optic nerve gliomas and neurofibromas, and increased risk of brain tumours [1, 2].

The NF1 gene product contains a GTPase activating protein (GAP) domain which is capable of downregulating the p21*ras* signal [3–5]: one exon encoding part of this GAP domain is characterised by the presence of a highly conserved phenylalanine–leucine arginine (FLR) motif [6]. Therefore, mutations in this region could affect the *ras* signalling pathway and contribute to tumorigenesis.

Interestingly, an altered lysine residue in the FLR exon of NF1 has been detected in three tumour types: colon adenocarcinoma, myelodysplastic syndrome and grade II astrocytoma (1 case out of 10 studied) and expression analysis demonstrated that GAP activities of these NF1 mutant proteins can be severely reduced [7].

It is well known that mutations of tumour suppressor genes, such as the p53 gene, can be involved in the development of tumours in the human nervous system [8]. However, the mutation reported by Li *et al.* [7] is the only one involving the NF1 gene in astrocytomas, the most frequent among brain tumours.

If NF1 mutations have a role in the neoplastic progression of astrocytomas one should expect to find them in glioblastomas (grade III astrocytomas), which represent the most malignant stage in such evolution.

In order to verify this hypothesis we have amplified the FLR exon in 18 cases of glioblastoma. Tumour DNA was prepared by phenol extraction [9] and two intronic oligonucleotide primers were used to amplify the FLR exon. The sense primer was NF1 (5′ CAAACCTTATACTCAATTCTCAACTC 3′) and the antisense primer NF2 (5′ AAGGGGAATTTAAGATAGCTAGATTATC 3′) [7]. Each polymerase chain reaction (PCR) mixture (100 μl) contained 2 μg of genomic DNA, 50 pmol of each primer, 200 μmol/l for each deoxynucleotide, 2 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris–Cl (pH 8.3) and gelatine 0.1% (w/v). Thirty-five cycles of amplification were carried out at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, with 5 min of initial denaturation, on a Trio Thermoblock incubator (Biometra). Direct sequencing was done by the authomatic method using "Taq Dydeoxy Terminator Cycle Sequencing Kit" and the Sequencer model 373A (Applied Biosystems). Primers used to amplify DNA fragments from the NF1 gene were also used for sequencing reactions.

Direct sequencing of both strands failed to identify any mutation. Therefore, if we consider our data together with those of Li *et al.* [7] only one out of the 28 astrocytomas examined is carrying a mutation of the FLR exon of NF1.

The number of patients examined can certainly be extended and the assessment of the role of NF1 alterations in astrocytomas requires a further study of the other domains in this large gene. Nevertheless, our data suggest that mutations of tumour suppressor genes others than NF1 may be crucial for the development of these highly fatal malignancies.

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Neurotoxicity Following Weekly Therapy with Folinic Acid and High-dose 5-Fluorouracil 24-h Infusion in Patients with Gastrointestinal Malignancies

Hans Josef Weh, Sybill Bittner, Mathias Hoffknecht and Dieter Kurt Hossfeld

IN RECENT months we have observed clinically relevant neurotoxicity in 4 out of 64 patients with various gastrointestinal malignancies treated weekly with folinic acid (FA) (500 mg/m^2) as a 1-h infusion and high-dose fluorouracil (5-FU) (2.600 mg/m^2) as a 24-h infusion [1].

The first patient, a 64-year-old male, had had several seizures of unknown aetiology in 1977, but since that time he has been seizure-free without medication. In May 1992 therapy with FA and 5-FU was started for an inoperable local relapse of a colon cancer. Six days after the fifth infusion the patient had a generalised seizure of grand mal type. Shortly thereafter, neurological examination and computed tomography of the brain were unremarkable. As the patient responded to the treatment, prophylactic anticonvulsant therapy with phenytoin was started and so far three additional 5-FU infusions have been given without any neurotoxicity.

The second patient, a previously healthy 60-year-old woman, had a left sided hemicolectomy in February 1990 for colon carcinoma. In June 1992 amyotrophic lateral sclerosis was highly suspected for muscular weakness with muscular atrophy and hyper-reflexia, sensations of discomfort in several muscles and visible fascicular twitchings of muscle fibres. Electroencephalogram (EEG) revealed signs of general increased activity. Cranial

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computed tomography (CCT) was normal. Since several hepatic metastases of the colon carcinoma were diagnosed at the same time, the neurological symptoms were considered to be possibly due to a paraneoplastic syndrome and therapy with FA/5-FU was started in September 1992.

At the end of the 5-FU infusion the patient developed various neurological symptoms with seizures, psychomotor epilepsy and local and temporal disorientation for several hours. She recovered completely within 24 h. EEG and CT were unchanged compared with findings in June 1992. Meanwhile, under phenytoin medication two further infusions of FA/5-FU have been given without any complications.

The third patient, a 64-year-old previously healthy male patient with inoperable hepatocellular carcinoma, received four infusions of FA/5-FU without any side effects. Three days after the fifth infusion, serious adverse effects occurred with diarrhoea grade 3, nausea grade 3, mucositis grade 2 and leukopenia grade 3. The patient was hospitalised for dehydration but in spite of parenteral hydration his clinical condition worsened. Six days after the last infusion of chemotherapy he developed a general seizure of grand mal type lasting for several minutes. Neurological examination some hours later was unremarkable as was the CT scan. In the following days stupor and coma developed without impairment of hepatic function and the patient died 17 days after the last chemotherapy. Autopsy was not performed.

The last patient, a 56-year-old female, received chemotherapy with FA/5-FU for carcinoma of the bile ducts with hepatic metastases. Twelve hours after the first 5-FU infusion the patient developed a severe headache. A transient blood pressure of 95/70 mmHg was found. Within hours a stroke-like picture developed with a left sided hemiplegia. CT scan and analysis of cerebrospinal fluid were unremarkable, but EEG revealed considerable right-sided disturbances.

The patient's clinical condition rapidly worsened and she expired 48 h after the end of the 5-FU infusion. Cerebral autopsy was completely unremarkable yielding no explanation for the clinical picture.

Neurotoxicity is a well known, but rather rare adverse effect of 5-FU [2, 3]. In the older literature the incidence ranges from 0.9 to 7% [2, 3]. It has been observed after 5-FU alone or in combination with allopurinol, folinic acid, thymidine and PALA [4–8]. The main symptoms are acute cerebellar ataxia, lethargy and seizures. Apparently, the only suspected risk factor for the occurence of neurotoxicity is the 5-FU dose intensity. The incidence of neurotoxicity seems to increase with higher 5-FU doses [2, 9]. Our findings with weekly FA/high-dose 5-FU also supports this assumption. The fact that in 2 of 3 patients with seizures after 5-FU previous neurological disturbances were known suggests that this fact may be—besides high-dose 5-FU intensity—another risk factor for this complication. Our finding that cytostatic therapy could be safely continued in patients with seizures when prophylactic anticonvulsant medication was used could be clinically important. Only the communication of other cases of neurotoxicity following this type of treatment may reveal the true incidence of this serious complication.

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EAP in Advanced Gastric Cancer

Ruggero Ridolfi, Donata Casadei Giunchi, Marinella Amadori, Maria Paola Innocenti, Roberta Maltoni and Dino Amadori

THE REPORT by Preusser *et al.* [1] stimulated interest in the treatment of advanced gastric cancer with a new chemotherapeutic regimen composed of etoposide, doxorubicin and cisplatin (EAP) [1].

Response rates ranging from 48 to 72% were reported by some authors [2–4] although these were associated with substantial toxicity. At lower toxicity levels, lower response rates of 0–15% were reported [4–7].

From January 1990 to December 1991 we treated 20 previously untreated advanced gastric cancer patients with EAP.

The patients were less than 70 years of age, were considered to have a life expectancy of >3 months and had histologically confirmed gastric cancer. There were 13 males and 7 females with a median age of 55 years (range 23–67).

6 patients had non-resectable disease and 14 had disease extension following surgery.

All patients received at least two cycles of treatment, with a maximum of six cycles in the case of response or stable disease.

2 patients (10%) obtained complete response (CR) and 1 (5%) partial response (PR) with a respective duration of 7, 6 and 7 months.

Severe toxicity resulted in 1 case of disseminated intravascular coagulation (DIC) (rapidly resolved), 3 cases of leuco-thrombocytopenia, 1 case of diarrhoea, 8 cases of nausea/vomiting and

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