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Case study

The case for DNA methylation based molecular profiling to improve diagnostic accuracy for central nervous system embryonal tumors (not otherwise specified) in adults

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ABSTRACT

Central nervous system primitive neuro-ectodermal tumors (CNS-PNETs), have recently been re-classified in the most recent 2016 WHO Classification into a standby catch all category, "CNS Embryonal Tumor, not otherwise specified" (CNS embryonal tumor, NOS) based on epigenetic, biologic and histopathologic criteria. CNS embryonal tumors (NOS) are a rare, histologically and molecularly heterogeneous group of tumors that predominantly affect children, and occasionally adults. Diagnosis of this entity continues to be challenging and the ramifications of misdiagnosis of this aggressive class of brain tumors are significant. We report the case of a 45-year-old woman who was diagnosed with a central nervous system embryonal tumor (NOS) based on immunohistochemical analysis of the patient's tumor at diagnosis. However, later genome-wide methylation profiling of the diagnostic tumor undertaken to guide treatment, revealed characteristics most consistent with *IDH*-mutant astrocytoma. DNA sequencing and immunohistochemistry confirmed the presence of *IDH1* and *ATRX* mutations resulting in a revised diagnosis of high-grade small cell astrocytoma, and the implementation of a less aggressive treatment regime tailored more appropriately to the patient's tumor type. This case highlights the inadequacy of histology alone for the diagnosis of brain tumours and the utility of methylation profiling and integrated genomic analysis for the diagnostic verification of adults with suspected CNS embryonal tumor (NOS), and is consistent with the increasing realization in the field that a combined diagnostic approach based on clinical, histopathological and molecular data is required to more accurately distinguish brain tumor subtypes and inform more effective therapy.

1. Introduction

Over the past decade, the omics era has revolutionized our understanding of central nervous system (CNS) tumors and revealed that many of these cancers are heterogeneous and not

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single disease entities, as first thought. In particular, central nervous system primitive neuro-ectodermal tumors (CNS-PNETs), which were first included as an entity in the 1993s edition of the WHO Classification of CNS tumors under 'medulloblastoma-like, outside of the cerebellum' [1], have now been classified in the most recent 2016 WHO Classification into a standby catch all category, "CNS Embryonal Tumor, not otherwise specified" (CNS embryonal tumor, NOS) based on epigenetic, biologic and histopathologic criteria [2–6].

The optimal treatment for CNS embryonal tumors (NOS), which are aggressive tumors, remains unknown. Historically, due to their histologic similarity to medulloblastoma, children with these tumors were treated with protocols designed for high-risk medulloblastoma, including upfront surgery followed by cranio-spinal irradiation (CSI) as well as platinum and alkylator-based chemotherapy [7,8]. This intensive chemo-radiation approach was also adopted for the treatment of adults with this disease, and remains the mainstay of therapy [9]. This is despite the acceptance that medulloblastoma and CNS embryonal tumors (NOS) are molecularly and genetically distinct [10–12], with different treatment outcomes associated not only with the different tumor types, but also age groups [13–15]. The latter may be due to biologic tumor heterogeneity, but is also likely to be influenced by a poorer tolerance of high dose chemotherapies in adults. It is hoped that therapy tailored to exploit tumor biology may reduce treatment burden and improve the prognosis for this disease, which currently stands at less than 50% survival five years from diagnosis in children, and is even lower in adults [15]. Here we describe the case of an adult female whose treatment would have been significantly altered with an improved understanding of the biology of her tumor.

2. Materials and methods

2.1. Informed consent

Consent was obtained from the patient on whom this case report was based.

2.2. Immunohistochemistry

Immunohistochemistry (IHC) was performed on formalin-fixed paraffin embedded tissue using the Roche Ventana Ultra platform (Roche Diagnostics, North Ryde, Australia). For ATRX detection, a rabbit polyclonal antibody (1:200 dilution) (Sigma-Aldrich, Castle Hill, Australia) was used with platform conditions: 92 min in cell conditioning solution at 95 °C, 120 min incubation with primary antibody at 36 °C, UV amplification for signal detection. For IDH1 detection, a H09 clone monoclonal antibody (1:100 dilution) (Dianova, Hamburg, Germany) was used with platform conditions: 64 min in cell conditioning solution at 95 °C, 28 min incubation with primary antibody at 36 °C, UV amplification for signal detection.

2.3. Methylation analysis

DNA methylation levels were quantified using the Illumina Infinium HumanMethylation450 array (450 k array, Illumina, San Diego, CA, USA) as previously described [16]. For comparison, 50 reference samples of different GBM subgroups were selected from a previously published cohort [17]. MGMT promoter methylation status was determined as previously described [18].

2.4. DNA sequencing

Sanger DNA sequencing of *IDH1* exon 4 and *IDH2* exon 4 was carried out on PCR amplified DNA extracted from formalin-fixed paraffin embedded tumor tissues. PCRs containing primers *IDH1* (F) 5′-CGGTCTTCAGAGAAGCCATT-3′ and *IDH1*(R) 5′-GCAAAATCA CATTATTGCCAAC-3′, or *IDH2*(F) 5′-AGCCCATCATCTGCAAAAAC-3′ and *IDH2*(R) 5′-CTAGGCGAGGAGCTCCAGT-3′ and 1.5 mM $MgCl_2$ were performed with an annealing temperature of 57 °C using a BioRad C1000 thermal cycler (BioRad, Gladesville, Australia). DNA sequences were compared to NCBI reference sequences *IDH1*: NG_023319.2 and *IDH2*: NG_023302.1.

3. Results

3.1. Case report

In 2013, a previously healthy 45-year old woman presented with a four-week history of headaches, short-term memory loss and personality change. She underwent an MRI scan of her brain and spine, which revealed an 82 mm irregular rim-enhancing solid-cystic mass, involving both frontal lobes [Fig. 1A]. Meningeal enhancement was also seen extending posteriorly to the hypothalamus. Due to clinical signs and symptoms consistent with raised intracranial pressure she underwent urgent maximal debulking surgery, with post-operative scans demonstrating near-total resection. Histopathological examination of the resection specimen showed a cellular neoplasm composed of poorly differentiated cells arranged in irregular aggregates and cords with spread to the leptomeninges and the Virchow-Robin spaces but notably not invading the cortex and white matter. The neoplastic cells had intermediate-sized, irregular, angulated nuclei with granular chromatin, small nucleoli and sparse amounts of cytoplasm. Mitotic figures were easily identified, and there was no microvascular proliferation or necrosis [Fig. 1B]. Immunohistological staining for synaptophysin, CD56, S100 [Fig. 1C] and MAP2 were positive in the tumor cells, and there was focal positivity for GFAP [Fig. 1D]. Staining for keratin, TTF-1, WT-1, CD45, CD99, EMA, Melan-A, HMB45, SMA, desmin, NeuN, pan-neurofilament and chromogranin-A were all negative, and the proliferative marker Ki-67 was positive in approximately 40% of tumor cells [Fig. 1E]. The IDH1(R132H) immunohistochemical stain was not performed in view of the morphological appearance of the tumor and the ATRX immunohistochemical stain was not available at the time of receipt of the original resection specimen. The histological findings were considered to be most consistent with CNS-PNET, WHO grade IV, now known as a CNS embryonal tumor (NOS) [6].

The patient underwent treatment with CSI (35 Gy with a 16 Gy boost to the tumor bed) and weekly vincristine. An MRI scan six weeks after completion of radiotherapy confirmed stable disease, and the patient went on to receive six of a planned eight cycles of platinum and alkylator-based chemotherapy. This initially consisted of cisplatin, vincristine and cyclophosphamide, although after the third cycle carboplatin was substituted for cisplatin due to grade 3 ototoxicity. Treatment was terminated early after the patient developed grade 3 peripheral neuropathy, and a further MRI scan at that time again showed stable disease.

The patient remained well for the next four months, but represented thirteen months from diagnosis with increasing fatigue, vagueness, weakness and ataxia. MRI at this point showed multiple new areas of leptomeningeal enhancement involving the brain stem, cerebellum and spinal cord, with cerebro-spinal fluid analysis revealing a markedly raised protein of 3.05 g/L, although malignant cells were not detected. An Ommaya reservoir was inserted and she received twice-weekly intrathecal methotrexate therapy for four weeks, and then weekly thereafter. Serial CSF analysis showed consistent reduction in protein levels to a minimum of 0.45 g/L, and an improvement in the patient's presenting symptoms. However, she quickly went on to develop progressive radic-

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