Brain-tumor cell kinetics correlated with survival

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In a review of 38 glioblastoma patients for whom fresh tissue kinetic data were available before any therapy was instituted, no correlation between the labeling index and survival time could be statistically determined. This relationship seems entirely consistent with the published theoretical determinants of tumor behavior: that is, altered ability for growth arrest and differentiation, constantly evolving mutant sublines, genetic instability, and an ever-changing metabolic and vascular environment.

KEY WORDS • glioblastoma multiforme • brain neoplasm • cell kinetics • prognosis • survival time

ALIGNANT gliomas present a difficult therapeutic problem and a limited median survival time. Estimates of occurrence of this entity range between 11,000 and 15,000 new tumors per year. Research by others has suggested that prognostication of survival time might be possible based on certain cell cycle parameters, and there has been speculation relative to the value of this prognostic method as it pertains to chemotherapeutic treatment protocols. Previous papers on the subject have tended to indicate that a single kinetic parameter (labeling index) would facilitate major subdivisions for biological classification which, in addition to prognosticating survival times, would indicate a degree of malignancy and perhaps replace the time-honored Kernohan grading system.

Preliminary studies in our laboratories indicated a much wider range of labeling indices for the pure glioblastoma (all histologically verified and peer-reviewed) than had previously been noted by other authors. It was our presumption that it would be unlikely for a single determinant to reflect the overall behavior of a tumor and thus indicate the degree of malignancy (and hence survival time). This presumption is reflected in previous publications by Hoshino and Wilson when they indicated "... while a high labeling index reflects actively proliferating tissue, equal labeling indices do not mean equal proliferative activity;" and "... even if the labeling indices of the two tumors are the same, their doubling times may differ dramatically because of differing growth fractions and rates of cell loss." Based on this presumption, an expanded experience with glioblastoma cell kinetics was reviewed (specifically, the labeling index and its correlation with survival time) in a homogeneous group of treated glioblastoma patients.

Clinical Material and Methods

The labeling indices of 172 patients harboring a variety of central nervous system (CNS) glial tumors were reviewed. From this group, 38 patients met the following criteria and were included in this study: 1) fresh tumor tissue for kinetic evaluation was available from the first intracranial procedure and prior to all therapy; 2) a histological diagnosis of glioblastoma multiforme was unequivocal and was reviewed by the Brain Tumor Cooperative Group (BTCG) pathology central office; 3) an adequate follow-up period was available, allowing a declaration of "time to treatment failure" based upon either a 30-point drop in Karnofsky performance status, or a combination of a 25% increase in tumor diameter as determined by computerized tomography (CT) scanning and a 20% drop in Karnofsky score; 4) the patient had received radiation therapy and some form of chemotherapy (more than two courses) as a participant in a BTCG national clinical trial; and 5) clinical treatment failure and death were related to CNS causes only. All records were reviewed to determine patient's age and sex, tumor location, postoperative Karnofsky score, time to failure declaration, labeling index, and total survival time. None of the patients were alive at the time of writing.
the most proliferative component. Cellular concentrations were recorded if each of their nuclei contained more than 3 grains/cell. Labeled cells having indices of less than 1% were developed and examined, and the average of the range of LI (%) was 0.20–4.8, 5.2–17.9. Postoperatively reviewed glioblastomas, the labeling indices ranged from 0.20% to 17.9%, with a median value of 4.40%. Median survival time for the 20 patients with labeling indices of less than 5% was 9.75 months while the 18 patients with labeling indices of greater than 5% survived a median of 9.5 months. Median time to failure declaration (based on the above criteria) was 7.1 months for patients with labeling indices of less than 5%, and 6.9 months for those with a labeling index of greater than 5% (Table 1). The labeling index was then plotted as an independent variable against total survival time and to declared failure. All data were subjected to standard statistical analysis, including linear regression analysis. No correlations were observed in any of the parameters alluded to above. Life table and survival probability curves for labeling indices above and below 5% again reflect the absence of any statistical differences (Fig. 1). Age, sex, and postoperative Karnofsky scores of these two groups of patients were not significantly different.

Although cell cycle times were measured and growth fractions were determined for eight of the 38 specimens (three with a labeling index of < 5% and five with a labeling index of > 5%), the insufficient number of samples made statistical evaluation invalid. However, an interesting gross difference between growth fractions for the two groups was appreciated (those with a labeling index of < 5% had a median 18.9% growth fraction and those with a labeling index of > 5% had a median 32% growth fraction).

Discussion

The concept of survival time prognostication in malignant glioma patients based upon cell kinetics, specifically the labeling index, was advanced by Hoshino and Wilson in a series of papers beginning in 1972.9–15,29 Their reports detail data collected from a heterogeneous group of glioma patients after either intra-arterial or intravenous injections of tritiated thymidine. Growth fractions and cell cycle times were calculated from these observations. The results showed that the labeling index in the glioblastoma multiforme group was greater than 5% in all cases except one, in which it was 4.5%. Labeling indices in “anaplastic astrocytomas” were 1% to 4%, with lower, more differentiated grades of tumor having indices of less than 1%. It was concluded by these authors that the most striking finding was the
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prognostic value of the labeling index. Furthermore, they reported that virtually all patients with a labeling index of greater than 5% (presumably all glioblastomas) died within 6 months of the operative procedure from which the labeling index was determined (median survival time 3 months). Labeling indices of less than 5% were associated with improved survival times (median survival time 13 months). They concluded "...the demonstrated prognostic value of labeling indices obtained at biopsy should stimulate the rapid development of automated methods for obtaining this parameter." Of interest was their observation of the presence of labeled cells in tumors reoperated months or years after the original labeling procedure, indicating a latent potential for cell differentiation and the possibility of movement of cells in and out of the nonproliferating pool.

Our results in a homogeneous population of glioblastoma patients seem at variance with these published conclusions about the predictive value and range of the labeling index and hence with the correlation between it and the degree of histological malignancy and subsequent survival time. This disparity is believed not to be due to the difference between in vivo and in vitro determinations of the labeling indices, inasmuch as when the technique used in this study was applied to other solid tumor systems it demonstrated no essential difference. Clearly, the data presented in this report indicate labeling indices for histologically confirmed, non-necrotic portions of the glioblastoma to fall in the range considered by Hoshino and Wilson to be part of the benign astrocytoma group (recognizing that some tissue sampling error may exist in all reported studies). Even then, in our series the two patients with the longest survival times (23.5 and 19.5 months) had labeling indices of 8.4% and 11.2%, respectively.

A partial explanation for the discrepancy between our findings and previous data may be in the mitotic index, where this parameter in mammalian tumors (both in animals and in man) is actually longer than those of their normal tissue counterparts (2 hours in some tumors, 45 minutes in the normal tissue). Tumor growth therefore must involve more than just cell proliferation. Furthermore, it has been shown that the determinant of tumor growth and survival times may not be related to the cell cycle time or the number of cells in division at any given time, but rather be related to the overall result of division (usually referred to as "net cell increase"). In tissue culture and perhaps in the human, growth of normal cells may be restricted by nutritional limitation or by cell-to-cell contact in a tightly compressed mass. Under such conditions, cells may stop dividing and enter a quiescent (Go) state. These cells may reenter the proliferating pool after decompression by an extensive cytoreductive effort, but this is yet to be documented. In addition to replenishment of nutrients, cells require specific growth factors (polypeptides and hormones) to accomplish reentry into the cell cycle. Chemically transformed cells will cease active mitosis when deprived of nutrition, but may remain in the G1 (arrested) phase. The simple addition of nutritional requirements in this setting seems to permit resumption of the active cycle state. Virally transformed cells continue to synthesize deoxyribonucleic acid (DNA) until their nutritional requirements are no longer met; they then become nonviable.

In addition to restriction by nutritional factors and cell-to-cell contact, control of cell growth in vivo involves the coupling of two processes: growth arrest and differentiation. Scott and Florine demonstrated five phases of these phenomena, and theorized that cells not only have the ability to undergo growth arrest and subsequent differentiation, but can also retain the potential to proliferate and expand within the tumor mass under the proper circumstances. Thus, tumor progression may be related to an acquired genetic liability that allows for selection of hardy, proliferative, variant sublines originating from a stem cell or small population of cells with a selective growth advantage over other abnormal cells. Continued selective proliferation coupled with genetic instability within the expanding tumor population then leads to the production of mutants, many of which die either as a result of metabolic and/or vascular disadvantage or perhaps as a result of some immunological factor. It seems reasonable to assume, however, that a variant with increasing aneuploidy, aberrant metabolic behavior, and increasing genetic abnormalities has a selective advantage over other tumor cells to ultimately become the predominant new subpopulation. This concept has been supported by Bigner in his examination of several existing glioblastoma cell lines and by the observations of Black, et al. Hence, glial neoplasms seem to have the capability of continued variation with increasing frequency within any given tumor, allowing a significant capacity for the generation of mutant sublines and resistance to the therapeutic modalities employed, none of which may be determined by a single pretreatment tissue kinetic sample. As a result, tumor repopulation and progressive malignancy may not be related to the percentage of cells undergoing division at any given time, but rather to the variant cell types produced and the number of cells surviving the division process (cell loss factor).

For a single determinant to have true prognostic value it must reflect not only the net cell increase, but mutant subtypes and the evolutionary process that selects for cellular and genetic instability as well as therapeutic resistance. As described above, Hoshino and Wilson were able to identify labeled cells several months and, in one case, several years after the in vivo labeling procedure. This may indicate the ability of some genetically unstable cells in malignant gliomas to retain their latent capacity for differentiation and repopulation of the tumor bed, having remained dormant for a protracted period of time.

Hence, it would appear that a single kinetic determination such as the labeling index cannot alone pre-
dict the many cellular options and biological variations alluded to above, nor prognosticate the degree of malignant glioma patients.

References

J. W. Bookwalter III, et al.