Molecular portrait of a rare case of metastatic glioblastoma: somatic and germline mutations using whole-exome sequencing

Despite the latest advances in surgery, radiological assessment, and radiotherapy treatment, the incidence of glioblastoma (GBM) is roughly comparable to that of mortality, and the prognosis is almost always related to intracranial progression after surgery or radio-chemotherapy. The incidence of extracranial metastases of GBM are rarely reported in the literature, and there is still no explanation for this hematological dissemination.

A 70-year-old man was referred to the Radiotherapy Department of Pisa University Hospital after partial excision of a WHO grade IV GBM. Microscopic examination showed pleomorphic astrocytic tumor cells with marked nuclear atypia, mitotic activity, microvascular proliferation, necrosis, and positive glial fibrillary acidic protein (GFAP) immunostaining. Shortly after the first visit, the patient reported lumbar spine pain. Radiological investigation revealed the presence of a lytic lumbar lesion. The total-body CT showed bone, lung, and liver tumor masses. In order to obtain a pathological diagnosis of extracranial disease, we decided to perform a biopsy of the sternal lesion (Fig. 1A). Histological examination showed pleomorphic cells, necrosis, and mitotic activity. Positive immunohistochemistry for GFAP and CD56 indicated a glial origin, while negative PanCk, LCA, and TTF1 results excluded epithelial, lymphoid, pulmonary, and thyroid origins. Cytological examination revealed GFAP-positive cells with hyperchromatic nuclei and poor cytoplasm (Fig. 1B).

Whole-exome sequencing was performed on paired GBM primary tumor and blood germinal DNA using the Ion Proton System (Life Tech). Filtering the data by high quality score, read depth, absence in dbSNP, mammalian conservation, and allele frequency 1%, we found that synonymous and missense gene mutations represented the most common types of variations in both GBM tumor and blood DNA. Additional filtering was performed to remove the entire mutational germinal load from the dataset to identify 70 GBM tumor-exclusive somatic mutations. We selected 8 of the most predominant mutations (higher allele count and read quality) that we assumed had emerged in an early stage of tumor progression: C8A-R30W, CRISP1-R162H, CTBP2-H788L, CTSK-V95L, DOCK9-M1635I, HSD17B7-S173N, PRSS1-Q209E, and TRIM29-V532I. All of these variations were confirmed in the GBM by Sanger sequencing.

In order to confirm the metastatic origin of the sternal lesions, we looked for at least one shared mutation within the 8 selected somatic mutations between GBM and sternal biopsy because the amount of starting material was not sufficient for a whole-exome analysis. We microdissected 100 GFAP-positive cells, taken after cytological preparation of the sternal lesion, and extracted DNA. The tumor-somatic C8A-R30W mutation was confirmed in DNA from the sternal biopsy while being absent in blood DNA (Fig. 1C). Sharing of the C8A-R30W mutation between the primary tumor and the sternal lesion confirms the latter as having a GBM metastatic origin.

The primary tumor data were also filtered for driver mutations. We found 4 variations in genes identified as tumor suppressors: RB1 deletion of 5 bases (Gln257fs), CREBBP stop mutation (Gln1027*), ARID1A one-base deletion (p.Val1867Alafs), and BRCA2 stop mutation (Gln2164*).

We finally performed a copy number variation (CNV) analysis, obtaining a prevalence of deletions in TP53, PTEN, ERBB2, TERT, RETL1, CDKN2A, and PHLDB1 as well as amplifications in BRCA2 using a log2 cutoff of 0.8. The only variation with significant variance, however, was the RETL1 deletion.

Although the reported incidence of extracranial GBM is 0.2%, this phenomenon may not be as rare as believed. The hypoxic and proliferative zone of the GBM has an angiogenesis-related breakdown of the blood-brain barrier, and GBM cells could have direct communication with the circulatory system. Thus, low levels of circulating GBM cells may be present in the early disease process of susceptible patients and ultimately lead to metastases in extracranial organs. The aggressive...
development of disease in this case was probably due to a specific genetic predisposition of the patient and the primary tumor. Indeed, some of the mutations found in germinal DNA disrupted the LZTR1 gene, known to be involved in cell self-renewal and growth. The primary tumor also carried 2 important inactivating mutations in the tumor suppressor RB1 and BRCA2 genes. In astrocytomas, alterations in RB1 and BRCA2 have been associated with increased tumor cell proliferation, decreased survival, and genomic instability. Furthermore, CNV analysis identified a significant deletion in the RTEL1 gene, which is critical for telomere replication and maintenance of genomic integrity.

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**Fig. 1.** (A) CT-guided biopsy of the sternal lesion (needle indicated by yellow arrow). (B) Gliarial fibrillary acidic protein-positive immunostaining in the cytological preparation of the sternal lesion. (C) DNA sequences showing the C8A-R30W mutation (C > T) that was present in both the glioblastoma (GBM) primary tumor and sternal metastasis DNA and absent in blood sample DNA.

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References


