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High expression of DNA topoisomerase IIα and Ki-67 antigen is associated with prolonged survival in glioblastoma patients

Markus Bredel[a,1], Maria Piribauer[b], Christine Marosi[b], Peter Birner[c], Brigitte Gatterbauer[d], Ingeborg Fischer[a], Thomas Ströbel[a], Karl Rössler[d], Herbert Budka[a], Johannes A. Hainfellner[a,*]

[a]*Institute of Neurology, University of Vienna, AKH 4J, Währinger Gürtel 18-20, POB 48, A-1097 Vienna, Austria*
[b]*Department of Internal Medicine I—Oncology, University of Vienna, A-1097 Vienna, Austria*
[c]*Institute of Clinical Pathology, University of Vienna, A-1097 Vienna, Austria*
[d]*Department of Neurosurgery, University of Vienna, A-1097 Vienna, Austria*

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Abstract

Assessment of tumour cell proliferation in glioblastoma (GB) has been a topic of considerable research interest over the past decade. However, the correlation of tumour proliferation and patient outcome has yielded controversial results. In this study, we examined immunohistochemically, using paraffin-embedded tissue, the expression of the proliferation-related markers DNA topoisomerase IIα (TIIα) and Ki-67 antigen in a cohort of 114 GB patients treated consecutively with surgery and radiochemotherapy, and correlated the expression with patient outcome. The TIIα labelling index (LI) ranged between 5.2 and 87.2% (median: 25.6%). Survival analysis disclosed an association between high TIIα expression levels and prolonged survival ($P=0.040$, log-rank test). TIIα expression correlates closely with Ki-67 labelling index ($R=0.927$, $P<0.001$), which itself is predictive of patient survival ($P=0.044$). However, in multivariate analysis, only the Karnofsky performance status remained predictive of patient survival. We conclude that high expression of TIIα and Ki-67 appears to be associated with a prolonged survival in our cohort of GB patients.

Keywords: Glioblastoma; Immunohistochemistry; Cell proliferation; Ki-67 antigen; Topoisomerase IIα; Survival

1. Introduction

Assessment of proliferative activity in glioblastoma (GB) has been the subject of considerable research interest during the last decade [1,2]. The monoclonal MIB-1 antibody, which labels the Ki-67 nuclear antigen expressed in the G_1, S, G_2 and M phases of the cell cycle, has been used extensively as an indicator of cellular proliferation in GBs [3]. Recently, immunohistochemical analysis of DNA topoisomerase IIα (TIIα) expression has been shown to reliably identify the percentage of cycling cells in human gliomas [4,5]. Monoclonal antibodies against this enzyme label cells in the S, G_2 and M phases of the cell cycle and also partly those in the G_1 phase [5].

To date, studies examining the relationship between immunohistochemical proliferation markers and patient outcome in human gliomas have generated controversial results. While in some studies the MIB-1 proliferation index was inversely related to patient survival [6–13], others have failed to show such a link [14–19]. TIIα expression in GBs has also been examined. However, a conclusive link between TIIα expression and outcome remains to be defined for GB patients, because of the small number of investigated patients [4,5,20].

In our study, we examined TIIα and Ki-67 expression immunohistochemically in a retrospective cohort of 114 GB patients treated consecutively at the University

* Corresponding author. Tel.: +43-1-40400-5507 or -5501; fax: +43-1-40400-5511.
E-mail address: johannes.hainfellner@akh-wien.ac.at (J.A. Hainfellner).
[1] Present address: Department of General Neurosurgery, Neurocenter, University of Freiburg, Germany.

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Hospital of Vienna between 1995 and 1999 and correlated the expression with patient survival.

2. Patients and methods

2.1. Clinical patient data

114 consecutive adult patients diagnosed with primary GB between 1995 and 1999 at the University Hospital of Vienna were retrospectively identified. In this population-based investigation, all patients who underwent initial surgery of primary GB were included. Patient age at diagnosis ranged from 35 to 78 years (mean age at primary surgery: 60 years). 37 patients underwent a gross total tumour resection and 67 a subtotal resection. In 10 patients, no information on the extent of resection was available. Adjuvant therapy generally consisted of combined radio- and chemotherapy. Before chemotherapy and radiotherapy, written informed consent was mandatory. 89 patients (78.1%) received adjuvant chemo- and radiotherapy, 3 patients (2.6%) only radiotherapy, and 1 patient (0.9%) only chemotherapy. In 11 patients (9.6%) no adjuvant therapy was administered, and no information on adjuvant therapy was available in 10 patients (8.8%). Chemotherapy was started 10–14 days after surgery. Eligibility criteria for the chemotherapy treatment were as follows: patients were required to be older than 18 years and to have a Karnofsky index $>60\%$. Adequate liver function (with asparate aminotransferase (SGOT), alanine aminotransferase (SGPT) and alkaline phosphatase levels 2 times below the normal range; bilirubin in the serum below 25.65 μmol/l) as well as renal function (with creatinine level 1.5 times below the normal range) and bone marrow function (leucocyte count $>3\times10^9$ cell/l, haemoglobin >100 g/l, platelet count $>100\times10^9$ cell/l) were required. Pregnant or nursing women, as well as patients with acute infections, were not eligible. Adequate contraception was mandatory. 88 patients received one of the nitrosourea-based regimens and 1 patient was treated with carboplatin and etoposide as first-line therapy. First-line chemotherapy consisted of six cycles. Due to tumour progression, 48/89 patients did not complete all six cycles. In patients older than 50 years and/or Karnofsky performance status of <70, 1-(2-choloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) was given orally at 100 mg/m^2 in 6–8 week intervals ($n=70$). Patients younger than 50 years and with Karnofsky performance status of >70 ($n=17$) received a combination of Dacarbazine (200 mg/m^2) and Fotemustine (100 mg/m^2) every 3 weeks intravenously (i.v.). One patient received 1-(4-amino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU) i.v. at 100 mg/m^2 in 6-week intervals. Postoperative irradiation was applied at a total dose of 66 Gy (2 Gy/fraction) or 51 Gy (3 Gy/fraction) within 5–6 weeks. Postoperative Karnofsky performance status was determined 10–14 days after surgery. Patient outcome was assessed using neuro-imaging, hospital and outpatient charts, and by telephone interview. Follow-up ranged from 5 to 1509 days (median: 397 days).

2.2. Tissue specimens and tumour histopathology

Haematoxylin-eosin stained sections of all tumour specimens were reviewed by a neuropathologist. All specimens were graded by standard criteria of the World Health Organization (WHO) and were confirmed to be GBs [21].

2.3. Immunohistochemistry

Immunohistochemical analysis was performed on paraffin-embedded tissue sections. The monoclonal antibodies JH2.7 (Neomarkers) against TIIα and MIB-1 (Dianova) against the Ki-67 antigen were used as primary antibodies. Sections were cut at a thickness of 4 μm and antigen retrieval was performed by boiling sections in citrate buffer (pH 6.0, 20 min for JH2.7; pH 6.6, 10 min for MIB-1). Antibody binding was visualised via the ABC technique and using DAB as chromogen.

JH2.7 and MIB-1 immunolabelling was assessed on subsequent sections. JH2.7 and MIB-1 binding was apparent as nuclear staining. In each specimen, a total of 500 tumour cell nuclei were evaluated in fields showing the highest density of TIIα and Ki-67 immunopositive cells. The fraction of labelled tumour cell nuclei was expressed as a percentage.

2.4. Statistical analysis

Spearman's coefficient of correlation, Kruskal–Wallis test, Mann–Whitney test, Chi-square test, and Fisher's Exact test were used as appropriate. Survival analysis was based on all of the patients, regardless of the adjuvant therapy administered. Since the vast majority of patients received both adjuvant radio- and chemotherapy it is impossible to distinguish between the effect of chemo- and radiotherapy in our collective. Univariate analysis of overall patient survival was performed as outlined by Kaplan and Meier [22]. Overall survival was defined from the day of operation until patient death. Death from a cause other than GB or survival until the end of the observation period were considered as censoring events. Multivariate survival analysis was performed using the Cox's proportional hazard model [23]. Patient age, postoperative Karnofsky performance status, extent of resection, and Ki-67 and TIIα labelling indices (LI) were entered into the Cox regression analysis.

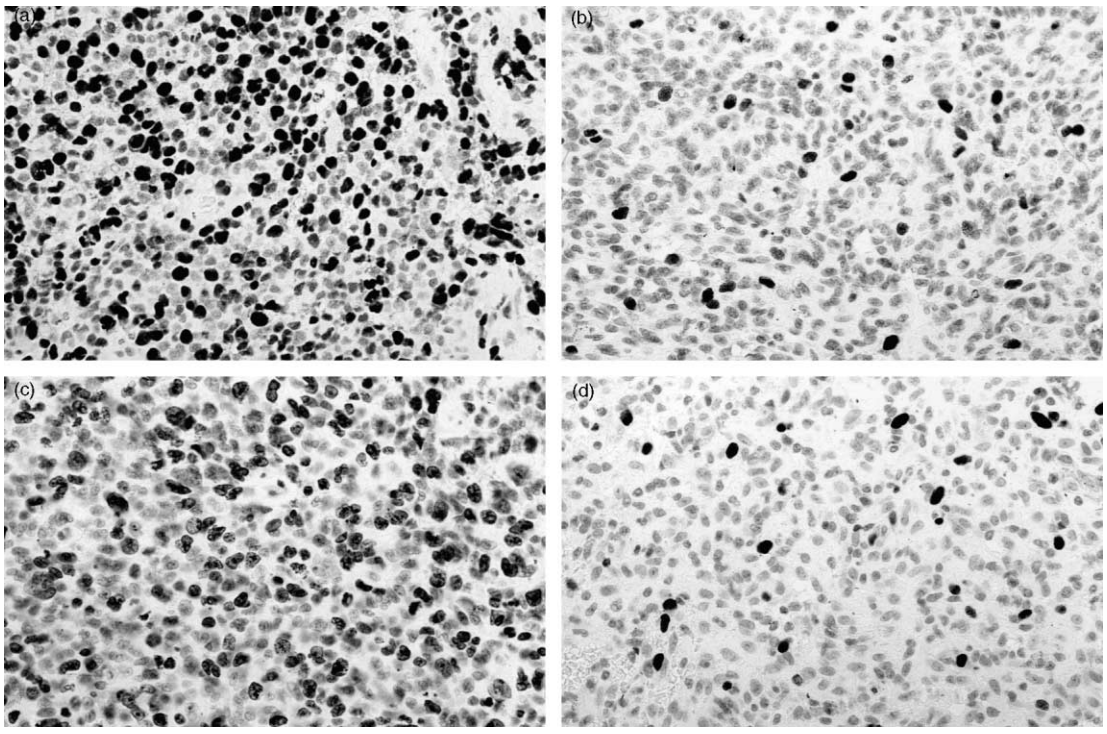

Fig. 1. Representative nuclear topoisomerase IIα (TIIα) and Ki-67 immunolabelling in two tumour specimens, one with increased (a) TIIα and (c) Ki-67 labelling index (LI); versus another with low (b) TIIα and (d) Ki-67 LI.

3. Results

3.1. TIIα and MIB-1 LIs

In 3/114 cases, immunohistochemical analysis was not possible due to a lack of material. In 111 cases, TIIα and MIB-1 LIs ranged from 5.2 to 87.2% and from 3.3 to 92.2%, respectively (Fig. 1). The mean TIIα LI± standard deviation (S.D.) was 29.9±16.8 (median: 25.6), the mean Ki-67 LI±S.D. was 29.8±16.5 (median: 26.7). The median was used to separate cases with low or increased Ki-67 or TIIα LIs. A sizeable portion of specimens ($n=57$; 51.4%) exhibited low TIIα LIs of $\leqslant 26.0$, and a slightly smaller portion ($n=54$; 48.6%) showed increased TIIα LIs of >26.0. Similarly, Ki-67 LI was low ($\leqslant 27.0$) in 58 patients (52.3%) and enhanced (>27.0) in 53 patients (47.7%). Fig. 1 shows representative immunohistochemistry results in a patient with increased TIIα and Ki-67 LIs, and in a patient with low LIs TIIα and Ki-67. An association between TIIα and Ki-67 LIs was apparent that approached high statistical significance ($R=0.927$, $P<0.001$, Spearman's coefficient of correlation) (Fig. 2).

3.2. Relationship between TIIα and Ki-67 LIs and clinical variables

There was no correlation between TIIα or Ki-67 labelling and the Karnofsky index, patient gender, tumour location and extent of resection ($P>0.05$). However, there was a very weak negative correlation between TIIα LI and patient age ($P=0.021$, $R=-0.219$, Spearman's coefficient of correlation), with higher TIIα LIs being present in younger patients. Ki-67 staining was not related to patient age ($P>0.05$). In another study analysing a smaller number of GB patients, an association between Ki-67 LI and patient age has been reported [24]. For a correlation with patient outcome, we divided the study cohort into two subgroups based

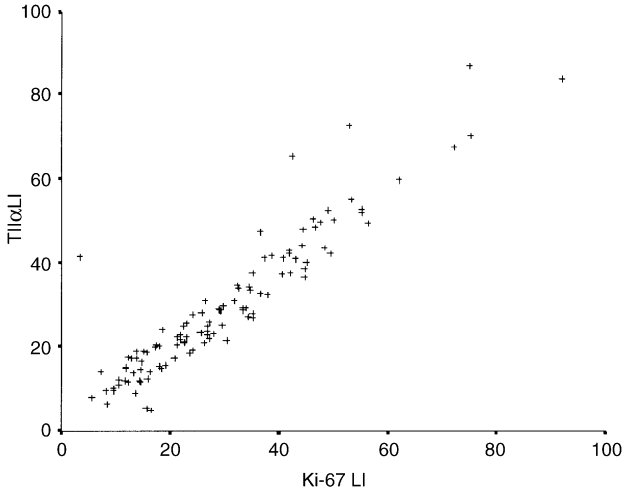

Fig. 2. Scatterplot showing the significant correlation between topisomerase IIα (TIIα) and Ki-67 labelling indices (LIs) in a cohort of 114 glioblastoma (GB) cases. The correlation coefficient determined was $R=0.927$ ($P<0.001$, Spearman's coefficient of correlation).

on the medians of the TIIα and Ki-67 LIs as explained above, and performed a univariate survival analysis. Patients with high TIIα or Ki-67 LIs had a significantly prolonged survival ($P=0.040$ and $P=0.044$, respectively, log-rank test) (Fig. 3). Median overall survivals among patients with high TIIα and Ki-67 levels were 58 and 57 weeks, respectively, compared with only 43 and 44 weeks, respectively, in the low-level groups. The 1-year survival rates for the TIIα and Ki-67 high-expression subgroups were 57.74 and 55.62%, respectively, compared with 39.41 and 41.27%, respectively, in the low-expression subgroups. Besides TIIα and Ki-67, high Karnofsky performance status (cut-off point: 80) and young patient age (cut-off point: 60 years) were also associated with prolonged survival ($P=0.0006$ and $P=0.00304$, respectively, log-rank test). At multivariate analysis, only the Karnofsky performance status remained predictive of patient survival ($P=0.008$, Cox's proportional hazard model).

4. Discussion

Constituting approximately 25% of all intracranial tumours, GB is the most common human brain tumour. This highly malignant neoplasm expands quickly and without therapy 95% of patients die within 3 months of diagnosis. Due to extensive invasion and diffuse infiltative growth to the surrounding areas complete surgical resection is not feasible. As a consequence, surgery and adjuvant therapies have extended median survival only up to 1 year. As in a host of other tumours, MIB-1 labelling of the Ki-67 antigen is highly indicative of the fraction of cycling cells in GBs. As expected, some clinicopathological studies showed an inverse relationship between patient survival and Ki-67 LI [6–13], whereas other studies failed to show such a link [14–19,25]. In this study, we examined the relationship between TIIα expression and patient outcome in a large consecutive series of GBs. TIIα expression and patient

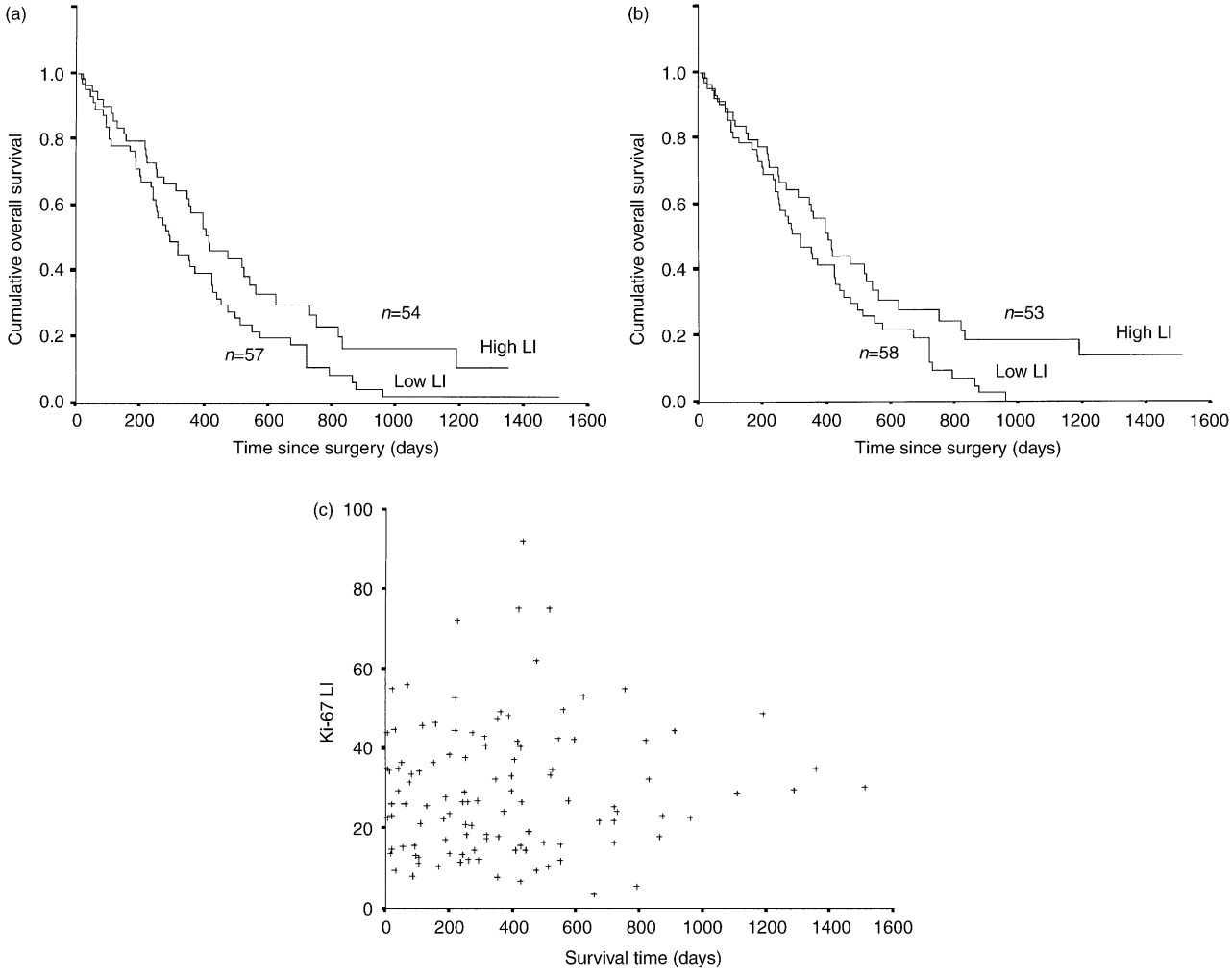

Fig. 3. The relationship between topisomerase (a) IIα (TIIα) and (b) Ki-67 labelling index (LI) and patient survival: Patients with increased TIIα or Ki-67 LIs (LI cut-off points: 26 and 27, respectively) showed a significantly prolonged survival compared with patients with low LIs ($P=0.040$ and $P=0.044$, respectively, log-rank test); (c) shows a scatterplot demonstrating the relationship between survival and Ki-67 LI (as a continuous variable).

outcome has so far been examined in only two small series of glioma patients that comprised both low-grade and high-grade tumours [4,5]. Our results show a significant correlation between prolonged patient survival and high TIIα and Ki-67 LIs. The data of our study suggest that a high percentage of cycling cells may render a GB more prone to the cytotoxic effects of radiotherapy and chemotherapy [26]. Hence, patients with highly proliferative tumours may profit particularly from their adjuvant treatment. Such an association of cell proliferation and survival in GB is reminiscent of the scenario in other highly proliferative tumour types, e.g. lymphomas [27].

The results of our survival analysis contrast with prior studies in GB [6–13]. A possible explanation are differences in applied therapeutic regimens. Another possible explanation are differences in the patient populations in the different studies.

In conclusion, the finding of note in the current study is the observation that high expression levels of TIIα and Ki-67 are associated with a prolonged survival in our cohort of GB patients. A plausible explanation is a particularly beneficial effect of adjuvant treatment in GB patients with highly proliferative tumours. However, such an interpretation needs confirmation by further studies.

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