OPEN

Citation: Oncogenesis (2013) **2**, e41; doi:10.1038/oncsis.2013.1

www.nature.com/oncsis

ORIGINAL ARTICLE

Three different brain tumours evolving from a common origin

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Despite an improved understanding of the molecular aberrations that occur in glioblastoma, the use of molecularly targeted therapies have so far been disappointing. We present a patient with three different brain tumours: astrocytoma, glioblastoma and gliosarcoma. Genetic analysis showed that the three different brain tumours were derived from a common origin but had each developed unique genetic aberrations. Included in these, the glioblastoma had *PDGFRA* amplification, whereas the gliosarcoma had *MYC* amplification. We propose that genetic heterogeneity contributes to treatment failure and requires comprehensive assessment in the era of personalised medicine.

Oncogenesis (2013) **2,** e41; doi:10.1038/oncsis.2013.1; published online 1 April 2012

Subject Categories: molecular oncology

Keywords: glioblastoma; genetics; personalised medicine; tumour; heterogeneity

INTRODUCTION

Glioblastoma is among the most aggressive brain tumours, affecting 3500 people per year in the UK.[1,2] Significant progress has been made in unravelling the genetic changes driving glioblastoma.[3–5] Despite this, trials using therapies to target these changes have thus far been disappointing.[6–8] One potential challenge to targeted therapy is tumour heterogeneity. A functional heterogeneity has been shown for glioblastoma stem-like cells with the demonstration that they can form endothelial cells contributing to tumour neovasculature.[9] Possibly of greater concern, there is increasing evidence of genetic heterogeneity in tumours. This was demonstrated in a recent study of clear-cell renal carcinoma, where 63–69% of all somatic mutations identified by exome sequencing could not be detected across all regions of the tumour.[10] We present a patient with three histologically different brain tumours for whom we have performed genome-wide copy number analysis along with *TP53, IDH1* and *IDH2* sequencing.

RESULTS

Case report

A 41-year-old male presented with weakness of the left arm and leg, poor coordination and difficulty in swallowing. The initial magnetic resonance imaging of the brain showed an enhancing mass in the right basal ganglia. Signal abnormality on the T2-dependent sequences extended into the right peduncle and also into the right side of the pons. The patient was commenced on dexamethasone 4 mg orally three times daily. A stereotactic biopsy of the right basal ganglia was performed and the histological examination confirmed a diagnosis of glioblastoma with regions of both grade II and IV tumour. The patient was treated with standard chemoradiation followed by three cycles of adjuvant temozolomide but his disease progressed.[11] The patient died 5 months after starting second-line chemotherapy and 10 months from diagnosis.

Imaging and histopathology

At *post-mortem*, three separate areas of the brain tumour were sampled, one area was from the central pons, one from the right basal ganglia and one from the left frontal region. The pontine lesion and right basal ganglia were part of the same tumour mass. Histopathological examination revealed that the pontine tumour was a glioblastoma (WHO grade IV), the right basal ganglia tumour was a fibrillary astrocytoma (WHO grade II) and the left frontal tumour was a gliosarcoma (WHO grade IV). Serial magnetic resonance imaging at diagnosis and during treatment and the corresponding H&E sections showed three regions of tumour: (i) glioblastoma, (ii) astrocytoma and (iii) gliosarcoma (Figure 1).

Genetic analysis of three tumours

In order to investigate the genetics underlying the three different histological groups, DNA from each tumour sample was screened for copy number change and loss of heterozygosity (LOH) using the Affymetrix 250K single-nucleotide polymorphism arrays (Affymetrix, Santa Clara, CA, USA). In addition, the tumour samples were analysed for mutations of *TP53*, *IDH1* and *IDH2*.[5,12] The results showed specific genetic changes common to all three tumours, as well as changes unique to each tumour. A model of tumour development based on DNA changes in the three tumours is proposed (Figure 2). All three tumours contained the *TP53* missense mutation c.817C>T (p.R273C) (Figure 3a) but no mutation of *IDH1* or *IDH2*. Each of the three tumours contained deletion of 9p23-21.3 encompassing *CDKN2A* and *CDKN2B* (Figure 3b), deletion of 15q13.3-22.31 (Figure 3c) and LOH of chromosomes 10, 17 and 19. The glioblastoma was characterised by amplification of 4q12 containing the receptor kinase genes *KDR* (*VEGFR*), *KIT* and *PDGFRA* (Figure 3d) and gain of *CDK4*, the astrocytoma by multiple rearrangements, whereas the gliosarcoma by amplification of 8q24.21 containing the oncogene *MYC* (Figure 3e).

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Received 19 November 2012; revised 20 December 2012; accepted 21 December 2012

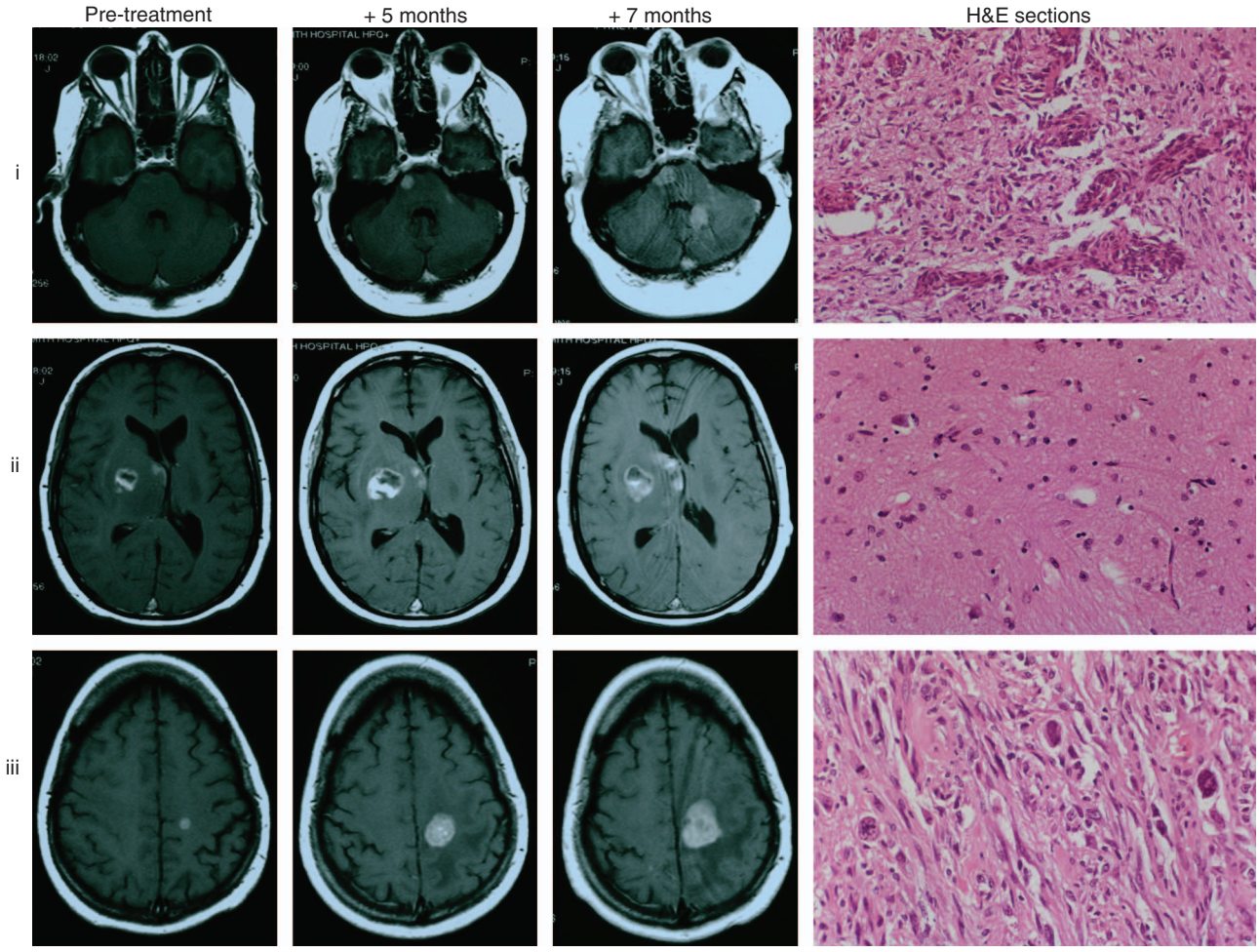

Figure 1. Serial magnetic resonance imaging and the corresponding H&E sections showing three regions of tumour in the same patient: (i) glioblastoma, (ii) astrocytoma and (iii) gliosarcoma.

DISCUSSION

The most significant advance in the treatment of glioblastoma has been the addition of temozolomide chemotherapy to radiotherapy, but the median survival in treated patients is 14.6 months.[11] There has been a focus on determining molecular markers that determine response in individual patients.[13–17] However, in designing trials with molecular targeted agents, tumour heterogeneity has not been addressed. If the target is not present throughout the tumour, how can the tumour respond to the treatment?[18]

Gliobastomas have been subdivided into 'primary' and 'secondary' glioblastoma.[19] The patients with primary glioblastoma are older with frequent activating mutations in *EGFR*. The secondary glioblastoma patients are younger and have tumours containing *TP53* mutation, which have developed from lower grade tumours.[12] More recently, this classification has been expanded based on genomic and expression changes defining four groups, with classical being defined by aberrations in *EGFR*, mesenchymal by *NF1*, proneuronal by changes in *PDGFRA* and *IDH1* and a neural group defined by neural markers.[5,20,21]

The glioblastoma in this case is defined as a secondary, proneural glioblastoma. This patient was typical of those with proneural tumours as he was relatively young and had poor survival despite aggressive treatment.[20] Gliosarcoma is distinguished histologically from glioblastoma by sarcomatous differentiation of the blood vessels. This diagnosis is made much less frequently than glioblastoma and the prognosis is worse.[22,23]

The shared origin of these three tumours was demonstrated by the identical p.R273C *TP53* mutation and the patterns of deletions. Deletion of 9p21.3 in tumours is common as this region contains the important tumour suppressor gene *CDKN2A*.[24,25] In this case, the region is deleted in all three tumours and the breakpoints for these deletions are the same. This would not be expected unless all three tumours were derived from the same origin. Again chromosome 15 contains a deletion with breakpoints common to all three tumours. In addition, there was a deletion at 11q14.1 and LOH of chromosomes 10, 17 and 19.

However, all the three tumours had additional unique mutations. The astrocytoma contained nonspecific changes in keeping with the greater radiation exposure. The gliosarcoma had a number of unique changes including amplification of 8q24.21-containing *MYC*. The glioblastoma had gain of *CDK4* and amplification of the receptor tyrosine kinase (RTK) *PDGFRA*. Three recent studies used FISH to investigate RTK gene amplification in glioblastoma. They demonstrated different subclones within the same tumour with differing and mutually exclusive *EGFR, MET* and *PDGFRA* amplification.[26–28]

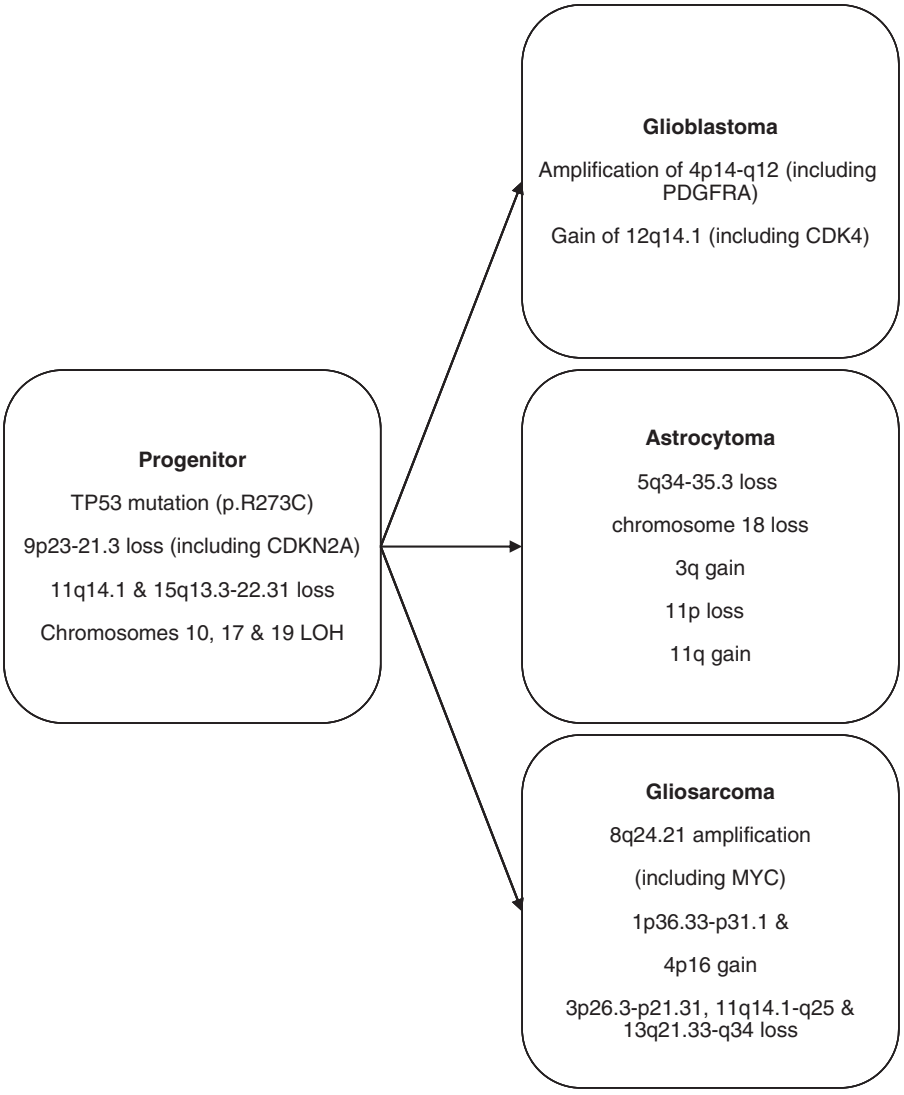

Figure 2. Model of tumour development based on DNA changes in the three tumours. (Note: chromosome 7 demonstrates gain in the gliosarcoma and astrocytoma and is rearranged in the glioblastoma. Chromosomes 2 and 5 are rearranged in the gliosarcoma).

This study raises questions over the current approach to diagnosis, tumour classification and therapies. We propose that a greater understanding of genetic heterogeneity is required. This will require more sensitive detection of mutations and rearrangements, more complete genetic assessment of the tumour mass and potentially, assessment of cell-free DNA where biopsy is problematic, for example, from cerebrospinal fluid or blood plasma.[29] This should be incorporated into a new tumour classification system and to guide the use of therapy ideally to those lesions present throughout the tumour burden. Alternatively, we will have to identify and target multiple lesions.

MATERIALS AND METHODS

Ethics approval

At the request of the patient and his wife, following the patient's death, a *post-mortem* examination was carried out and samples from the brain tumours were removed for research purposes. Approval for the use of patient material in research was obtained from Riverside Research Ethics Committee (RREC 3059 and RREC 3344).

Sample preparation

At *post-mortem*, samples were taken for histopathological analysis and the remaining was stored at $-80\,^{\circ}C$.

Affymetrix 250K single-nucleotide polymorphism array analysis

Tumour DNA was analysed for structural variations using the Affymetrix 250K Nsp array following standard Affymetrix protocols. Affymetrix 250K Nsp results were analysed for copy number changes and LOH using the Copy Number Analyser for GeneChip software package.[30] Tumour data was normalised against that of normal male DNA. All copy number figures show data smoothed using a running mean of 50 single-nucleotide polymorphisms. Regions of LOH potentially masked by contaminating normal DNA were identified using the AsCNAR algorithm.[31]

Sequencing

All exons of *TP53* and exon 4 of both *IDH1* and *IDH2* (including the mutational hotspots, R132 in *IDH1* and R172 in *IDH2*) were sequenced for mutations. Target exons were amplified by PCR from tumour DNA, followed by bidirectional direct sequencing using the dideoxy chain termination method on an ABI 3730 DNA Sequencer (Applied Biosystems,

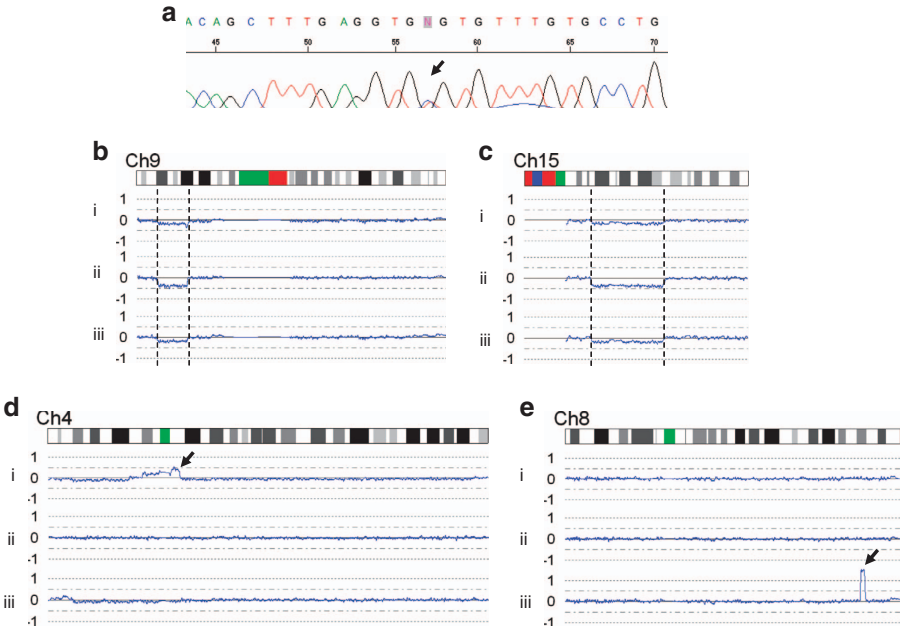

Figure 3. Genetic analysis of the 3 tumours: (i) glioblastoma, (ii) astrocytoma and (iii) gliosarcoma. Panel (**a**) shows the *TP53* missense mutation c.817C>T (p.R273C), this was present in all three tumours (astrocytoma shown here). Panel (**b**) shows the 9p23–21.3 loss including *CDKN2A* and *CDKN2B* in all three tumours. Panel (**c**) shows the loss 15q13.3–15q22.31 in all tumours. Panel (**d**) shows a region of gain including 4q12 in the glioblastoma only. Among the genes in this region are the receptor kinase genes *KDR* (VEGFR), *KIT* and *PDGFRA*. Panel (**e**) shows amplification of 8q24.21 containing the oncogene *MYC* in the gliosarcoma.

Foster City, CA, USA). Results were screened by eye using the Applied Biosystems Sequence Scanner Software v1.0.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Bryan Young and Tracy Chaplin-Perkins who ran the Affymetrix arrays. This work was supported by The Brain Tumour Charity, Mothers and Daughters and The National Brain Appeal.

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