Long-Term Survival of a Patient with Glioblastoma

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Glioblastoma (GB) accounts for 50-60% of all intracranial gliomas and has a median survival of 13.6 months even with aggressive surgical resection, radiation and chemotherapy[1]. This dismal prognosis is compounded by the failure of novel therapies to yield more than minimal prognostic improvements. A major advance was adjuvant temozolamide treatment but this increased median survival in multi-centre trials by only 2.5 months[2-4].

Prognostic indicators have been identified for GB and include young age, female sex, good Karnofsky performance status, absence of focal neurological deficits and complete surgical resection[1,5-9]. Histological characteristics such as the presence of oligodendroglial components and giant cells have also been associated with improved survival[7,9,10]. Genetic and chromosomal alterations have been examined as prognostic indicators and therapeutic targets. Methyl-guanine-methyl-transferase (MGMT) hypermethylation is the best established of these and predicts both survival and response to alkylating agents including procarbazine/lomustine/vincristine and temozolamide[2,7]. Less well accepted markers of favourable prognosis include p53 mutation, epidermal growth factor receptor amplification and losses of chromosomes 1p and 19q[8,11-13] whereas controversial markers of poor prognosis include gains of chromosome 7, loss of chromosome 9p/10p/10q and mdm2 overexpression[8,11,12].

Despite a poor prognosis, 2% of patients with GB survive more than three years[5,6,14-16]. It might be expected that these patients would have identifiable molecular markers; however, no distinct markers consistently present in long-term survivors have been established and the mechanism behind the improved survival remains unknown.

Case Report

A 49-year-old male presented with a three-month history or worsening ataxia and nausea. On neurological examination, the patient had mild right upper limb intention tremor and dysdiadochokinesis. Magnetic resonance imaging (MRI) revealed an irregular, contrast-enhancing mass in the right cerebellar hemisphere measuring about 4 cm in diameter (Figure 1). A paramedian suboccipital craniectomy was performed and resection attempted. The mass was markedly vascular and abutted on the tentorium superiorly. The other margins were quite well defined but after partial resection and loss of several liters of blood, the resection was aborted. The patient was sedated and intubated overnight and resection was completed relatively easily the next day. Complete resection was confirmed by absence of enhancement around the postoperative resection cavity on MRI one day later.

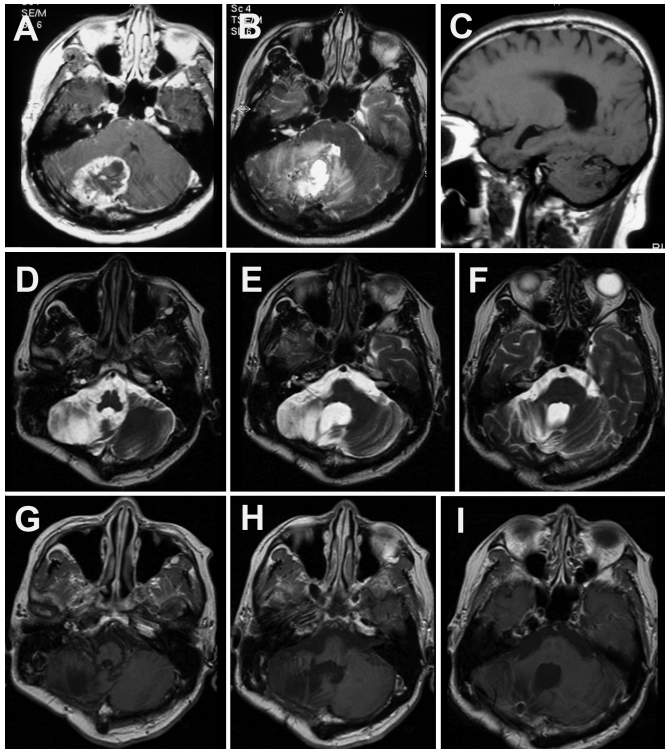

Figure 1: *Preoperative axial T1 weighted with gadolinium (A), T2 axial (B) and T1 sagittal (C) without gadolinium magnetic resonance images (MRI) show an irregular, enhancing intraaxial right cerebellar mass with surrounding hyperintensity on T2 image and prominent flow voids on the sagittal image. A series of axial T2 (D-F) and T1 (G-I) MRIs with gadolinium obtained eight years after surgical resection, radiation and chemotherapy show an area of encephalomalacia but no enhancement showing no evidence of tumor recurrence.*

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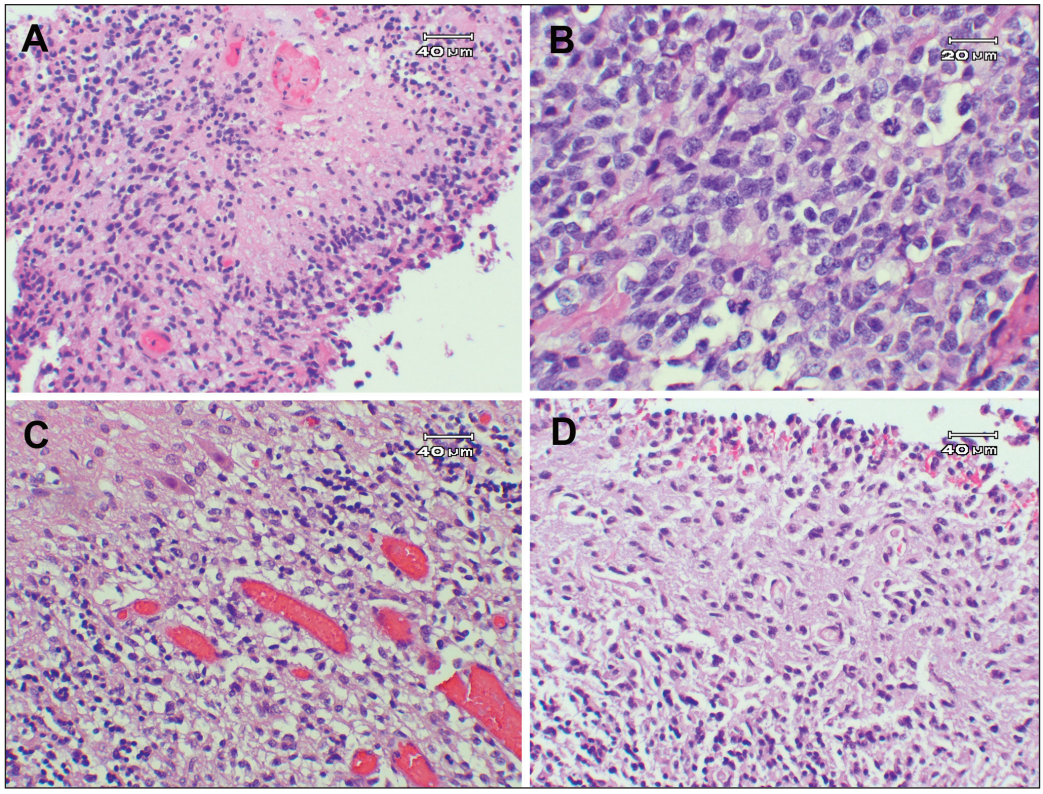

Figure 2: *Histopathology of GB with long-term survival shows necrosis with pseudopallisading (A) and prominent mitotic activity (B). Anaplastic astrocytes also can be seen infiltrating into the internal granule cell (C) and molecular layers (D) of the cerebellum (hematoxylin and eosin).*

Histology revealed a highly cellular infiltrative glial neoplasm with moderate nuclear pleomorphism, several multinucleated forms, numerous mitoses and multiple foci of necrosis and endothelial hyperplasia. There were occasional perinuclear halos and variable amounts of eosinophilic cytoplasm (Figure 2). There was immunoreactivity to glial fibrillary acidic protein but not to S100. The Ki67 labeling index was estimated to be 20%. A pathological diagnosis of GB with subtle oligodendroglial features was made, although the tumor was thought to be a GB and not an anaplastic oligodendroglioma. The patient had fractionated external beam radiotherapy performed based on computed tomographic simulation fused with the preoperative MRI. The target volume (10.5 by 12 by 7 cm) was defined to include the tumor volume on the preoperative MRI plus a 1.5 to 2 cm margin. This volume was treated with 4800 cGy in 24 200 cGy fractions administered five days per week. After this a boost volume (8.5 by 9.5 by 6.5 cm) with a reduced margin was treated to a total dose of 6000 cGy in 30 fractions over six weeks. A three dimensional conformal plan with three-field arrangement of right posterior, left posterior and right anterior oblique portals of 6 and 18 MV photons was used for the entire treatment. This was followed by an 18-month course of chemotherapy with procarbazine, lomustine and vincristine. Recovery was complete and the patient returned to an active practice of surgery.

Follow-up over the next eight years showed no enhancement at the resection site and no change in hyperintensity on T2 and FLAIR images. The patient did not manifest any clinical or radiologic evidence of recurrence (Figure 1). Seven and a half years after surgery he developed biopsy proven prostate carcinoma which was local and has not been treated yet.

Five years after the surgical recurrence, the long recurrence-free survival prompted review of the pathology by the same pathologist who did not know the patient's condition but the diagnosis remained GB. Independent pathologic review confirmed this. Fluorescence in-situ hybridization analysis of the patient's tumor also was performed on stored paraffin-embedded tissue. Human RPC1-11 BAC library-derived probes (Research Genetics, Huntsville, AL) specific for chromosomal regions 1p32, 1q42, 19p13, and 19q13 were used to detect loss of heterozygosity at chromosomes 1p and 19q. The tumor was found to possess normal 1p dosage and loss of heterozygosity at chromosome 19q. Immunohistochemistry for O6 methylguanine-DNA methyltransferase (MGMT) did not demonstrate positive staining.

O-6-methylguanine-DNA methyltransferase was also assessed by polymerase chain reaction (PCR)[17]. Genomic DNA was isolated from 10 µm thick paraffin sections using a QIAamp DNA mini kit (Qiagen Sciences, Maryland, USA). Bisulfite treatment of 1µg of DNA was performed using EZ DNA

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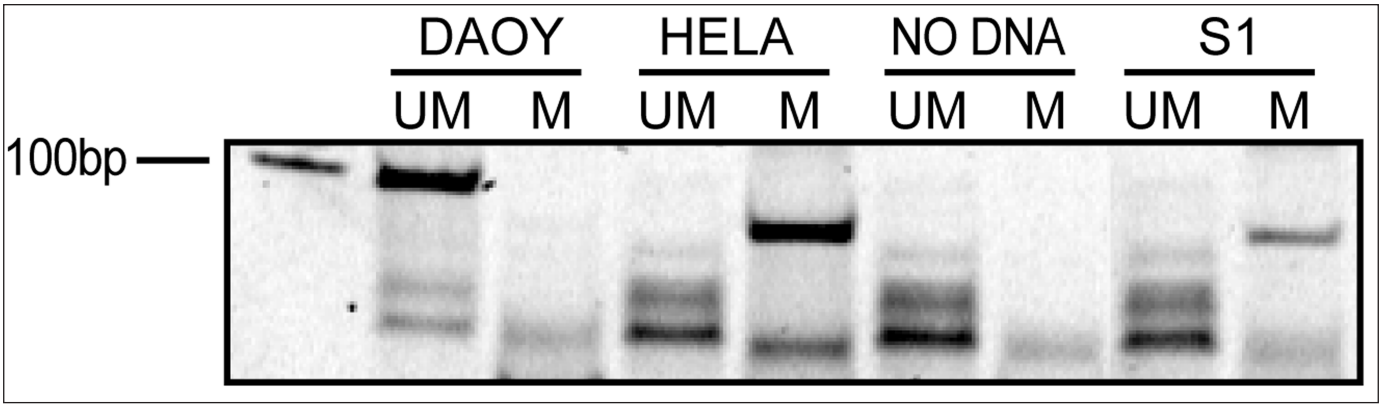

Figure 3: *MGMT methylation by PCR. DAOY cells were unmethylated (UM) whereas HELA cells and the tumor sample (S1) show methylation (M).*

Methylation Gold Kit (Zymo Research, Orange, CA, USA). Methylation specific polymerase chain reaction was performed using specific primers to distinguish methylated and unmethylated DNA. Sequences of primers were: unmethylated forward 5'TTTGTGTTTTGATGTTTGTAGGTTTTTGT 3', unmethylated reverse 5'AACTCCACACTCTTCCAAAAACA AAACA 3'; methylated forward 5' TTTCGACGTTCGTAGG TTTTCGC 3'; methylated reverse GCACTCTTCCGAAAAC GAAACG 3'. Methylated and unmethylated PCR products were visualized on a Criterion precast gel 10% TBE (Bio-Rad, Hercules, CA, USA). Control DNA was treated concomitantly and consisted of genomic DNA isolated from Hela cells for the methylated form and from DAOY cells for the unmethylated form. This showed that MGMT was methylated (Figure 3).

Discussion

Long-term survivors of GB constitute rare exceptions to the dismal prognosis otherwise associated with this tumor. The study of these patients may provide an understanding of how to both predict and prolong survival of patients with GB. Factors contributing to long-term survival include patient characteristics, histological attributes, treatments and genetics. However, the infrequent occurrence of such survivors and the heterogeneity of their tumors have not led to definition of any specific characteristic associated with long-term survival.

The patient described here is a rare example of eight year progression-free survival following GB resection and adjuvant therapy. Superficially, this patient possessed many characteristics associated with good prognosis including young age, good pre-morbid functioning and gross total resection. Some histological features present in this case also have been suggested to improve prognosis including the presence of giant cells and oligodendroglial features. However, the other features were the loss of heterozygosity of chromosome 19q without loss at 1p and methylation of MGMT.

Combined loss of heterozygosity of chromosome 1p and 19q was originally found to be a useful marker of survival and susceptibility to alkylating agents in oligodendroglioma, oligoastrocytoma and oligodendroglial components of GB[4,18-22]. The prognostic significance of combined loss of 1p and 19q in patients with GB, however, is controversial with studies both refuting and supporting its role in prognosis[8,11,13,23]. It also is controversial whether tumors that resemble GB histologically but have combined loss of 1p and 19q should be considered GB or anaplastic oligodendroglioma[24]. Losses of specific regions chromosome 1p appear to predict improved survival in GB particularly in the centromeric regions associated with the NOTCH2 gene[23,25]. The impact of a solitary loss of heterozygosity at 19q remains unclear as few studies have considered its impact on survival independent of 1p loss. However, it is clear that loss of heterozygosity at 19q occurs much more commonly among long-term survivors of GB and that it often occurs without 1p loss[11,23].

There are some limitations to the single locus technology used here to assess loss of heterozygosity. It is possible that partial allelic loss of 1p could have been missed. Polymerase chain reaction-based techniques using a larger number of probes, or technology such as CGH might demonstrate loss of 1p, which would suggest the tumor arose from oligodendroglia. The routine microscopy of the tumor also suggested some features of oligodendroglial lineage. Glioblastomas with oligodendroglial features, loss of 1p and 19q are described and associated with longer survival[24].

This tumor also did not have immunoreactivity to MGMT but the more specific PCR reaction did show methylation of MGMT. Epigenetic silencing of MGMT by methylation of the promoter is associated with response to temozolamide chemotherapy in patients with malignant astrocytoma and glioblastoma[2]. It also may be associated with longer survival in patients who are treated with other types of chemotherapy[26].

Another unique feature of this case is location of the GB in the cerebellum. Only 1% of GB are located in the cerebellum[27,28]. Why they are less common here than would be expected based on brain volume, and to some extent astrocyte numbers, is unknown. A brief review of the literature suggests that cerebellar GB have similar male predominance, peak age incidence in sixth decade of life, propensity for local recurrence and similar poor prognosis to supratentorial GB[27-29].

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