Mutations of PIK3CA in Anaplastic Oligodendrogliomas, High-Grade Astrocytomas, and Medulloblastomas

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Abstract

The phosphatidylinositol 3’-kinase pathway is activated in multiple advanced cancers, including glioblastomas, through inactivation of the PTEN tumor suppressor gene. Recently, mutations in PIK3CA, a member of the family of phosphatidylinositol 3’-kinase catalytic subunits, were identified in a significant fraction (25–30%) of colorectal cancers, gastric cancers, and glioblastomas and in a smaller fraction of breast and lung cancers. These mutations were found to cluster into two major “hot spots” located in the helical and catalytic domains. To determine whether PIK3CA is genetically altered in brain tumors, we performed a large-scale mutational analysis of the helical and catalytic domains. A total of 13 mutations of PIK3CA within these specific domains were identified in anaplastic oligodendrogliomas, anaplastic astrocytomas, glioblastoma multiforme, and medulloblastomas, whereas no mutations were identified in ependymomas or low-grade astrocytomas. These observations implicate PIK3CA as an oncogene in a wider spectrum of adult and pediatric brain tumors and suggest that PIK3CA may be a useful diagnostic marker or a therapeutic target in these cancers.

Introduction

The phosphatidylinositol 3’-kinases (PI3Ks) are a family of enzymes that increase intracellular pools of phosphatidylinositol 3,4,5-trisphosphate (PIP3) by phosphorylating its chemical precursor, phosphatidylinositol 4,5-bisphosphate (1, 2). PIP3 levels are tightly controlled by stringent PI3K regulation and via the PTEN PIP3 phosphatase, which converts PIP3 back to phosphatidylinositol 4,5-bisphosphate. PTEN-inactivating mutations have been found in approximately 30% of primary glioblastomas (3, 4), and recent studies showed an inverse correlation between PTEN expression and Akt activation in clinical glioblastoma tissue (5). Because PI3Ks and PTEN have opposing effects on the PIP3 pool, and PTEN-inactivating mutations appear to contribute to oncogenesis, it is reasonable to postulate that increased PI3K activity via gain of function mutations or gene amplification might have similar oncogenic effects. In fact, the genomic region containing PIK3CA is amplified in ovarian cancer, cervical cancers, and some brain tumors (6–8), although whether the target of these amplicons is PIK3CA rather than an adjacent gene has not been determined. Recently, a large-scale mutational analysis of all major classes of PI3K catalytic subunits within several tumor types revealed somatic mutations in the catalytic subunits in a significant fraction (25–30%) of colorectal cancers, gastric cancers, and glioblastomas and in a smaller fraction (<10%) of breast and lung cancers (9). All mutations were identified in a specific catalytic subunit, PIK3CA. Even more remarkably, the mutations clustered in hot spots within the helical (exon 9) and catalytic (exon 20) domains. These results suggested that PIK3CA may represent one of the most highly mutated oncogenes among gastrointestinal and high-grade glial tumors. To investigate the potential oncogenic role of PIK3CA in a more diverse sampling of brain neoplasms, we performed a large-scale mutational analysis on the six adult and pediatric malignant neoplasms, including glioblastoma multiforme, anaplastic astrocytoma, low-grade infiltrative astrocytoma, anaplastic oligodendroglioma, ependymoma, and medulloblastoma.

Materials and Methods

Tissue Samples and Genomic DNA. Brain tumor cell lines, xenografts, frozen primary tumor samples, and blood were obtained from the Duke University Brain Tumor Center Tissue Bank. Primary tumor samples were obtained and frozen at the time of surgery from patients who were treated at the Duke University Medical Center, after obtaining written consent. Acquisition of tissue specimens was approved by the Duke University Health System Institutional Review Board and performed in accordance with Health Insurance Portability and Accountability Act regulations.

Choice of Brain Tumors. Six brain tumor types were selected for mutational analysis. Included were the most common infiltrative astrocytic tumors, glioblastoma multiforme (WHO grade IV), anaplastic astrocytoma (WHO grade III), and low-grade infiltrative astrocytoma (WHO grade II). We also included anaplastic oligodendroglioma (WHO grade III). Medulloblastoma and ependymoma, the most common malignant brain tumors of childhood, were included.

PCR and Sequencing. Primer sequences and PCR conditions have been described previously (9). PCR products were sequenced by Agencourt Bioscience Corp. (Beverly, MA).

Results and Discussion

PIK3CA mutational analysis was performed on exon 9 and exon 20 of PIK3CA by PCR of genomic DNA from brain tumor samples followed by direct sequencing. We specifically examined exons 9 and 20 of PIK3CA because recent screening of a large number of colon cancers (n = 234) revealed four fifths of the observed mutations to be clustered in these two exons (9).

Analysis of 285 selected brain tumors identified mutations in 3 of 21 (14%) anaplastic oligodendrogliomas, 4 of 78 (5%) medulloblastomas, 5 of 105 (5%) glioblastomas, and 1 of 31 (3%) anaplastic astrocytomas. No mutations were observed in 24 low-grade astrocytomas or 26 ependymomas (Table 1). Among these alterations, 11 were located at positions previously observed to be altered in colorectal cancers (9), whereas two alterations affected residues not known to be mutated. All but one of the mutations were shown to be heterozygous. We also evaluated whether the mutations were somatically acquired (i.e., tumor specific) by examining the sequence of PIK3CA in genomic DNA from normal tissue of the relevant patient.
PIK3CA mutations

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Anaplastic oligodendroglioma</th>
<th>Glioblastoma multiforme</th>
<th>Medulloblastoma</th>
<th>Anaplastic astrocytoma</th>
<th>Low-grade astrocytoma</th>
<th>Ependymoma</th>
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<td>Exon 9</td>
<td>G1624A</td>
<td>E542K</td>
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<td>Y1021N</td>
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<td>H1047L</td>
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<td>1</td>
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<td>1</td>
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<td>1</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

* Corresponding normal tissue were available.

Corresponding normal tissue was available for 7 of the 13 tumors, and in all 7 cases, the mutations were observed to be somatic.

Four independent lines of evidence suggest that the observed PIK3CA mutations are functionally important during tumor progression rather than representing passenger or nonfunctional alterations. First, as described above, most of the missense changes observed in this study correspond to residues that have been reported to represent bona fide somatic mutations in colorectal cancers (9). In addition, in vitro studies have shown the H1047R mutant to have increased lipid kinase activity (9). Second, the ratio of nonsynonymous to synonymous mutations is a reliable indicator of selection during in vitro studies have shown the H1047R mutant to have increased lipid kinase activity (9). Second, the ratio of nonsynonymous to synonymous mutations is a reliable indicator of selection during tumor progression because silent mutations are unlikely to exert a selective growth advantage. There were no somatic synonymous mutations detected in this study. Therefore, the ratio of nonsynonymous to synonymous mutations was much higher than the expected 2:1 ratio for nonselected passenger mutations. Third, the prevalence of the mutations located in coding regions of PIK3CA was ~40 per Mb of tumor DNA, more than 40 times higher than the background mutation frequency of nonfunctional alterations observed in the genome of cancer cells [~1 per Mb (10)]. Finally, all of the mutated residues we identified were highly conserved evolutionarily, with retention of identity in mouse, rat, and chicken.

Furthermore, we performed a mutational analysis of PTEN in a subset of the samples. We identified PTEN mutations in 15 of 22 glioblastoma multiforme cell lines, 2 of 18 glioblastoma multiforme primary tumors, and 0 of 7 anaplastic astrocytomas. No PTEN mutations were identified in any of the 13 tumors with PIK3CA mutations, indicating that PIK3CA mutations in brain tumors occur only in tumors that do not carry PTEN mutations.

Proto-oncogenes can be activated by point mutation or gene amplification. To determine whether PIK3CA gene dosage might contribute to oncogenic activity in brain tumors, we measured PIK3CA amplification in four categories of brain tumors by quantitative real-time PCR. No evidence of significant gene amplification (>5-fold) was shown in 60 medulloblastomas, 50 glioblastomas, 21 anaplastic oligodendrogliomas, or 14 anaplastic astrocytomas (data not shown). Furthermore, no significant increase of PIK3CA expression was observed in 42 medulloblastomas or 21 glioblastomas by quantitative real-time PCR, suggesting that gene amplification and overexpression were not significant mechanisms of PIK3CA activation among these tumors, as was found in colorectal cancer (9).

Among the brain cancers we analyzed, only glioblastomas have been shown previously to have alterations in PIK3CA (9). Samuels et al. (9) examined 15 glioblastomas and found 4 PIK3CA mutations. One mutation was present in exon 20, whereas the other three were scattered in different exons outside the hot spot regions. The overall decreased incidence of PIK3CA mutations in our glioblastoma samples may be consistent with the finding in the study by Samuels et al. (9) of an increased incidence of non-hot spot mutations among glioblastomas. In addition, the mutation frequency might also depend on the selection of samples. One suggestive explanation for a difference may reflect on the age distribution of PIK3CA mutations seen in our samples. It was noted that four of six patients with PIK3CA mutations in high-grade astrocytomas (WHO grade III and IV) were diagnosed with the disease at a relatively young age (19, 36, 49, and 53 years). Only one was diagnosed with glioblastoma multiforme at age 65 years, and for another, the patient age at diagnosis was unknown. Furthermore, after performing additional mutational analyses on 53 glioblastoma multiforme tumors in patients older than 55 years, no further PIK3CA mutations were identified in exon 9 or 20. One possibility for this difference is that PIK3CA mutations occur less frequently in primary glioblastomas than in secondary glioblastomas, the latter of which are known to evolve through different genetic alterations and affect a younger age group (11). Further support for this possibility is the fact that PTEN mutations occur predominantly in primary glioblastomas (12) and are uncommonly found in secondary glioblastomas (13). It could be postulated that the PI3K pathway is in fact activated in some secondary glioblastomas, through gain of function of PIK3CA mutations rather than PTEN mutations.

The absence of mutation in low-grade astrocytoma and the presence of a single mutation among 31 anaplastic astrocytomas suggest that PIK3CA abnormalities might occur at relatively later stages of glioma progression. Similar to our findings, PIK3CA mutations were previously found to occur at a relatively late stage of colon cancer progression (9).

Four PIK3CA mutations were identified in 78 medulloblastomas, suggesting that in addition to the involvement of the hedgehog/patched and Wnt signaling pathways (14, 15), PI3K and its upstream and downstream factors might also play important roles in medulloblastoma tumorigenesis.

Three of 21 anaplastic oligodendrogliomas contained PIK3CA mutations in exon 9 or exon 20. To our knowledge, the identified PIK3CA mutations represent the first oncogene-specific mutations in anaplastic oligodendrogliomas. Although oligodendrogliomas affect younger adults, they tend to be more indolent and have an overall better prognosis than astrocytic tumors (16). Accurate diagnosis is a key to the management of oligodendrogliomas. However, prognosis can be somewhat variable. Evidence from several retrospective studies suggests that allelic losses of 1p and 19q serve as molecular markers of response to chemotherapy and radiation therapy and are an indicator of prolonged survival in patients with oligodendrogliomas (17). The discovery here of relatively common PIK3CA mutations in anaplastic oligodendroglioma gives new insight into the molecular...
Our observations of PIK3CA mutations in multiple brain tumor types extend the recent observations of PIK3CA mutations in glioblastomas. In particular, we found mutations of the PIK3CA gene in a substantial proportion of anaplastic oligodendrogliomas, as well as in high-grade astrocytomas and medulloblastomas. The consistency of hot spot mutations in PIK3CA across diverse tumor types suggests a possible approach to targeted therapy. One could envision the development of agents acting as highly selective antagonists of the mutant alleles products, sparing normal cells exhibiting wild-type PIK3CA activity. Our findings suggest that PIK3CA is an important oncogene in common malignant brain tumors and has the potential to make a significant impact on the future of cancer therapeutics.

Acknowledgments

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References