Concurrence of chromosome 6 chromothripsis and glioblastoma metastasis

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The authors report an unusual case of a widely metastatic glioblastoma. DNA copy number microarray profile of the resected specimen revealed complex rearrangements found throughout chromosome 6, a phenomenon known as chromothripsis. Such chromothripsis pattern was not observed in 50 nonmetastatic glioblastoma specimens analyzed. Analysis of the 1000+ gliomas profiled by The Cancer Genome Atlas (TCGA) data set revealed one case of chromosome 6 chromothripsis resembling the case described here. This TCGA patient died within 6 months of undergoing tumor resection. Implications of these findings are reviewed in the context of the current literature.

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KEY WORDS glioblastoma; metastasis; chromothripsis; oncology

GENOMIC rearrangements are characterized by joining of segments of DNA that are discontinuous in the normal genome. Most solid tumor genomes can harbor tens to hundreds of these rearrangements scattered throughout the altered genome.¹⁴ Interestingly, recent advances in high-throughput genomics and informatics have revealed that in 2%–3% of solid tumors, hundreds of chromosomal rearrangements can be found clustered in one or a few chromosomal regions.²³ The term chromothripsis has been coined to capture this phenomenon, because the clustering of rearrangements suggests that the region involved was “shattered” into little pieces at a point during tumor evolution and rejoined in a manner that failed to recapitulate the normal genome.^{8,23,36} Typically, these rearrangements lead to inactivation of genes or noncoding RNAs that are present in the involved region, though in rare instances the genomic rearrangement

can result in gene activation or gain of novel functions.^{8,23} Cells that survive such a catastrophic event emerge with a highly rearranged genome that can confer selection advantages.^{20,23,24}

Here we report the case of an unfortunate patient who suffered from a widely metastatic glioblastoma. DNA copy number microarray (OncoScan, Affymetrix) profile of the resected specimen revealed chromothripsis of chromosome 6. Such a chromothripsis pattern was not found in 50 nonmetastatic glioblastomas treated at the senior author's institution. One comparable case of chromosome 6 chromothripsis was seen in 1000+ gliomas within The Cancer Genome Atlas (TCGA) data set, and the patient with this condition died within 6 months of undergoing tumor resection. Given previous reports demonstrating that the metastatic potential of cancer cells is suppressed by transfer of chromosome 6^{21,26,27,39} and enhanced by inactivation

ABBREVIATIONS TCGA = The Cancer Genome Atlas.

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FIG. 1. Resection of cerebellar anaplastic oligoastrocytoma, with postoperative pseudomeningocele. Preoperative (A) and postoperative (B) T1-weighted contrast-enhanced axial MR images. Axial (C) and sagittal (D) T1-weighted contrast-enhanced MR images acquired 3 weeks after tumor resection demonstrating a cervical pseudomeningocele (arrows).

of key chromosome 6 genes,^{2,6,11,29,34,40} and given that chromothripsis often inactivates genes present in regions involved, we propose chromothripsis of chromosome 6 as a mechanism contributing to glioblastoma metastasis.

Case Report

This patient is a 23-year-old man who underwent resection of a hemorrhagic cerebellar lesion 6 months prior to presentation (Fig. 1A and B). Postoperative findings were notable for a pseudomeningocele (Fig. 1C and D) that spontaneously resolved after a month. Pathological examination of this cerebellar lesion indicated an anaplastic oligoastrocytoma, and the patient was started on a course of temozolomide therapy. Surveillance imaging 6 months after the initial procedure revealed two extracranial, heterogeneously enhancing masses (Fig. 2A and B) located at the site of the previous pseudomeningocele. The lesions were resected and the pathology revealed glioblastoma (Fig. 2C–E and G) with invasion of the surrounding musculature (Fig. 2E and F). Greater than 90% of the tumor cells from these specimens stained positive for Ki 67 (Fig. 2H). Isocitrate dehydrogenase 1 immunostaining was negative.

The patient was treated with involved-field irradiation, but he developed multifocal metastatic lesions including

FIG. 2. Development, resection, and pathological analysis of cervical glioblastoma. A: Axial postcontrast MR images 6 months postresection demonstrating a new cervical mass in the area of the previous pseudomeningocele. B: Postoperative contrast-enhanced axial MR image of the cervical lesion. C: Necrosis with pseudopalisading is evident at lower power. H & E, original magnification $\times 200$. D: Numerous mitotic figures, including atypical forms, are featured amid the striking astrocytic pleomorphism. H & E, original magnification $\times 400$. E: The high-grade glial neoplasm involves adjacent soft tissue including skeletal muscle. H & E, original magnification $\times 100$. F: Tumor cells flank individual skeletal muscle cells (arrows). H & E, original magnification $\times 400$. G: Immunoreactivity confirms glial nature of tumor. GFAP, original magnification $\times 400$. H: Immunolabeling demonstrates high proliferative index. Ki 67, original magnification $\times 400$. Figure is available in color online only.

additional lesions in the neck musculature (distant to the previous lesions) and multiple spinal (osseous and leptomeningeal) lesions (Fig. 3A–C). CT of the chest, abdomen, and pelvis revealed no metastatic involvement of other organs. Avastin therapy was initiated, and the patient died 2 months later.

The resected primary lesion was characterized by targeted sequencing of 47 cancer-pertinent genes (Supplemental Table 1) and by DNA (OncoScan) copy number microarray analysis.⁹ Targeted sequencing revealed a guanine-to-adenine transition at nucleotide 1633 of the phosphoinositol 3 kinase alpha subunit, resulting in a glu-

FIG. 3. Rapid development of multifocal neuroaxis metastases. Sagittal postcontrast thoracic (A) and lumbar (B) spine MR images obtained 2 months after resection of cervical glioblastoma, demonstrating multiple, new osseous and leptomeningeal lesions. Sagittal postcontrast brain MR image (C) obtained at the same time demonstrating a new right pontine lesion, with multiple supratentorial leptomeningeal lesions and a right cerebellar recurrence also found. Multiple cervical spine leptomeningeal and lumbar nerve root lesions were also seen on MRI (not shown).

tamic acid to lysine substitution at amino acid position 545 (*PIK3CA* c.1633G>A (p.E545 K)). This amino acid position, which localizes to the helical domain, is a known mutational hotspot within *PIK3CA*. This is the most frequently observed *PIK3CA* mutation in multiple cancer types and has been shown to result in increased kinase activity and induce tumor formation in multiple experimental systems.^{13,19,38}

The OncoScan profile revealed deletions at 1q, 10q (encompassing the *PTEN* locus), 12q, 13q, 14q, 15q, 16p, 16q, 17p (encompassing *TP53*), 18q, and 21q, and chromothripsis of chromosome 6 (Fig. 4). We compared the OncoScan results of this metastatic glioblastoma to those derived from 50 consecutive nonmetastatic glioblastomas treated at the same institution and found chromothripsis of chromosome 6 to be the only genomic feature unique to this metastatic glioblastoma. Analysis of the 1000+ low-grade gliomas and glioblastomas in the TCGA data set

revealed only one case of chromosome 6 chromothripsis of comparable severity (Fig. 5). This TCGA patient died within 6 months of resection.

Discussion

While the interpretation of “N of 1” studies warrants extreme caution, thoughtful consideration of these studies has yielded insights that have led to significant scientific advances.²² It is in this context that we present our finding of chromosome 6 chromothripsis in a metastatic glioblastoma specimen. Despite the aggressively infiltrative nature of high-grade glial tumors, secondary metastasis to non-CNS sites is exceedingly rare, especially to the skin and soft tissue. In our literature search, we have identified a total of 19 such cases^{1,4,5,7,10,12,15,17,18,25,28,30–33,37} (Table 1). Given the rarity of the phenomenon, it is of no surprise that its etiology remains poorly understood. While seeding secondary to surgical manipulation has been suggested as a potential mechanism,³⁵ and this process may explain the tumor recurrence at the site of the postoperative pseudomeningocele in our patient, we cannot exclude the possibility of microscopic systemic metastasis because of the subsequent development of distant soft-tissue and multifocal osseous lesions. In fact, rare cases of glioblastoma metastasis in the absence of surgical manipulation^{3,16} suggest that the inherent genomic landscape of the cancer may be contributory to its metastatic potential.

Consistent with this hypothesis, genomic analysis of the metastatic glioblastoma specimen revealed a highly unusual genomic rearrangement pattern that was not observed in 50 consecutive nonmetastatic glioblastomas treated at the same institution—the chromothripsis of chromosome 6. TCGA analysis confirmed the rarity of chromosome 6 chromothripsis in clinical glioblastoma specimens. While there are no clinical annotations of glioblastoma metastasis for this TCGA patient, the poor clinical course (6-month overall survival after resection) is consistent with an aggressively infiltrative tumor.

These findings are particularly intriguing in the context of a substantiated body of literature that microcell-mediated transfer of chromosome 6 into human cancer cell lines suppresses metastatic potential.^{21,26,27,39} Moreover, inactivation or loss of key genes present on chromosome 6 greatly facilitates the metastatic potential of cancer cells.^{2,6,11,29,34,40} Since chromothripsis most commonly results in inactivation of genes or noncoding RNAs present in the involved region, our findings suggest that chromothripsis of chromosome 6 may inactivate key metastasis suppressor genes and facilitate glioblastoma metastasis. While the proposed hypothesis is intriguing, it is conceivable that other mutational events, including those not characterized by our gene panel or DNA copy number microarray, play contributory roles to glioblastoma metastasis. As such, clinical and experimental validations of the findings presented in this case report are warranted.

Conclusions

Chromothripsis of chromosome 6 may facilitate the metastatic potential of glioblastoma cells.

FIG. 4. OncoScan chromosomal analysis results. Karyotype (A), log-ratio (B), and B-allele (C) frequency plots demonstrate chromosome 6 chromothripsis. Multiple copy number abnormalities and several large regions of loss of heterozygosity (LOH) were also found, including loss of 1q, deletion of distal 10q including the *PTEN* locus, and 17p LOH including the *TP53* locus. Also noted were deletions of 13q, 14q, 15q, and 16q; copy gains were noted at distal 12q and proximal 18q. No high copy gains or regions of amplification were identified. chr = chromosome. Figure is available in color online only.

FIG. 5. TCGA analysis for chromosome 6 chromothripsis. The number of copy number aberration (CNA) breaks was examined for the 1000+ low-grade gliomas and glioblastomas in the TCGA data set. Each chromosome was normalized by the total number of CNA breaks in all other chromosomes. One single case of chromosome 6 chromothripsis of comparable severity to the presented case was identified (**lower**). Break count for chromosome 6 in this case was magnified (**upper**). Figure is available in color online only.

TABLE 1. Summary of cases of glioblastoma metastasis to the skin

Authors & Year	Age (yrs), Sex	Primary Histopathology (WHO grade)	Site of Primary Malignancy	Location of Metastasis
Carvalho et al., 1991	26, F	Primary GBM (IV)	Rt temporal lobe	Scalp & cervical lymph node
Vural et al., 1996	40, M	Primary GBM (IV)	Rt temporoparietal	Cervical subcutaneous tissue
	49, M	Primary GBM (IV)	Lt cerebral hemisphere	Scalp
Houston et al., 2000	32, M	Primary GBM (IV)	Lt temporal lobe	Scalp
Figueroa et al., 2002	35, M	Primary GBM (IV)	Lt temporal lobe	Scalp
Santos et al., 2003	42, M	Primary GBM (IV)	Lt frontoparietal	Scalp
Allan, 2004	60, M	Primary GBM (IV)	Unknown	Scalp
Bouillot-Eimer et al., 2005	60, M	Primary GBM (IV)	Lt parietal lobe	Scalp
Jain et al., 2005	49, M	Primary GBM (IV)	Rt temporoparietal	Scalp
Schultz et al., 2005	74, F	Primary GBM (IV)	Lt temporal lobe	Scalp
Saad et al., 2007	13, M	Primary GBM (IV)	Lt frontal lobe	Scalp & temporal bone
Mentrikoski et al., 2008	58, F	Primary GBM (IV)	Lt frontal lobe	Scalp
	41, M	Anaplastic oligodendroglioma (III)	Unknown	Scalp
Miliaras et al., 2009	63, M	Primary GBM (IV)	Lt frontoparietal	Scapular subcutaneous tissue
Senetta et al., 2009	48, F	Primary GBM (IV)	Rt frontoparietal	Scalp
	53, F	Primary GBM (IV)	Lt frontal lobe	Scalp
Jusúé Torres et al., 2011	63, F	Primary GBM (IV)	Rt frontal lobe	Scalp
Guo et al., 2012	19, F	Primary GBM (IV)	Pons	Cervical subcutaneous tissue
Ginat et al., 2013	62, M	Primary GBM (IV)	Lt frontal lobe	Scalp
Present case	23, M	Anaplastic oligoastrocytoma (III)	Rt cerebellar hemisphere	Cervical subcutaneous tissue

GBM = glioblastoma.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Chen, Rennert, Hoshide, Signorelli, Sack.

Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: Chen, Rennert, Hoshide, Signorelli, Sack. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Chen.

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