene or peptide would be useful for the clinical level.

**67. LIGAND-INDEPENDENT ACTIVATION OF THE EGFRvIII: A NATURALLY OCCURRING MUTATION OF THE EGFR COMMONLY EXPRESSED IN GLIOMA**  
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Mutations of the epidermal growth factor receptor (EGFR) gene are found at a relatively high frequency in glioma, with the most common being the de2-7 EGFR (or EGFRvIII). This mutation arises from an in-frame deletion of exons 2-7, which removes 267 amino acids from the extracellular domain of the receptor. Despite being unable to bind ligand, the de2-7 EGFR is constitutively active at a low level. Transfection of human glioma cells with the de2-7 EGFR has little effect in vitro, but when grown as tumor xenografts this mutated receptor imparts a dramatic growth advantage. We

have now mapped the phosphorylation pattern of de2-7 EGFR, both in vivo and in vitro, using a panel of antibodies unique to the different phosphorylated tyrosine residues. Phosphorylation of de2-7 EGFR was detected constitutively at all tyrosine sites surveyed both in vitro and in vivo, including tyrosine 845, a known target in the wild-type EGFR for src kinase. There was a substantial upregulation of phosphorylation at every tyrosine residue of the de2-7 EGFR when cells were grown in vivo compared to the receptor isolated from cells cultured in vitro. Upregulation of phosphorylation could be mimicked in vitro by the addition of specific components of the ECM such as collagen via an integrin-dependent mechanism. Since this increase in in vivo phosphorylation enhances de2-7 EGFR signaling, this observation explains why the growth enhancement mediated by de2-7 EGFR is largely restricted to the in vivo environment. In a second set of experiments we analyzed the interaction between EGFRvIII and ErbB2. Co-expression of these proteins in NR6 cells, a mouse fibroblast line devoid of ErbB family members, dramatically enhanced in vivo tumorigenicity of these cells compared to cells expressing either protein alone. Detailed analysis of these xenografts demonstrated that EGFRvIII could heterodimerize and transphosphorylate the ErbB2. Since both EGFRvIII and ErbB2 are commonly expressed at gliomas, this data suggests that the co-expression of these two proteins may enhance glioma tumorigenicity.

**68. LONG-TERM SURVIVAL IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED IN PHASE 2 STUDIES WITH ANP**

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The purpose of this study was to determine the frequency of long-term survivals in patients with glioblastoma multiforme (GBM) treated in FDA and Institutional Review Board monitored phase 2 studies with ANP (anti-neoplastons A10 and AS2-1). One hundred seventy-three patients with GBM who could be evaluated were accrued to FDA-monitored phase 2 trials. Seventy-nine patients were admitted to the study protocols (SP), and an additional group of 94 patients were treated under special exception (SE) because of low Karnofsky performance status (KPS), below 60. Ninety-eight percent of patients failed prior surgery, radiation therapy, and/or chemotherapy. ANP was given intravenously daily in escalating doses. The median duration of ANP administration was 4 months for SP and 3 months for SE, and the average dosage of A10 was 6.18 (SP) and 6.92 (SE) and of AS2-1 was 0.26 (SP) and 0.25 (SE) g/kg/d. Responses were assessed by gadolinium-enhanced MRIs and PET scans (as necessary). Long-term survival was defined as patients surviving 3 years after initial diagnosis. There was 15.5% long-term survival in the SP group and 7.1% in the SE group. The maximum survival in the SP group was more than 12 years and in the SE group was more than 10 years. Survival was significantly reduced in the SE group, which consisted of patients with lower KPS. The data indicate that more than 15% of evaluable patients with GBM treated with ANP in phase 2 studies were long-term survivors. The results are significantly worse in a group of patients with lower KPS, but compare favorably with radiation therapy and chemotherapy.

**69. RESPONSES OF THE ADULT MAMMALIAN CENTRAL NERVOUS SYSTEM TO EXPERIMENTAL INTRACRANIAL GLIOMA**

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In the brain, tumor cells and normal cells are the interacting elements of a two-part system connected by competition for physical space, extracellular matrix components, and secreted soluble factors. Our goal is to understand how normal brain cells respond to, and feed back onto, tumor cells. In the present experiments, U251 human glioma cells were injected into cortices of adult nude mice to induce experimental glioma. Brains were examined over time when tumors were 0.5 to 4 mm in diameter and occupying an increasing portion of the frontal area. Markers of cell type and status are examined by immunofluorescence in horizontal sections. Nestin immunoreactivity (ir) was detected in tumor and host cells and separated using species-specific antibodies (Chemicon; MAB353 for mouse, MAB5326 for human). On the ipsilateral side, nestin ir was observed in host cells along both lateral and medial ventricular walls, in cells positioned between the tumor mass and the pia, in cells dispersed around the tumor and just inside its edge, and in the corpus callosum and adjacent tumor. Some nestin-immunoreactive (nestin-ir) cells, especially those in the parenchyma lateral to the ventricle and inside the tumor mass, had the morphology of neural progenitor cells (NPCs). Other nestin-ir cells, particularly those interposed between the