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ORIGINAL ARTICLE

Genomic characterization of recurrent high-grade astroblastoma

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Astroblastomas are rare primary brain tumors, diagnosed based on histologic features. Not currently assigned a WHO grade, they typically display indolent behavior, with occasional variants taking a more aggressive course. We characterized the immunohistochemical characteristics, copy number (high-resolution array comparative genomic hybridization, OncoCopy) and mutational profile (targeted next-generation exome sequencing, OncoPanel) of a cohort of seven biopsies from four patients to identify recurrent genomic events that may help distinguish astroblastomas from other more common high-grade gliomas. We found that tumor histology was variable across patients and between primary and recurrent tumor samples. No common molecular features were identified among the four tumors. Mutations commonly observed in astrocytic tumors (*IDH1/2*, *TP53*, *ATRX*, and *PTEN*) or ependymoma were not identified. However one case with rapid clinical progression displayed mutations more commonly associated with GBM ($NF1^{N1054H/K63*}$, $PIK3CA^{R38H}$ and ERG^{A403T}). Conversely, another case, originally classified as glioblastoma with nine-year survival before recurrence, lacked a GBM mutational profile. Other mutations frequently seen in lower grade gliomas (*BCOR, BCORL1, ERBB3, MYB, ATM*) were also present in several tumors. Copy number changes were variable across tumors. Our findings indicate that astroblastomas have variable growth patterns and morphologic features, posing significant challenges to accurate classification in the absence of diagnostically specific copy number alterations and molecular features. Their histopathologic overlap with glioblastoma will likely confound the observation of long-term GBM "survivors". Further genomic profiling is needed to determine whether these tumors represent a distinct entity and to guide management strategies.

Keywords Astroblastoma, aCGH, NGS, GBM, SETD2

Introduction

Astroblastoma is a rare and controversial central nervous system (CNS) tumor. Since the earliest descriptions by Bailey and Cushing, the entity remains predominantly described in the literature as isolated reports or small case series (1–3). Diagnostic classification relies on identifying "astroblastic" pseudorosettes and perivascular hyalinization within a relatively well-circumscribed mass (4,5). However, these features are not universally present, nor are they specific to astroblastoma. Previous immunohistochemistry and electron microscopy studies have proposed both astrocytic and ependymal origins for astroblastomas, with possible differentiation from tanycytes or ependymal astrocytes (6–10). Recent reports have suggested that the distinctive histologic features of astroblastoma may reflect a special phenotype among astrocytic gliomas (11). Moreover, while astroblastoma is thought to be an indolent tumor with good prognosis, aggressive, infiltrative variants with

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recurrence have also been described (4,7,12,13). The 2016 WHO Classification of Tumors of the Central Nervous System describes malignant astroblastomas as having focal or multiple foci of high cellularity, anaplasia, increased mitotic activity (>5 mitoses per HPF), elevated proliferative index (typically >10%), microvascular proliferation, and necrosis. The nonspecific histologic features of astroblastoma have led to further confusion as to their clinical distinction from glioblastoma (GBM). Therefore, the diagnosis of astroblastoma continues to pose a significant diagnostic and prognostic dilemma.

A growing body of evidence has demonstrated that human brain tumors display characteristic molecular signatures consistent with tumor type. Previous efforts to analyze genomic alterations in astroblastoma have been limited. Gain of chromosome arm 20q (4 cases), gain of chromosome 19 (3 cases), and rare losses of chromosome 10, X, and gain of 9q were described in a series of 7 cases (14). A recent report of 3 cases described MGMT promoter hypermethylation, with one case displaying molecular signature suggestive of a GBM (including loss of 10q, mutations in PTEN and TP53) (11). We sought to determine if recurrent genomic aberrations in astroblastoma could be detected using high-resolution array comparative genomic hybridization (aCGH) and next-generation targeted exonic sequencing that had not previously been applied to these tumors. Furthermore we sought to determine whether their profiles overlapped with known profiles of GBM or lower grade gliomas that have been recently well defined.

Materials and methods

Clinical cohort and patient demographics

The cohort was initially defined by systematic archival review for the term “astroblastoma” from 1995 to 2015, i.e. cases where (1) a clinical diagnosis of astroblastoma rendered, or (2) astroblastoma was mentioned in the pathology report as part of a differential diagnosis. The search revealed four patients. All tumors had high-grade features and three had paired primary and recurrent samples.

Three neuropathologists independently reviewed each case (TAB, MA, KLL) as part of this research study (research re-review) and confirmed the presence of features of astroblastoma. These features included perivascular astroblastomatous rosettes, hyalinized vessels, and more block-like processes than are typical for diffuse gliomas. One case (Case 3) upon re-review was reclassified as GBM with astroblastomatous features. Clinical variables and patient demographics were collected (Table 1). This study was approved by the Dana-Farber/Brigham and Women’s Cancer Center Institutional Review Board. Patients were consented to minimal risk research or studied using waiver of consent following IRB approval.

Immunohistochemistry

Immunohistochemistry was performed using commercially available antibodies following standard protocols for paraffin-embedded formalin-fixed tissue. The slides were incubated with antibodies for EMA, GFAP, OLIG2, and SOX2 and developed using 3,3′-diaminobenzidine (DAB) as the chromagen (Sigma Chemical Co., St. Louis, MO) before counterstaining with hematoxylin.

Genomic profiling

Genome-wide copy number analysis was performed using array comparative genomic hybridization (aCGH) and the 1 × 1M Agilent SurePrint G3 Human CGH Microarray chip as previously described (15,16). Next-generation targeted exome sequencing (Oncopanel) of 300 cancer-related genes was performed using Illumina-based methods as previously described (17). The OncoPanel assay surveys exonic DNA sequences of 300 cancer genes and 113 introns over 35 genes for detection of rearrangements; DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer.

The 300 genes are: ABL1, AKT1, AKT2, AKT3, ALK, ALOX12B, APC, AR, ARAF, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AURKA, AURKB, AXL, B2M, BAP1, BCL2, BCL2L1, BCL2L12, BCL6, BCOR, BCORL1, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRD4, BRIP1, BUB1B, CADM2, CARD11, CBL, CBLB, CCND1, CCND2, CCND3, CCNE1, CD274, CD58, CD79B, CDC73, CDH1, CDK1, CDK2, CDK4, CDK5, CDK6, CDK9, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK2, CIITA, CREBBP, CRKL, CRLF2, CRTC1, CRTC2, CSF1R, CSF3R, CTNNB1, CUX1, CYLD, DDB2, DDR2, DEPDC5, DICER1, DIS3, DMD, DNMT3A, EED, EGFR, EP300, EPHA3, EPHA5, EPHA7, ERBB2, ERBB3, ERBB4, ERCC2, ERCC3, ERCC4, ERCC5, ESR1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXT1, EXT2, EZH2, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FAS, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FKBP9, FLCN, FLT1, FLT3, FLT4, FUS, GATA3, GATA4, GATA6, GLI1, GLI2, GLI3, GNA11, GNAQ, GNAS, GNB2L1, GPC3, GSTM5, H3F3A, HNF1A, HRAS, ID3, IDH1, IDH2, IGF1R, IKZF1, IKZF3, INSIG1, JAK2, JAK3, KCNIP1, KDM5C, KDM6A, KDM6B, KDR, KEAP1, KIT, KRAS, LINC00894, LMO1, LMO2, LMO3, MAP2K1, MAP2K4, MAP3K1, MAPK1, MCL1, MDM2, MDM4, MECOM, MEF2B, MEN1, MET, MITF, MLH1, MLL (KMT2A), MLL2 (KTM2D), MPL, MSH2, MSH6, MTOR, MUTYH, MYB, MYBL1, MYC, MYCL1 (MYCL), MYCN, MYD88, NBN, NEGR1, NF1, NF2, NFE2L2, NFKBIA, NFKBIZ, NKX2-1, NOTCH1, NOTCH2, NPM1, NPRL2, NPRL3, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PARK2, PAX5, PBRM1, PDCD1LG2, PDGFRA, PDGFRB, PHF6, PHOX2B, PIK3C2B, PIK3CA, PIK3R1, PIM1, PMS1, PMS2, PNRC1, PR AME, PRDM1, PRF1, PRKAR1A, PRKCI, PRKCZ, PRKDC, PRPF40B, PRPF8, PSMD13, PTCH1, PTEN, PTK2, PTPN11, PTPRD, QKI, RAD21, RAF1, RARA, RB1, RBL2, RECQL4, REL, RET, RFWD2, RHEB, RHPN2, ROS1, RPL26, RUNX1, SBDS, SDHA, SDHAF2, SDHB, SDHC, SDHD, SETBP1, SETD2, SF1, SF3B1, SH2B3, SLITRK6, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMC3, SMO, SOCS1, SOX2, SOX9, SQSTM1, SRC, SRSF2, STAG1, STAG2, STAT3, STAT6, STK11, SUFU, SUZ12, SYK, TCF3, TCF7L1, TCF7L2, TERC, TERT, TET2, TLR4, TNFAIP3, TP53, TSC1, TSC2, U2AF1, VHL, WRN, WT1, XPA, XPC, XPO1, ZNF217, ZNF708, ZRSR2. Intronic regions are tiled on specific introns of ABL1, AKT3, ALK, BCL2, BCL6, BRAF, CIITA, EGFR, ERG,