Expression of p53 and p21 in Primary Glioblastomas

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Background and Purpose: Primary glioblastomas (GBMs) are highly radioresistant, and in contrast to secondary GBMs, they bear wild-type (wt) p53 protein, which is stabilized in a proportion of these tumors. Therefore, it was investigated in vivo whether p53 expression has prognostic value in patients undergoing radiochemotherapy. Additionally, the authors tried to identify, in vitro, subgroups of primary GBM with different susceptibilities to irradiation, on the basis of their p53 and p21 responses to ionizing radiation.

Material and Methods: Tumor tissue samples from 31 patients suffering from primary GBM undergoing a combined radiochemotherapy with topotecan were investigated. The percentage of cells expressing p53 protein was determined immunohistochemically. Additionally, primary cultures from eleven primary GBMs were established and investigated. p53 and p21 expressions were evaluated before irradiation with 10 Gy and at 2 and 8 h after irradiation. p53 protein expression was measured by Western analysis and p21 mRNA expression by reverse transcription-polymerase chain reaction (RT-PCR).

Results: The percentage of p53-positive cells within the tumor specimens obtained from the 31 patients ranged from 0% to 28%, the median value being 4.3%. No significant correlation with disease-free survival or overall survival was found. In vitro, p53 protein was detected in seven of eleven cultures from primary GBM. After irradiation a decrease in p53 protein expression was seen in six of the seven p53-positive cultures. Half of the cultures (two of four) without basal p53 expression showed an increase in p53 expression after irradiation. Basal overexpression of p21 was detected in six of the eleven cultures; in four out of six irradiation led to a decrease in p21 expression. In all cell lines (five of eleven) initially showing absent p21 expression, irradiation induced p21 expression. Despite these responses, G1 arrest was not detectable in any of the GBM cultures.

Conclusion: p53 protein expression in vivo does not correlate with the outcome of patients with primary GBM. Therefore, p53 protein content per se does not appear to be a helpful prognostic factor for prognosis-adapted therapy in primary GBM. By contrast, primary GBM cells in vitro show different and independent responses in their p53 and p21 pathways to ionizing radiation. The failure of G1 arrest seems to be due to a functional defect in the p53 pathway, either because p21 was not induced or because of an unidentified defect downstream from p21.

Key Words: Glioblastoma · p53 · p21 · Prognostic factors


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**Introduction**

Despite many advances in oncology in recent years, the prognosis of patients with glioblastoma (GBM) remains distressingly poor. Operative resection forms the basis of treatment but it is not feasible to effectively resect beyond the limits of macroscopically visible tumor without drastically compromising neurologic function. Adjuvant radiotherapy, however, provides an improvement in the prognosis, increasing the median survival time to about 1 year [13, 14, 24, 28, 30, 39]. The limited efficacy of radiation treatment is believed to arise from the poor response and apoptosis to ionizing radiation in the tumor cells [17]. The search for novel therapeutic strategies in GBM requires clearer insight into the mechanisms involved in the cellular response to ionizing radiation.

A distinction between different GBMs on clinical grounds has long been recognized. Approximately 75–80% of GBMs arise de novo, without history of progression from a grade II or III glial tumor, and are referred to as primary GBMs. These tumors are genetically characterized by a loss of chromosome 10 and epidermal growth factor receptor (EGFR) gene amplification occurring predominantly in older patients and exclusively in GBMs, being demonstrable in neither grade II nor grade III astrocytomas [25, 38]. By contrast, approximately 20–25% of GBMs show progression from astrocytoma or anaplastic astrocytoma and are clinically referred to as secondary GBMs. This second genetic subgroup of GBM displays mutations in the tumor suppressor gene TP53 [31]. Notably, amplification of EGFR almost never occurs in GBMs with TP53 mutations [33], thus creating two mutually exclusive genetic groups. Histologically, primary and secondary GBMs cannot be distinguished from each other, as both exhibit tumor necrosis, vascular endothelial proliferation, and cellular atypia.

The mutant TP53 present in secondary GBMs readily explains their radioresistance. Primary GBMs, however, are equally radioresistant [2, 8, 22], a fact not easily explicable on the grounds of their known genetic alterations. An intriguing finding in approximately 30% of primary GBMs is an accumulation of wild-type (wt) p53 protein [35]. Although the functional significance of this stabilized p53 protein is not understood, it raises the possibility that p53 functions abnormally in at least some primary GBMs, despite the presence of an intact TP53 gene. Thus, a functional defect in p53 or its pathway might account for the genetic instability and radioresistance of primary GBMs [32].

The tumor suppressor gene TP53 has been shown to play a pivotal role in bringing about an arrest at the G1/S transition [5, 41]. Activation of p53 by DNA damage leads to the transcriptional activation of p21, an inhibitor of the cyclin-dependent kinases regarded as the “engine of the cell cycle”, responsible for driving the cell through the various phases of the cycle when bound to its respective cyclin [6]. Since G1 arrest can be induced by the overexpression of p21 and, furthermore, is abrogated in irradiated p21-deficient cells [3, 40], p21 seems to be the key effector of the G1 arrest in response to DNA damage. However, the question of whether p21 can be induced independently of p53 is not fully answered. While some groups showed that p21 is solely dependent on p53 for its induction, for example in human peripheral neuroepithelial tumor cell lines [15], other groups maintain that p21 can be induced independently of p53 [18].

Therefore, the aim of this project was to determine the impact of p53 status on intracellular pathways in primary GBM cells, and G1 arrest in vitro. Additionally, we investigated the value of p53 expression on survival rates in vivo in primary GBM treated with simultaneous radiochemotherapy.

**Material and Methods**

**Patients and Methods**

A total of 31 patients with histologically confirmed supratentorial primary GBM without a known history of low-grade tumor were enrolled in the study. A total dose of 60 Gy in daily fractions of 2.0 Gy with simultaneous chemotherapy with 0.5 mg topotecan (Hycamtin®) intravenously administered 1 h prior to each irradiation was given as described previously [10]. The first follow-up occurred 6 weeks after therapy had been completed. Subsequent follow-ups were scheduled every 3 months. In addition to clinical investigations and monitoring of indices, a radiologic examination was undertaken to detect possible relapses. Disease progression was defined according to WHO criteria by either an increase of at least 25% in tumor size, any new tumor identified by CT or MRI scan, or neurologic worsening.