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Mutations in *CIC* and *FUBP1* Contribute to Human Oligodendroglioma

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Abstract

Oligodendrogliomas are the second most common malignant brain tumor in adults and exhibit characteristic losses of chromosomes 1p and 19q. To identify the molecular genetic basis for this alteration, we performed exomic sequencing of seven tumors. Among other changes, we found that the *CIC* gene (homolog of the *Drosophila* gene *capicua*) on chromosome 19q was somatically mutated in six cases and that the *FUBP1* gene [encoding far upstream element (FUSE) binding protein] on chromosome 1p was somatically mutated in two tumors. Examination of 27 additional oligodendrogliomas revealed 12 and 3 more tumors with mutations of *CIC* and *FUBP1*, respectively, 58% of which were predicted to result in truncations of the encoded proteins. These results suggest a critical role for these genes in the biology and pathology of oligodendrocytes.

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Supporting Online Material
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Oligodendrogliomas (ODs) account for 20% of brain tumors in adults and, as their name suggests, they consist primarily of cells resembling oligodendroglia (1, 2). These tumors generally arise in the white matter of the cerebral hemispheres, commonly in the frontal lobes. Well-differentiated ODs can evolve into high-grade "anaplastic" ODs, although it is often difficult to clearly distinguish these two types from each other or from other brain tumors (1, 2). Because this distinction is important for the management of patients, molecular biomarkers for ODs are of great interest.

To date, the best biomarker for ODs is loss of heterozygosity (LOH) of chromosomes 1p and 19q (2–4). Assessment for LOH events is now commonly performed in patients with ODs because of their important implications for therapeutic responses (2–4). The chromosome losses occur in 50% to 70% of tumors and are often associated with a pericentromeric translocation of chromosomes 1 and 19, producing marker chromosome der(1;19) (q10;p10) (2–6). This translocation is unbalanced, leaving the cells with one copy of the short arm of chromosome 1 and one copy of the long arm of chromosome 19. The functional basis for most cancer translocations involves one of the genes residing near the breakpoints, producing fusions that alter the gene's product. In contrast, the der(1;19) (q10;p10) breakpoints are in gene-poor centromeric regions and are always associated with LOH (4, 5). This suggests that the basis for the t(1;19) translocation is the unmasking of a tumor suppressor gene(s) on either chromosome 1p or 19q (2–4, 7, 8). This is supported by the fact that some tumors lose only chromosome 1p sequences, while others lose only chromosome 19q sequences (2–4, 7, 8).

To identify this putative tumor suppressor gene(s), as well as to increase understanding of OD pathogenesis, we sequenced the coding exons of 20,687 genes in DNA from seven anaplastic ODs using the Illumina HiSeq platform (9). The clinical characteristics of the patients and their tumors are listed in table S1. The average distinct coverage of each base in the targeted regions was high (135-fold) and 94% of the bases were represented by at least ten distinct reads (table S2). Loss of heterozygosity (LOH) of chromosomes 1p and 19q was confirmed using common single nucleotide polymorphisms (SNPs) identified as heterozygous in DNA from corresponding normal cells (Fig. 1 and fig. S1).

We have previously described methods for the accurate identification of somatic mutations in next-generation sequencing data from Illumina instruments (10). Using these stringent criteria to avoid false positive calls, we identified a total of 225 non-synonymous somatic mutations, affecting 200 genes among the seven tumors (table S3). There were an average of 32.1 ± 10.7 non-synonymous somatic mutations per tumor (table S2), similar to the number found in the most common type of adult brain tumor [glioblastoma, 35.6 non-synonymous somatic mutations per tumor (11)].

There were a number of notable mutations identified in these seven tumors. We identified three tumors with mutations in *PIK3CA*, encoding the catalytic subunit of the PI3Kα enzyme, and one tumor with a mutation in *PIK3R1*, encoding the regulatory subunit (table S3). The *NOTCH1* gene was mutated in two tumors and at least one of these was inactivating (a 1 bp deletion), consistent with the recently described tumor suppressor role for this gene (12). Finally, the *IDH1* (isocitrate dehydrogenase 1) gene was mutated in all seven tumors at the same residue, resulting in an amino acid substitution of His for Arg at codon 132, as expected for this tumor type (13, 14).

One of the major goals of this study was the investigation of the target gene(s) on chromosome 1 or 19. By analogy with other tumor suppressor genes (15, 16) we expected that the residual copy of the target gene(s) would contain mutations in most tumors with LOH of the relevant region. On chromosome 1p, there were eight somatically mutated

genes, but only two with mutations in more than one tumor: *FUBP1* (Far Upstream Element [FUSE] Binding Protein 1) and *NOTCH2* (table S3). On chromosome 19q, there were three genetically altered genes identified, two of which were mutated in a single tumor each. The third, *CIC* (homolog of the *Drosophila capicua* gene), was mutated in six of the seven tumors. In each of these six cases, the fraction of mutant alleles was high (80.5 ± 10.7%), consistent with loss of the non-mutated allele. The mutations were confirmed to be homozygous by Sanger sequencing (Fig. 2A).

To validate these results and determine the spectrum of *FUBP1*, *NOTCH2*, and *CIC* mutations in ODs, we examined tumor DNA from an additional 27 tumors and matched normal cells. No additional mutations of *NOTCH2* were found, but *FUBP1* and *CIC* mutations were identified in 3 and 12 of the additional cases respectively and generally (14 of 16 mutations) appeared to be homozygous (Fig. 2B and table S4). The probability that these mutations were passengers rather than drivers was $<10^{-8}$ for both genes [binomial test (17)]. All *FUBP1* mutations and more than 25% of the *CIC* mutations were predicted to inactivate their encoded proteins, as they altered splice sites, produced stop codons, or generated out-of-frame insertions or deletions (Fig. 2B and table S4). This type of mutational pattern is routinely observed in tumor suppressor genes such as *TP53* or *FBXW7* (18) but is never observed in bona fide oncogenes.

The *capicua* gene was discovered in a screen for mutations affecting the anteroposterior pattern of *Drosophila* embryos (19). In *Drosophila*, the protein encoded by *CIC* has been shown to be a downstream component of receptor tyrosine kinase (RTK) pathways that includes EGFR, Torso, Ras, Raf, and mitogen-associated protein kinases (MAPKs) (20). In the absence of RTK signaling, cic, in combination with other transcription factors such as Groucho (Gro), blocks transcription by binding to canonical octameric elements in regulatory regions (21). RTK signaling blocks the function of cic via MAPK-mediated phosphorylation or docking, resulting in degradation of cic and the consequent activation of the genes it normally represses (22). The most highly conserved functional domain of the cic protein is the HMG (high mobility group) box responsible for its binding to DNA. Importantly, 8 of the 11 missense mutations we observed in ODs were located in this domain (Fig. 2B).

In addition to the high conservation of *CIC* sequences among metazoans, the human cic protein contains nine consensus phosphorylation sites for MAPK (23). This suggests that human cic functions similarly to its *Drosophila* counterpart. This hypothesis is supported by mass spectroscopic studies that have shown human cic protein to be phosphorylated within 10 min of EGF treatment of HeLa cells (24).

The protein encoded by *FUBP1* binds to single stranded DNA, in particular the far-upstream element (FUSE) of *MYC*, a well-studied oncogene (25). Although overexpression of *FUBP1* can stimulate *MYC* expression (25), it has also been shown that FUBP1 protein participates in a complex with PUF60 that negatively regulates *MYC* expression (26). Our data, showing that *FUBP1* is inactivated by mutations, are consistent with the idea that *FUBP1* mutations lead to *MYC* activation in these tumors by relieving the negative effects of the FUBP1-PUF60-*FUSE* complex.

There are only a small and statistically insignificant number of point mutations of *FUBP1* or *CIC* recorded in the COSMIC database (18). However, *CIC* has been shown to be translocated in two cases of Ewing's sarcoma-like tumors that harbored t(4;19)(q35;q13) translocations. Unlike the mutations observed in ODs, the translocations in these two cases seemed to activate the cic protein by fusing it to the C-terminus of DUX4, conferring oncogenic properties to the new protein (27).

Overall, 23 mutations of *CIC* or *FUBP1* were identified in the 34 tumors analyzed in this study. Notably, of the 27 cases with 19q loss, 18 cases (67%) contained intragenic mutations of *CIC*, while none of the 8 ODs without 19q loss contained *CIC* mutations (table S1). As our mutational screens would not detect some types of inactivating mutations (e.g., large deletions or promoter mutations) or epigenetic alterations, the fraction of tumors with detectable *CIC* and *FUBP1* mutations is likely an underestimate of their actual contribution. To evaluate the prevalence of *CIC* and *FUBP1* mutations, we sequenced 92 tumors of the nervous system and 206 non-nervous system tumors and found only 3 missense mutations in *CIC* (breast, prostate, and medulloblastoma) and no truncating alterations.

The identification of inactivating mutations of *CIC* or *FUBP1* in a substantial fraction of ODs is expected to provide important insights into the pathogenesis of these tumors as well as help refine methods currently used for their diagnosis, prognosis, and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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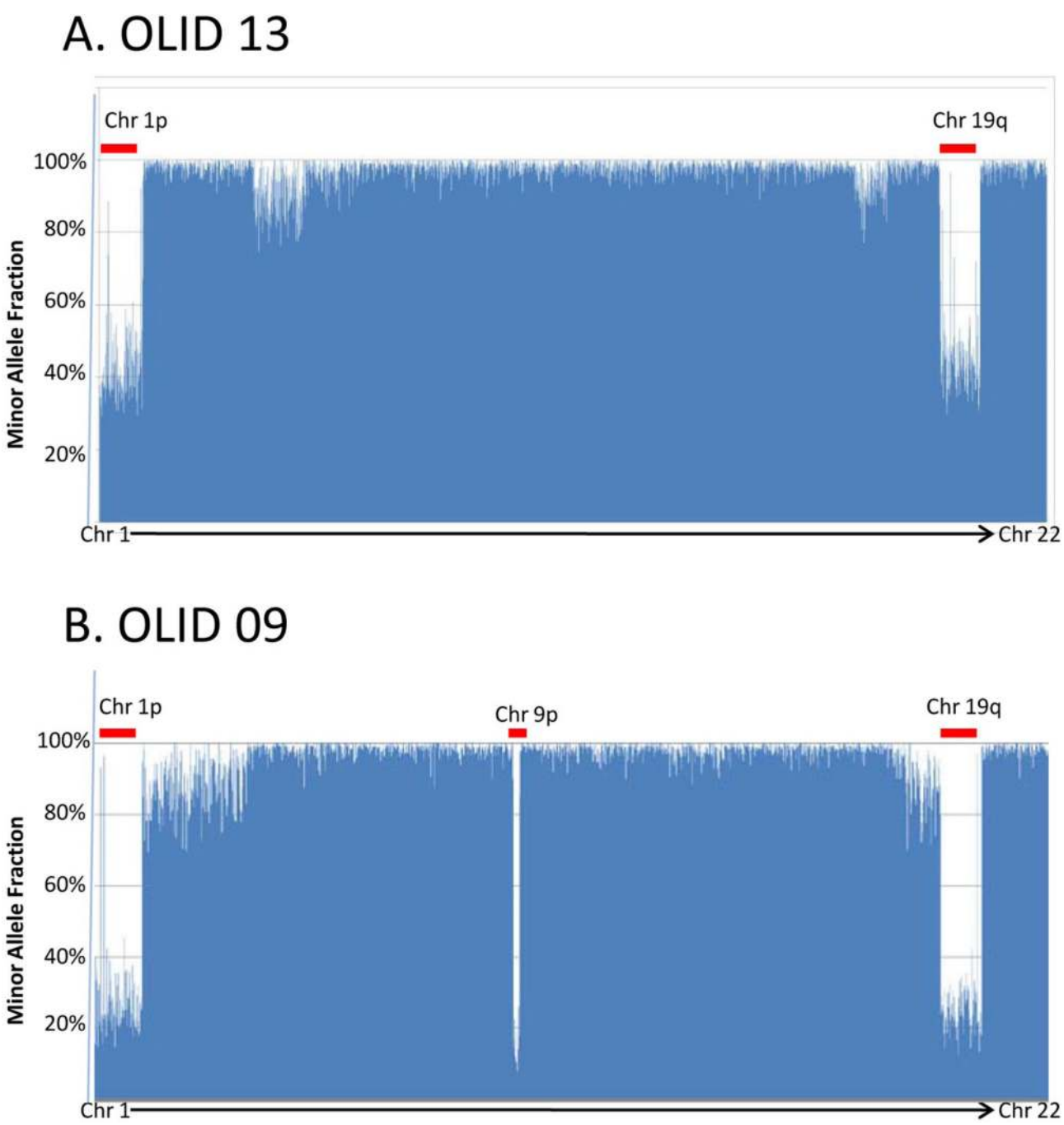

Fig. 1.

Loss of heterozygosity (LOH) maps of two representative tumors. (**A**) In tumor OLID 13, the estimated LOH on chromosome 1 extends from base 901,779 to base 148,526,024 and the estimated LOH on chromosome 19 extends from base 18,116,940 to base 62,357,562. (**B**) In tumor OLID 09, the estimated LOH on chromosome 1 extends from base 1,844,406 to base 110,751,800, the estimated LOH on chromosome 9 extends from base 108,032 to base 20,875,240 and the estimated LOH on chromosome 19 extends from base 18,545,563 to base 62,923,619. The "minor allele" of each SNP represents the allele that was less common in the tumor. If both alleles of the SNP were represented by an equal number of tags, the minor allele fraction would be represented as 100% on the y-axis. The remaining

signals in the regions exhibiting LOH represent contaminating non-neoplastic cells in the samples. Partial allelic skewing (e.g., on chromosome 2 in OLID 13) reflects losses of the relevant region in a subfraction of the neoplastic cells within the tumor.

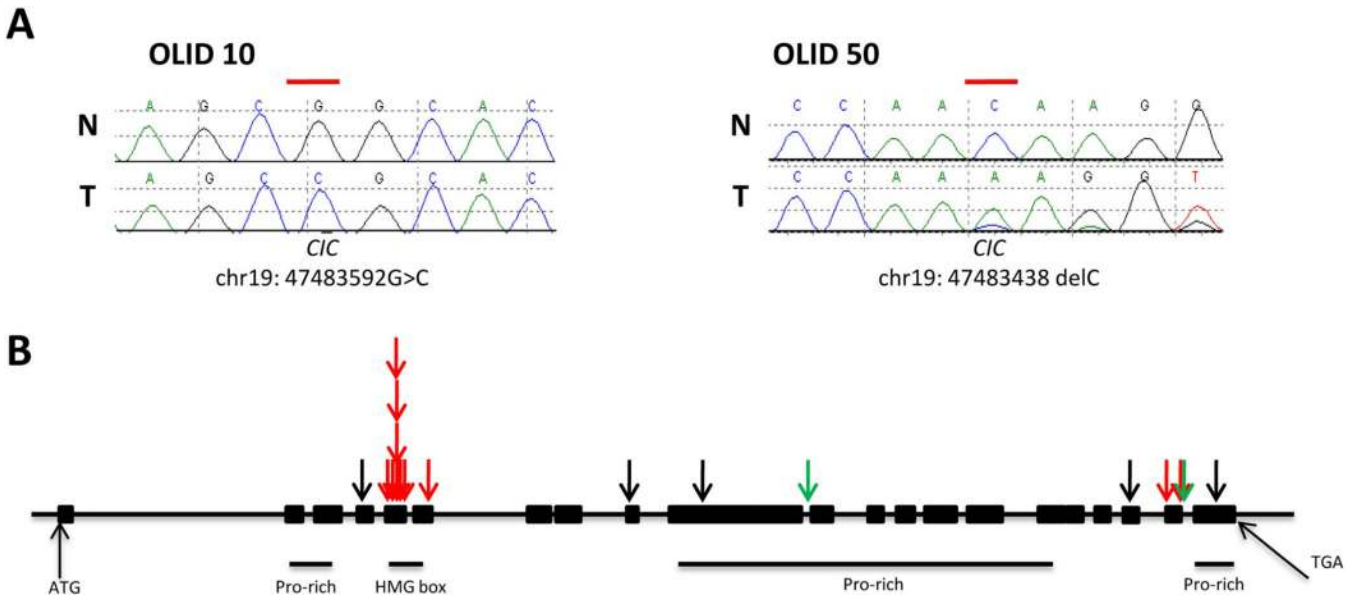

Fig. 2.

Mutations in *CIC* (**A**) Sanger sequencing chromatograms showing representative *CIC* mutations in the indicated tumors. T, DNA from tumor; N, DNA from matched normal tissue. The mutated bases are overlined with a red bar. (**B**) Mutation distribution of *CIC* mutations. Red arrows represent missense mutations substitutions, black arrows represent insertions or deletions, and green arrows represent splice site alterations. See tables S3 and S4 for details. The black boxes denote exons, Pro-rich denotes the proline-rich domains, HMG denotes the high mobility group domain, and the start and stop codons are indicated.