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Case report

Patient-derived tumor organoid predicts drugs response in glioblastoma: A step forward in personalized cancer therapy?

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ABSTRACT

Despite significant medical advances, glioblastoma multiforme (GBM) remains a formidable therapeutic challenge. Advent of targeted capture sequencing and patients-derived organoid cultures may hold the key to scientifically sound individualized treatment approaches. We report on our initial experience of using the combination of these two technologies to create a tailored approach of systemic therapies for a patient with GBM, which challenges the conventional treatment paradigm, as well as specifically highlighting the complexities of such an approach and the potential for a more favorable treatment outcome.

1. Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. The standard therapy for GBM is maximal surgical resection followed by radiotherapy with concurrent and adjuvant temozolomide (TMZ). In spite of the extensive treatment, the disease is associated with poor clinical outcome. The average overall survival is in the range of 12–15 months from the date of initial diagnosis [1]. Whilst the reasons behind this poor prognosis is multifactorial, a lack of an effective personalized treatment approach to address the genomic heterogeneity seen in this disease remains a critical challenge. With the advent of target capture sequencing for identification of genomic alterations, coupled with patient-derived organoid cultures which allows for real-time perpetuation of genomically representative cells of ex-vitro drug testing, we now have the technical capabilities of providing an individualized treatment approach with educated therapeutic decisions [2]. In the following report, we demonstrate the feasibility of such an approach through a case of GBM that was recently treated in our centre. Clinical Research Ethics approval was received from the Joint CUHK-NTEC CREC, Hong Kong.

2. Case presentation

A 58 years-old male university professor presented with a 2-month history of on-and-off headache. Subsequent investigations including magnetic resonance imaging (MRI) of the brain showed an extensive, heterogeneous infiltrative mass lesion in the right temporal lobe, extending to the right parietal lobe with involvement of the cortical, subcortical and deep periventricular/-subependymal regions. There was also compression of the right lateral ventricle and resultant dilatation of the ipsilateral temporal horn, as well as a midline shift of 1.0 cm to the left side. Craniotomy with subtotal resection was performed. Formal pathology assessment confirmed the diagnosis of GBM, IDH wild-type and ATRX-retained (Fig. 1A–E). The MGMT methylation status was negative. Post-operative MRI showed presence of residual tumor in the posterior-medial and anteromedial margins of the right temporal surgical bed. He then received radiotherapy of 60 Gy plus concomitant temozolomide (TMZ) over a period of 6 weeks in accordance to the NCIC-EORTC protocol [1]. Reassessment MRI on completion of the concomitant phase of treatment revealed further progres-

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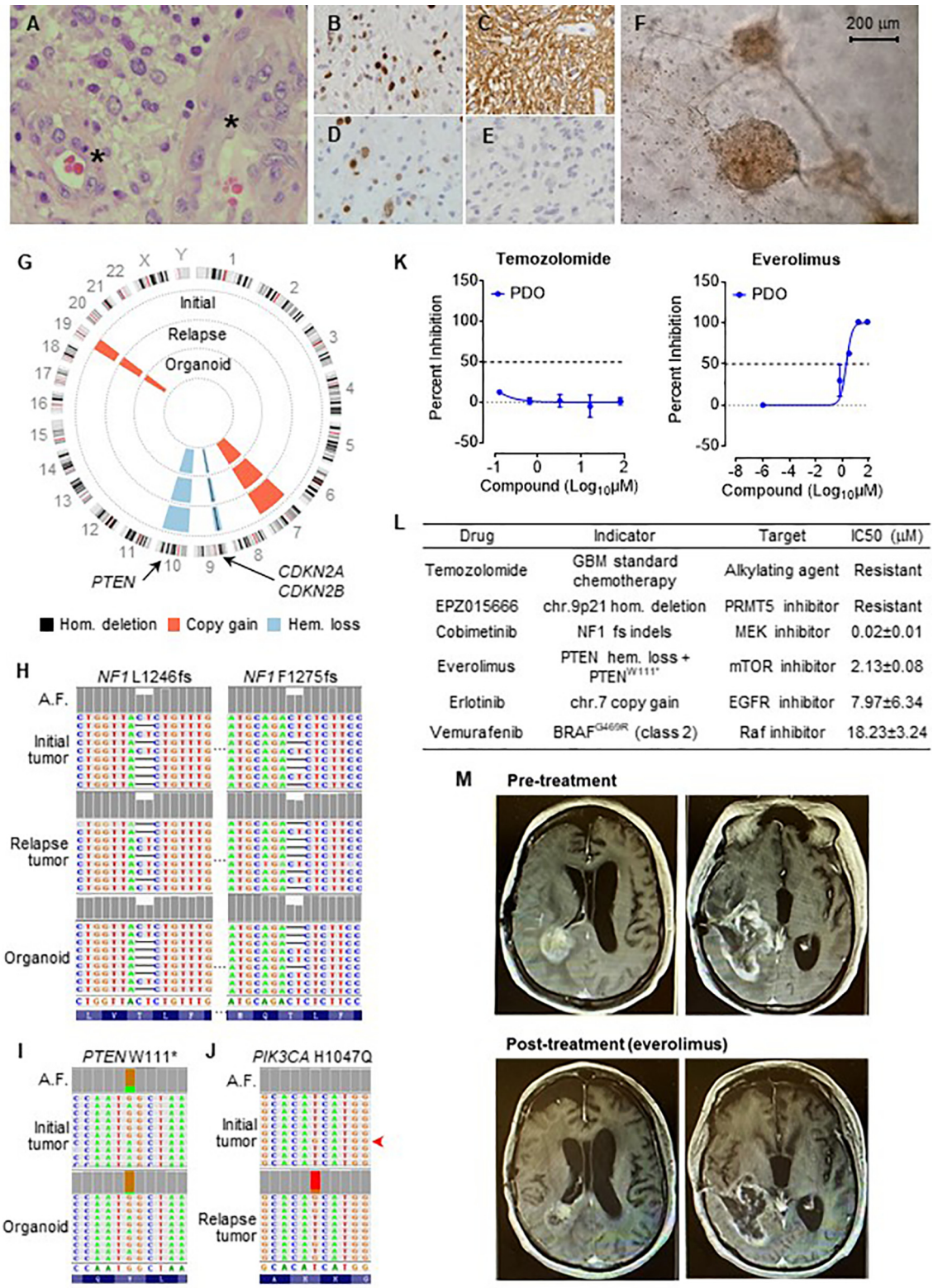

Fig. 1. A–F. Representative H&E, IHC stains and patient-derived tumor organoid of a right temporal IDH-wildtype glioblastoma from a 57 year-old man. (A) The tumor shows pleomorphic hyperchromatic astrocytic cells with endothelial proliferation (asterisk). (B) Tumor cells show high proliferation index, (C) strong cytoplasmic GFAP expression, (D) occasional nuclear expression of OLIG2, and (E) negative for IDH1 mutation. (400×). (F) PDO in Matrigel often shows cytoplasmic processes that protrude and connect cluster of organoids. G–J. Initial-relapse tumor pair and cultured PDO shared same clonal origin. (G) Circos plot demonstrating CNV profiles. Each track represents one sample. Region with red color represents copy gain, whereas blue represents hemizygous loss and black represents homozygous deletion. Major clonal events maintained in initial and relapse tumors, and PDO cultured from initial tumor. (H) IGV plot showing 2 somatic indels in *NF1*. Indels are directly represented by aligned gapped reads. (I) Nonsense mutation of *PTEN* maintained in PDO and (J) clonal enrichment of PIK3CA hotspot mutation detected in relapse tumor. Red arrow indicates variant allele detected in initial tumor. Alignments for somatic mutation, indel and copy number variations were processed against germline variants from the corresponding sequencing of patient's blood DNA. All mutations were manually checked to exclude putative germline variants and sequencing errors. Hom. deletion, homozygous deletion; Hem. loss, hemizygous loss; A.F., allelic frequency. K-L. Ex-vivo PDO responses to anticancer agents. (K) PDOs were subjected to chemotherapeutic agent temozolomide and genome-guided targeting by everolimus. Absence of cytotoxic effect to temozolomide in PDO concurred with patient's response. Effect from Everolimus was more profound. (L) Summary table of drug sensitivity profile of PDO. Selected DA-approved drugs showed varying sensitivity profile. (M) MRI images showed tumor response from post-operative treatment with everolimus. A volumetric reduction by 41.4% was recorded at 1 month post-treatment.

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sion of residual temporo-occipital GBM. He subsequently presented with worsening left sided hemiparesis and confusion. A second debulking surgery on the relapse tumor was performed.

Tumor organoids were cultured from patient's intra-operatively tissue from first surgery (Fig. 1F). Dissociated cells from collagenase digestion were seeded in Matrigel, and cultured for organoids in neurobasal medium according to the method described [3]. The clonal resemblance of the initial-relapse pair, and patient-derived organoid (PDO), was established through target capture sequencing of a custom panel, which also provided information for druggable candidates. Two frameshift indels of *NF1* shared across all samples, together with a highly consistent profile of copy number alterations, accentuated on a shared clonal origin (Fig. 1G-H). Heterozygous *PTEN* copy loss along with $PTEN^{W111^{*}}$ nonsense mutation in the initial tumor suggested a bi-allelic loss of *PTEN* function that likely induced mTOR signaling. Meanwhile, intratumoral heterogeneity at first clinical presentation was evident through low frequency of hotspot $PIK3CA^{H1047Q}$ mutations detected in the initial tumor but enriched at relapse (Fig. 1I-J). Together, these genetic abnormalities underscored activation of the PI3K/AKT/mTOR pathway, which is common in GBM [4].

Next, we tested a panel of genome-guided drug candidates on cultured PDO to predict drug sensitivity. It was interesting to note that TMZ resistance in the patient was recapitulated in his tumor-derived PDO (Fig. 1K). We further tested FDA-approved anti-cancer drugs associated with either *PTEN* loss/$PTEN^{W111^{*}}$ such as mTOR inhibitor everolimus or NF1 frameshift such as MEK inhibitor cobimetinib. PDO showed higher cytotoxic sensitivity to cobimetinib and everolimus compared to other drugs (Fig. 1L). Since efflux of cobimetinib by P-gp may have implications for treatment of patients with brain tumors [5], everolimus was opted as a candidate.

The patient was initially started on everolimus 5 mg daily which was further stepped up to 10 mg daily. Reassessment imaging after four weeks of treatment showed the residual enhancing tumor to be less bulky with partial relief of mass effect on the right lateral and third ventricles, and midline return of structures. This improvement allowed the successful tapering of steroids. Interestingly, during a temporary drug hold for the management of an infective condition, there was evidence of rapid disease progression and clinical deterioration. On completion of treatment and resolution of his infective condition, everolimus was resumed. Subsequent imaging confirmed interval reduction in size and contrast enhancement, in association with significant reduction in perilesional oedema and associated mass effect of his GBM recurrence. Serial findings were indicative of partial treatment response (Fig. 1M). This illustrates the strong dependency of this tumor on the *PTEN* pathway for growth. Identification of this dependency has revealed an actionable target for personalized treatment.

3. Discussion

The aforesaid case confirmed the technical feasibility of using PDO in ex-vivo drug sensitivity testing to guide further treatment. Moving forward, there is a need to validate this individualized therapeutic approach through prospective translational research and clinical trials in order to establish its superiority compared with conventional treatment paradigms.

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