Brain Pathology ISSN 1015-6305

RESEARCH ARTICLE

KIAA1549-BRAF Fusions and IDH Mutations Can Coexist in Diffuse Gliomas of Adults

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Keywords
1p19q loss, BRAF mutation, deregulation of the Ras-RAF-ERK signaling pathway, diffuse gliomas, IDH1 mutation, KIAA1549/BRAF fusion gene.

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Received 1 March 2012
Accepted 9 May 2012
Published Online Article Accepted 17 May 2012

doi:10.1111/j.1750-3639.2012.00603.x

Abstract

KIAA1549-BRAF fusion gene and isocitrate dehydrogenase (IDH) mutations are considered two mutually exclusive genetic events in pilocytic astrocytomas and diffuse gliomas, respectively. We investigated the presence of the *KIAA1549-BRAF* fusion gene in conjunction with IDH mutations and 1p/19q loss in 185 adult diffuse gliomas. Moreover $BRAF^{v600E}$ mutation was also screened. The *KIAA1549-BRAF* fusion gene was evaluated by reverse-transcription polymerase chain reaction (RT-PCR) and sequencing. We found IDH mutations in 125 out 175 cases (71.4%). There were *KIAA1549-BRAF* fusion gene in 17 out of 180 (9.4%) cases and $BRAF^{v600E}$ in 2 out of 133 (1.5%) cases. In 11 of these 17 cases, both IDH mutations and the *KIAA1549-BRAF* fusion were present, as independent molecular events. Moreover, 6 of 17 cases showed co-presence of 1p/19q loss, IDH mutations and *KIAA1549-BRAF* fusion. Among the 17 cases with *KIAA1549-BRAF* fusion gene 15 (88.2%) were oligodendroglial neoplasms. Similarly, the two cases with $BRAF^{v600E}$ mutation were both oligodendroglioma and one had IDH mutations and 1p/19q co-deletion. Our results suggest that in a small fraction of diffuse gliomas, *KIAA1549-BRAF* fusion gene and $BRAF^{v600E}$ mutation may be responsible for deregulation of the Ras-RAF-ERK signaling pathway. Such alterations are more frequent in oligodendroglial neoplasm and may be co-present with IDH mutations and 1p/19q loss.

INTRODUCTION

Mutations in the gene encoding human cytosolic NADP+-dependent isocitrate dehydrogenase (IDH) have been reported to be very frequent, approaching 70%–80% in World Health Organization (WHO) grades II and III diffuse gliomas such as astrocytomas, oligodendrogliomas and oligoastrocytoma (21, 22). Moreover, the occurrence of *IDH1* mutations in WHO grade IV glioblastomas (GBMs) identify such lesions as "secondary" GBM in contrast to "primary" GBM lacking such molecular alterations (14). On the other hand, the WHO grade I pilocytic astrocytomas (PAs), while showing absent or rare *IDH* mutations, frequently disclose a tandem duplication at 7q34, resulting in the fusion of *BRAF* and *KIAA1549* genes, and the production of a chimeric protein with constitutive BRAF activity. This event results in the activation of the extracellular-signal-regulated kinases (ERK)/mitogen-activated protein kinase (MAPK) pathway and promotes G2/M transition in the cell cycle. These two events have so far been considered as mutually exclusive and molecular analysis of these two genetic alterations has been used as a sensitive and highly specific method of distinguishing PAs from diffuse gliomas (2, 5, 9, 10, 12, 15, 18). However, the concept that *IDH* mutations and *BRAF-KIAA1549* fusion gene are mutually exclusive molecular events is derived from a large study based exclusively on fluorescence *in situ* hybridization (FISH) (11). In addition, most of the tumors with *BRAF-KIAA1549* fusion gene were PAs in the pediatric population, whereas tumors with the *IDH* mutation were mostly diffuse gliomas in adults (11). In contrast to adults, *IDH* mutations are rare in pediatric gliomas, irrespective of histological type (1, 16). Therefore, it is possible that the apparent mutual exclusion between presence of the *BRAF-KIAA1549* fusion gene and *IDH* mutation may merely reflect the existence of different classes based on histological subtype (PA vs. diffuse gliomas) and age (children vs. adults). It is not clear whether this mutual exclusion exists in diffuse gliomas in adults. The serendipitous observation of few cases of oligodendrogliomas with the *KIAA1549-BRAF* fusion

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common in both PAs and diffuse gliomas. However, according to these authors, the nature of gains in the *BRAF* region differs between PAs and diffuse gliomas on the basis that they were able to detect by FISH the fusion of *BRAF* and *KIAA1549* predominantly in PAs, and only in a single instance in a diffuse grade II glioma. A major criticism of this study is that although FISH analysis is a powerful method to detect chromosomal alteration and particularly chromosomal gain or loss, it may be subject to laboratory variability when dealing with more subtle alterations such as fusion genes (7). In FISH analysis, the probes used can detect genomic duplications involving the 3′ region of BRAF (the same region involved in the KIAA1549-BRAF fusion), but are not specific for this rearrangement. Moreover, a threshold of 15% is established by evaluation of normal tissue and is used as a minimum for determining BRAF duplication. Cases showing a lower percentage of positive cells are considered not duplicate (20). On the basis of these considerations, our study based on detection of the fusion gene by RT-PCR assay followed by direct sequencing of the PCR product has allowed to detect such rare molecular alteration in a large cohort of adult diffuse gliomas.

Another interesting and previously unreported finding of the present study was the occurrence of 1p/19q loss in 6 of 10 cases in which *IDH* mutations and *KIAA1549-BRAF* fusion were copresent. All these "triple-positive" cases were oligodendroglial tumors (five oligodendrogliomas and one oligoastrocytoma) (Figure 3). In this study, we also found $BRAF^{V600E}$ mutation in two out of 133 diffuse gliomas (1.5%). They both were oligodendrogliomas, and in one of them such mutation was associated with IDH mutations and 1p/19q loss. This is consistent with the results of a previous study on 162 low-grade diffuse gliomas, which showed that only one oligodendroglioma had a $BRAF^{V600E}$ mutation (17).

Recent studies have found a high frequency of CIC mutations in oligodendrogliomas in associations with IDH mutations and 1p/19 anomalies (3, 23). The significance that these alterations, together with KIAA1549-BRAF fusion gene and $BRAF^{V600E}$, coexist more frequently in oligodendroglial neoplasms needs further investigations.

The clinical significance of *BRAF* alterations in diffuse gliomas remains unclear. The observed deregulation of the Ras-RAF-ERK signaling pathway in nonpilocytic gliomas is attributed to its upstream positive regulators, including epidermal growth factor receptor and platelet-derived growth factor receptor, which are known to be highly active in the majority of diffuse gliomas. Our results, however, suggest that in a small percentage of diffuse gliomas, such deregulation might be related to 7q34 rearrangements, resulting in a novel in-frame *KIAA1549-BRAF* fusion gene like that found in PAs. In addition, we demonstrate that such alterations can be associated with *IDH* mutations and 1p/19q loss.

Future studies should expand on these observations and continue to define the heterogeneous genetic and biological features of adult gliomas and the role of *BRAF* alterations in the pathogenesis and clinical behavior of these tumors.

ACKNOWLEDGMENTS

Manila Antonelli is supported by a grant from "Fondazione Italiana per la Lotta al Neuroblastoma" and Loredana Moi by a grant from "Il Fondo di Giò Onlus."

REFERENCES

1. Antonelli M, Buttarelli FR, Arcella A, Nobusawa S, Donofrio V, Oghaki H, Giangaspero F (2010) Prognostic significance of histological grading, p53 status, YKL-40 expression, and IDH1 mutations in pediatric high-grade gliomas. *J Neurooncol* **2**:209–215.
2. Bar EE, Lin A, Tihan T, Burger PC, Eberhart CG (2008) Frequent gains at chromosome 7q34 involving BRAF in pilocytic astrocytoma. *J Neuropathol Exp Neurol* **9**:878–887.
3. Bettegowda C, Agrawal N, Jiao Y, Sausen M, Wood LD, Hruban RH *et al* (2011) Mutations in CIC and FUBP1 contribute to human oligodendroglioma. *Science* **333**:1453–1455.
4. Bissola L, Eoli M, Pollo B, Merciai BM, Silvani A, Salsano E *et al* (2002) Association of chromosome 10 losses and negative prognosis in oligoastrocytomas. *Ann Neurol* **52**:842–845.
5. Forshew T, Tatevossian RG, Lawson AR, Ma J, Neale G, Ogunkolade BW *et al* (2009) Activation of the ERK/MAPK pathway: a signature genetic defect in posterior fossa pilocytic astrocytomas. *J Pathol* **2**:172–181.
6. Hawkins C, Walker E, Mohamed N, Zhang C, Jacob K, Shirinian M *et al* (2011) BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res* **14**:4790–4798.
7. Horbinski C, Miller CR, Perry A (2011) Gone FISHing: clinical lessons learned in brain tumor molecular diagnostics over the last decade. *Brain Pathol* **1**:57–73.
8. Idbaih A, Marie Y, Lucchesi C, Pierron G, Manié E, Raynal V *et al* (2008) BAC array CGH distinguishes mutually exclusive alterations that define clinicogenetic subtypes of gliomas. *Int J Cancer* **8**:1778–1786.
9. Jones DT, Kocialkowski S, Liu L, Pearson DM, Bäcklund LM, Ichimura K, Collins VP (2008) Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res* **21**:8673–8677.
10. Jones DT, Kocialkowski S, Liu L, Pearson DM, Ichimura K, Collins VP (2009) Oncogenic RAF1 rearrangement and a novel BRAF mutation as alternatives to KIAA1549:BRAF fusion in activating the MAPK pathway in pilocytic astrocytoma. *Oncogene* **20**:2119–2123.
11. Korshunov A, Meyer J, Capper D, Christians A, Remke M, Witt H *et al* (2009) Combined molecular analysis of BRAF and IDH1distinguishes pilocytic astrocytoma from diffuse astrocytoma. *Acta Neuropathol* **3**:401–405.
12. Lin A, Rodriguez FJ, Karajannis MA, Williams SC, Legault G, Zagzag D *et al* (2012) BRAF alterations in primary glial and glioneuronal neoplasms of the central nervous system with identification of 2 novel KIAA1549: BRAF fusion variants. *J Neuropathol Exp Neurol* **1**:66–72.
13. Louis D, Ohgaki H, Wiestler OD, WK Cavanee editors (2007) *WHO Classification of Tumors: Pathology and Genetics of Tumours of the Nervous System*. IARC Press: Lyon.
14. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H (2009) IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. *Clin Cancer Res* **15**:6002–6007.
15. Pfister S, Janzarik WG, Remke M, Ernst A, Werft W, Becker N *et al* (2008) BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest* **5**:1739–1749.
16. Pollack IF, Hamilton RL, Sobol RW, Nikiforova MN, Lyons-Weiler MA, LaFramboise WA *et al*; Children's Oncology Group (2011) IDH1 mutations are common in malignant gliomas arising in adolescents: a report from the Children's Oncology Group. *Childs Nerv Syst* **1**:87–94.
17. Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C *et al* (2011) Analysis of BRAF V600E mutation in 1.320 nervous system tumors reveals high mutation frequencies in

pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. *Acta Neuropathol* **3**:397–405.
18. Sievert AJ, Jackson EM, Gai X, Hakonarson H, Judkins AR, Resnick AC *et al* (2009) Duplication of 7q34 in pediatric low-grade astrocytomas detected by high-density single-nucleotide polymorphism-based genotype arrays results in a novel BRAF fusion gene. *Brain Pathol* **3**:449–458.
19. Tatevossian RG, Lawson AR, Forshew T, Hindley GF, Ellison DW, Sheer D (2010) MAPK pathway activation and the origins of pediatric low-grade astrocytomas. *J Cell Physiol* **3**:509–514.
20. Tian Y, Rich BE, Vena N, Craig JM, Macconaill LE, Rajaram V *et al* (2011) Detection of KIAA1549-BRAF fusion transcripts in formalin-fixed paraffin-embedded pediatric low-grade gliomas. *J Mol Diagn* **6**:669–677.
21. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol* **4**:1149–1153.
22. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W *et al* (2009) IDH1 and IDH2 mutations in gliomas. *N Engl J Med* **8**:765–773.
23. Yip S, Butterfield YS, Morozova O, Chittaranjan S, Blough MD, An J *et al* (2012) Concurrent CIC mutations, IDH mutations, and 1p/19q loss distinguish oligodendrogliomas from other cancers. *J Pathol* **1**:7–16.