Genetic Aberrations Leading to MAPK Pathway Activation Mediate Oncogene-Induced Senescence in Sporadic Pilocytic Astrocytomas

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Abstract

Purpose: Oncogenic BRAF/Ras or NF1 loss can potentially trigger oncogene-induced senescence (OIS) through activation of the mitogen-activated protein kinase (MAPK) pathway. Somatic genetic abnormalities affecting this pathway occur in the majority of pilocytic astrocytomas (PA), the most prevalent brain neoplasm in children. We investigated whether OIS is induced in PA.

Experimental Design: We tested expression of established senescence markers in three independent cohorts of sporadic PA. We also assessed for OIS in vitro, using forced expression of wild-type and V600E-mutant BRAF in two astrocytic cell lines: human telomerase reverse transcriptase (hTERT)-immortalized astrocytes and fetal astrocytes.

Results: Our results indicate that PAs are senescent as evidenced by marked senescence-associated acidic β-galactosidase activity, low Ki-67 index, and induction of p16INK4a but not p53 in the majority of 52 PA samples (46 of 52; 88.5%). Overexpression of a number of senescence-associated genes [CDKN2A (p16), CDKN1A (p21), CEBPB, GADD45A, and IGFBP7] was shown at the mRNA level in two independent PA tumor series. In vitro, sustained activation of wild-type or mutant BRAF induced OIS in both astrocytic cell lines. Loss of p16INK4a in immortalized astrocytes abrogated OIS, indicative of the role of this pathway in mediating this phenomenon in astrocytes. OIS is a mechanism of tumor suppression that restricts the progression of benign tumors. We show that it is triggered in PAs through p16INK4a pathway induction following aberrant MAPK activation.

Conclusions: OIS may account for the slow growth pattern in PA, the lack of progression to higher-grade astrocytomas, and the high overall survival of affected patients. Clin Cancer Res; 17(14): 4650–60. ©2011 AACR.

Introduction

Primary brain tumors are the second most common type of cancer in children (after leukemia) and the leading cause of cancer-related mortality and morbidity in young patients (1, 2). Astrocytomas account for 50% of all central nervous system (CNS) tumors and are commonly regrouped into low-grade (WHO grade I and II, LGA) or high-grade (WHO grade III and IV, HGA) tumors (3). Pilocytic astrocytomas (PA) are WHO grade I tumors. They are the predominant histologic subtype in LGA and the most prevalent CNS neoplasm in childhood, accounting for 23% of all pediatric brain tumors (2). They occur sporadically throughout childhood and in 15% to 40% of children affected with neurofibromatosis type 1 (NF1; ref. 4). These tumors arise throughout the CNS but have a predilection for the cerebellum and the optic pathways (5). PAs are slow growing tumors, which often harbor a cystic component. They exhibit distinct features, readily distinguishable from other LGAs, including an improved clinical course and prognosis in children, limited proliferation index, and no TP53 mutations or platelet-derived growth factor (PDGF) A/PDGFRα amplification (6–10). Maximal
surgical resection is the mainstay of therapy, and failure to achieve it remains the main therapeutic concern (3). Although cerebellar PAs are usually readily amenable to complete surgical resection, residual tumor in other less anatomically accessible locations can require adjuvant therapies for tumor control (9, 11). Thus, despite an excellent 5-year overall survival rate of more than 90% and progression-free survival rate of more than 65% for PA (1, 11, 12), affected children may experience significant morbidity due to the tumor location and/or the side effects of adjuvant therapies in case of residual disease.

Several consecutive articles, including from our groups, recently showed aberrant activation of the mitogen-activated protein kinase (MAPK) pathway to be the main molecular abnormality in PA (13–18). Indeed, gene fusions and activating mutations involving key regulators of the MAPK pathway have been found in the majority of PAs tested. Duplication of 7q34 leading to in-frame KIAA1549-BRAF fusions and constitutive BRAF activation have been identified in up to 65% of sporadic PAs tested (13–18). KRAS activating mutations affecting codons 12, 13, and 61 have been identified in approximately 4% to 7% of PAs (13, 19–21) and similarly low incidence rates have been found for the V600E BRAF activating mutation in PA (13, 14, 16, 17). RAF1 fusions leading to constitutive activation of RAF1 have been detected in 4 PAs to date (13, 15) and a novel BRAF activating mutation was also recently identified at low frequency in PA (15, 22, 23). Moreover, in NF1 patients, NF1 gene inactivation leads to increased Ras activity and subsequent MAPK pathway activation. All of these observations converge to indicate a major role of MAPK pathway activation in the clinical course and biology of PA.

Senescence is a physiologic phenomenon characterized by a permanent cell-cycle arrest. Oncogene-induced senescence (OIS) was first reported when the expression of oncogenic Ras in primary human or rodent cells resulted in a permanent G1 arrest and was accompanied by accumulation of p53 and p16 (24). Collectively, investigators have subsequently observed that mutations in KRAS, BRAF, PTEN, and NF1 can trigger cellular senescence in vivo and have implied that OIS is a mechanism of tumor suppression that restricts the progression of benign tumors in the absence of additional cooperating mutations (25–28). Human melanocytic nevi, which are common benign lesions of cutaneous melanocytes that rarely progress to melanoma, constitute an intriguing example. Somatic mutations leading to constitutive activation of BRAF (V600E) are present in up to 80% of melanocytes from this benign tumor and lead to growth arrest of cells through OIS (27, 29). Human neurofibromas developing in the context of neurofibromatosis type I also show senescence in vivo due to loss of NF1 activity (25).

The natural history of PA is unique, as these tumors are self-containing, almost never progress to higher-grade astrocytomas, and their initial growth is usually followed by a decrease in tumor activity, after which they enter a dormant phase and either remain quiescent or restart cycle(s) of growth followed by dormancy, similar to what is observed in OIS in vitro. On the basis of these observations, we hypothesized that activation of the MAPK pathway leads to OIS in PA, potentially accounting for the benign nature of these tumors. Thus, we investigated a number of established senescence markers in 52 primary sporadic pediatric PAs, including expression levels of p16<sup>INK4a</sup>, p53, and KI-67, a marker of proliferation index, in archival tissues and senescence-associated acidic β-galactosidase (SA-β-Gal) activity in fresh tissue samples where available (30). Gene expression profiling in 2 independent PA cohorts also strongly supported the presence of an activated senescence program in PA.

In addition, we overexpressed wild-type or V600E mutant BRAF in human immortalized astrocytes and in fetal astrocytes and assessed for OIS induction in vitro in these surrogate astrocytic models. Our results indicate that MAPK activation triggers OIS in PA. As MAPK activation is likely the major abnormality driving proliferation in PA, OIS may be responsible for the tendency of PA for spontaneous growth arrest. Furthermore, the absence of additional genetic alterations and the presence of functional cell-cycle control mechanisms could explain the lack of transformation of PA into higher-grade tumors, accounting for the relatively benign course of this grade I tumor when considering overall survival.

Materials and Methods

Sample characteristics and pathologic review

All samples were obtained with informed consent from patients after approval of the Institutional Review Board of the respective hospitals they were treated in and were
Table 1. Patient characteristics and genetic alterations affecting the MAPK pathway (Montreal cohort)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>24 M; 28 F</td>
</tr>
<tr>
<td>Age [average and (range), years]</td>
<td>6.22 (0.33–18)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
</tr>
<tr>
<td>Posterior fossa</td>
<td>28 (53.8%)</td>
</tr>
<tr>
<td>Optic pathway</td>
<td>8 (15.4%)</td>
</tr>
<tr>
<td>Brainstem</td>
<td>7 (13.5%)</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>4 (7.7%)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>5 (9.6%)</td>
</tr>
<tr>
<td>MAPK pathway aberration</td>
<td></td>
</tr>
<tr>
<td>KIAA1549-BRAF fusion</td>
<td>37 (71.2%)</td>
</tr>
<tr>
<td>V600E BRAF</td>
<td>2 (3.8%)</td>
</tr>
<tr>
<td>SRGAP3-RAF1</td>
<td>0</td>
</tr>
<tr>
<td>KRAS mutation</td>
<td>2 (3.8%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>11 (21.2%)</td>
</tr>
</tbody>
</table>

Independently reviewed by senior pediatric neuropathologists according to the WHO guidelines (3, 31). Patients’ characteristics in the Montreal, Cambridge, and Heidelberg series are detailed in Table 1 and Supplementary Tables S1 and S2.

Cell lines, antibodies, and transfections

Human telomerase reverse transcriptase (hTERT)-immortalized human astrocytes (kind gift of Dr A Guha, Labatt Brain Tumour Research Centre, Toronto, Ontario, Canada) were grown as previously described (32). Human fetal astrocytes were obtained from 18- to 22-week-old fetal brain specimens provided by the Human Fetal Tissue Repository (Albert Einstein College of Medicine, Bronx, NY; collaboration with Dr Jacques Antel, Montreal Neurologic Institute) following approved guidelines from the Canadian Institutes of Health Research and processed as described in Supplementary Table S1. Primary antibodies were from Cell Signaling Technology (GFAP/Myc-tag/β-actin) and from Santa Cruz Biotechnology (pERK/BRAF/p53/p16INK4a). Cells were transfected with wild-type Myc-tagged BRAF or V600E BRAF (a kind gift of Dr Richard Marais, Cancer Research, London, UK) as previously described (33). Transfection efficiency was assessed using immunofluorescence and Western blot analysis as previously described (33).

BRAF tandem duplication screening

Reverse transcriptase-PCR to detect the BRAF fusion transcripts was carried out as previously described (16).

Gene expression profiling

Cambridge series. Samples were analyzed on the Illumina HT12 v3 expression platform. Selected genes were assessed for differential expression between tumor and control samples, using a 2-tailed Mann–Whitney U test.

Heidelberg series. Samples were analyzed on the Illumina WG6 v3 expression platform. The mean of the normal brain expression was subtracted from the expression value for each sample and for each gene of interest, to give a log2 fold-change value. Detailed overview is provided in Supplementary Table S1.

Analysis of SA-β-Gal activity

Cells were plated in a 6-well plate at 50% confluence overnight prior to the assay. A detailed overview of the experimental procedure is provided in Supplementary Table S1. The appearance of a blue color was determined under the microscope as previously described (27, 30).

Immunofluorescence, immunohistochemical analysis, Western blotting, and cell-cycle analysis

Immunofluorescence and immunohistochemical staining and Western blot analysis were carried out and scored as previously described (24, 28, 34). Detailed overview of the experimental procedures and data analysis using the FlowJo software are provided in Supplementary Table S1. All experiments were carried out at least 3 independent times in triplicate and at least 40,000 cells were acquired from each sample for fluorescence-activated cell-sorting analysis.

Results

Genetic alterations in the MAPK pathway in PA included in the Montreal cohort

We first confirmed the nature of the genetic abnormality affecting the MAPK pathway in the cohort of 52 sporadic PAs from the Montreal series investigated in this study. To this end, we used specific exonic primer pairs for PCR amplification of KIAA1549-BRAF fusion products. Fusion transcripts were detected in 37 of 52 (71.1%) PA samples (24 of 28 posterior fossa, 4 of 7 brainstem, 5 of 8 optic pathway, 1 of 4 cerebrum, and 3 of 5 spinal PAs). We also sequenced BRAF exons 11 and 15 in 50 of 52 PAs and identified the hotspot V600E mutation in 2 of the 13 tumors negative for KIAA1549-BRAF fusion and 1 in a PA with KIAA1549-BRAF fusion. We then screened for mutations of exons 2 to 7 of RAF1, exons 2 to 3 of KRAS and NRAS, and exon 13 of PTPN11, and for SRGAP3-RAF1 fusion, and identified 2 samples with mutated KRAS (2 posterior fossa) in the 13 samples that were negative for KIAA1549-BRAF fusion or V600E BRAF mutation (Table 1, Supplementary Table S2). These data, as well as overall survival and event-free survival in our patient cohort (Supplementary Fig. S1), are in concordance with current literature, showing the preponderance of KIAA1549-BRAF fusions as MAPK activating events in PA (1, 11, 12, 35).

PAs express senescence markers

Activation of the INK4a/ARF locus by oncogenes is well documented as a tumor-suppressive mechanism, notably through induction of OIS. Senescent cells have been shown to display increased expression of p16INK4a, p21CIP1, or p53
while having a low to null mitotic index (36–38). The p16^{INK4a} protein is a major tumor suppressor, often highly expressed in senescent cells \textit{in vitro} and inactivated in a variety of human cancers, including 30% to 70% of HGGs (39, 40). OIS is characterized by cycle arrest, which is accompanied by the induction of both p16^{INK4a} and SA-β-Gal activity, a commonly used senescence marker (27, 28, 37). We investigated whether senescence was triggered in PA \textit{in vivo} using SA-β-Gal activity and levels of p16^{INK4a}, p53, and Ki-67 (a marker of cellular proliferation). Fresh PA tumor samples from 6 consecutive patients including lesions from the cerebellum (N = 4), brainstem (N = 1), and spinal cord (N = 1) were markedly positive for SA-β-Gal (Fig. 1; Montreal cohort). Five of these samples...
(PA 2-3-7-12) had KIAA1549-BRAF fusions and 1 (PA 37) had no identified genetic abnormality of the MAPK pathway (Table 1, Supplementary Table S2). We then investigated fixed material from the 52 PA samples included in our cohort, including these 6 samples for which we obtained fresh material at initial surgery, for the expression of p16INK4a, p53, and Ki-67 by using specific antibodies targeting these proteins. Staining for p16INK4a showed markedly increased expression in 46 of 52 (89.5%) PAs compared with normal brain (Fig. 2, Table 2). PAs were positive for p16INK4a regardless of their anatomic location and regardless of the presence or type of the genetic alteration affecting the MAPK pathway. Immunopositivity for p16INK4a was both cytoplasmic and nuclear. Its extent varied widely, in terms of both the fraction of positive cells and its intensity (Table 2), and was found predominantly in the nonpilocytic component, particularly in those PAs that had the classical biphasic "loose-dense" pattern (Fig. 2). Six PAs had p16INK4a staining in 5% of cells or less counted across any field (Fig. 2, Table 2). These PAs had an active MAPK pathway as shown by the positive pERK staining on the respective slides (Supplementary Fig. S2B).

No PAs showed increased nuclear p53 expression consistent with previous publications indicating absence of TP53 mutations in PA (reviewed in ref. 9). As also expected in PA, Ki-67 index was low in all cases (1%–2%; rarely up to 5%). The MAPK pathway was triggered in all PA cases (14), as shown by the positivity of the antibody against the phosphorylated (activated) forms of ERK1/2 (pERK; Supplementary Fig. S2B; ref. 14). Slides from normal control brain stained on the respective slides (Supplementary Fig. S2B). No PAs were negative for p16INK4a, p53, and Ki-67 expression (Supplementary Fig. S2A, Table 2).

Taken together, these data show that at the protein level, PAs display features of OIS, and these are now widely accepted markers of this central role for both CDKN2A (p16) and CDKN1A (p21). Several key publications show that in culture possess at least 2 independent mechanisms limiting their life span and leading to senescence: First, a telomere-dependent pathway registers the cumulative number of cell divisions and triggers senescence following short-term overexpression of BRAFWT or BRAFV600E induces features typical of OIS in two independent astrocytic cell line models

A wealth of data support a model in which somatic cells in culture possess at least 2 independent mechanisms limiting their life span and leading to senescence: First, a telomere-dependent pathway registers the cumulative number of cell divisions and triggers senescence following

### Table 2. Levels of Ki-67, nuclear p53, and p16<sup>INK4a</sup> staining in all 52 primary PAs and in control brain samples

<table>
<thead>
<tr>
<th></th>
<th>All PA samples (N = 52)</th>
<th>KIAA1549-BRAF fusion (N = 37)</th>
<th>V600E BRAF (N = 2)</th>
<th>KRAS (N = 2)</th>
<th>Unknown (N = 11)</th>
<th>Control brain (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67 &lt;1%</td>
<td>9 (18.8%)</td>
<td>7 (13.4%)</td>
<td>0</td>
<td>0</td>
<td>2 (18%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>1%–5%</td>
<td>39 (86.5%)</td>
<td>29 (78.4%)</td>
<td>2 (100%)</td>
<td>2 (100%)</td>
<td>7 (64%)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;5%</td>
<td>2 (3.8%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>p53 0%</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1%–20%</td>
<td>42 (80.7%)</td>
<td>32 (86.5%)</td>
<td>0</td>
<td>2 (100%)</td>
<td>8 (72.7%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>&gt;20%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>p16 &lt;10%</td>
<td>6 (11.5%)</td>
<td>3 (8%)</td>
<td>0</td>
<td>0</td>
<td>3 (27%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>10%–50%</td>
<td>30 (57.7%)</td>
<td>20 (54%)</td>
<td>2 (100%)</td>
<td>2 (100%)</td>
<td>6 (54.5%)</td>
<td>0</td>
</tr>
<tr>
<td>≥50%</td>
<td>16 (30.8%)</td>
<td>14 (37.8%)</td>
<td>0</td>
<td>2 (18%)</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2. p16INK4A is induced in the majority of PAs. Immunohistochemical analyses of p16INK4A (p16) were done on 52 pediatric PA samples (Montreal cohort) and control brains (CB) from age-matched children. A representative staining of 3 CBs and 18 PAs from different regions within the brain is shown. PA1–PA15 had marked p16 induction, whereas PA16–PA18 and the CB had limited p16 staining.
Moreover, forced BRAFWT and BRAFV600E expression in unchanged (Fig. 4C) compared with the empty vector. NHA and fetal astrocytes, whereas p53 levels remained p16INK4a in cells from the astrocytic lineage, similar to expression levels induce OIS and increased levels of G1–M phase as shown by increased G1 and decreased S-phase following cell-cycle analysis (Fig. 4D).

Tumor samples also had a senescence. These results show that forced overexpression of WT and mutant BRAF induces p16INK4a expression, SA-β-Gal activity and cell-cycle arrest in 2 astrocytic cell lines. The maintenance of telomere length through hTERT immortalization of NHA argues for an active oncogene-driven senescence process, rather than a loss of replicative potential in these cells, which can normally be serially passaged successfully.

**Loss of p16 abrogates the senescent features of NHA cells stably overexpressing BRAFWT or BRAFV600E**

To better understand the cellular responses to expression of BRAF, we sought to generate stable NHA clones over-expressing BRAFWT or BRAFV600E. In several independent experiments, we observed unusually low transfection efficiency and were able to select only 4 clones overexpressing BRAFWT from 75 that were screened and 2 clones over-expressing BRAFV600E from 60 screened. Interestingly, when we investigated p16INK4a and p53 levels in clones, we observed that p16INK4a expression was lost in all BRAF-overexpressing clones whereas p53 expression remained unchanged (Fig. 5A). This suggests that inactivation of p16INK4a in NHA may allow the cells to bypass BRAF-induced senescence and to divide enough to constitutively incorporate the DNA plasmid and become transformed. In keeping with this hypothesis, sustained expression of BRAFWT and BRAFV600E did not induce senescent-associated features in NHA, as was seen following transient overexpression. Indeed, cells had no morphologic changes indicative of senescence, no significant induction of SA-β-Gal activity (Fig. 5B), showed foci of transformation (Fig. 5C), and were able to grow and form colonies in anchorage-independent conditions (soft agar) compared with EV transfectants (Fig. 5D). These data indicate that p16INK4a may protect against BRAF-driven proliferation and that its loss may diminish the physiologic protection provided by this cell-cycle regulator through induction of senescence.

**Discussion**

We show in vivo and in vitro that PAs display classical hallmarks of senescence, suggesting that MAPK pathway activation, whatever the genetic defect at its origin, drives OIS partly through the induction of p16INK4a pathway activation in astrocytes. Fresh samples from pediatric PAs were invariably positive for SA-β-Gal, and we observed a clear induction of p16INK4a protein in 46 of 52 (89.5%) primary pediatric PA samples. Tumor samples also had a low mitotic index indicative of a limited growth potential and no increased p53 nuclear expression, suggesting that it is primarily the INK4a/ARF pathway that is being triggered in these tumors through induction of p16INK4a (48). Similar to what we observed in vivo, we show that transient BRAFWT or mutant BRAFV600E over-expression in human astrocytes (NHA and fetal astrocytes) induced morphologic changes evocative of senescent cells, which was accompanied by the induction of p16INK4a.
Figure 4. Transient overexpression of wild-type (WT)-BRAF and V600E BRAF induces OIS in immortalized astrocytes (NHA) and fetal astrocytes (FA). A, transfection efficiency at 48 hours of NHA and FA following transient overexpression of Myc-tagged (red staining) WT-BRAF (top, green staining for BRAF) and V600E BRAF (bottom). 4', 6-Diamidino-2-phenylindole (DAPI) staining (blue) is provided to show the total number of cells per field. B, morphologic changes and induction of SA-β-Gal activity were seen in FA (top) and in NHA (bottom) cells transiently overexpressing WT-BRAF or mutant V600E BRAF compared with empty vector controls (EV). The percentage of cells showing positive SA-β-Gal activity following transient BRAF (WT and V600E) induction was statistically significantly increased compared with EV controls (P < 0.001, Fisher's exact test). C, a mean of 4-fold induction in p16INK4a expression was seen in NHA (left) and FA (right) following overexpression of either WT-BRAF or mutant BRAF. D, Cell-cycle analysis of NHA and fetal astrocyte cell lines transiently overexpressing WT or mutant V600E BRAF shows an increase in G1 and a decrease in the S-phase in these cells compared with cells transfected with EV. This is suggestive of a G1–M cell-cycle arrest induced by BRAF overexpression in both astrocytic cell lines. Results are representative of 3 independent experiments carried out in triplicates for each cell line.
SA-β-Gal activity, and cell-cycle arrest. Conversely, we also show that loss of p16INK4a expression in NHA abrogated OIS, promoted cellular transformation, and increased proliferation following sustained WT-BRAF or V600E expression in vitro. This is of particular interest given a recent study reporting deletion of p16 in a subset of clinically aggressive PAs (49). Moreover, in mice, loss of p16INK4a allowed cultured astrocytes to grow without senescing (37). These data are concordant with our results on the role for p16INK4a pathway activation in the induction of OIS in PAs.

Our data may provide a rationale for the lack of progression to higher-grade tumors of PAs, in contrast to WHO grade II and III astrocytomas. Senescence is not triggered by a single, linear series of events but, instead, is regulated by a complex signaling network including the p53 and retinoblastoma (Rb) tumor suppressor pathways, which serve as critical cell-cycle checkpoints that mediate both replicative and oncogene-induced senescence. In contrast to PAs, nearly all grade II to IV astrocytomas, exhibit alterations in genetic loci related to the p53 and Rb pathways governing G1 arrest, including loss of the INK4a/ARF or Rb loci or gain of CDK4 (37, 38).

In this study, 6 PA samples from the Montreal cohort of 52 samples showed limited p16INK4a expression. These samples had marked pERK positivity, limited proliferation index, no p53 nuclear staining, and did not belong to a given age group. Three harbored KIAA1549-BRAF fusions and were typical cerebellar PAs. In nevi, additional factors to p16INK4a contribute to the protection of melanocytes against BRAFV600E-driven proliferation (27). Senescence in these 6 PAs as well as in all of the other PA samples investigated in this study could be additionally triggered through other mechanisms. Indeed, at the transcriptional level, a number of genes associated with OIS showed significant upregulation in PA compared with normal brain. These included CDKN2A (p16) and CDKN1A (p21), which are widely accepted markers of OIS (24, 41), as well as IGFBP7, CEBPB, and GADD45A, which have also been associated with MAPK-induced OIS (42–46, 50). Furthermore, telomerase activity has been shown to be low in LGA and telomere attrition has been shown to increase with recurrence in LGA including PA (51). Together, these observations indicate that there may be a more widespread senescence program that is activated in PA more than solely p16INK4a induction and that there may be a degree of functional redundancy in these tumors.

In addition to benign melanocytic nevi, the V600E BRAF mutation has been identified in the majority of melanomas. It is unclear to date as to what leads to cellular transformation in this tumor type, as senescence can still be partly triggered in tumor cells (29). Limiting signals affecting induction and levels of OIS may be dictated not only by the initiating genetic alteration but also by the threshold (intensity and duration) of the signaling and/or the tissue type (cell of origin, microenvironment including the stroma and immune response). The lack of progression to higher-grade tumors in senescent PAs may be due to the nature of the oncogenic aberration, as KIAA1549-BRAF fusions predominate and the V600E mutation accounts for only 4% to 7% of all genetic abnormalities in PAs, or to any of these other factors.
other factors. Additional studies using larger cohorts of patients or engineered mouse models generated to that effect (26) are needed to tease out the impact of these variables in PA.

In summary, our study suggests that aberrant MAPK signaling leads to OIS in PAs. This can explain the dichotomy between activation of an oncogenic pathway, which was recently found to be constitutively activated in 75% to 100% of PAs, and their tendency toward growth arrest. This unique phenomenon is at least partly due to the induction of \( p16^{ink4a} \) in cells and may account for the lack of progression to higher-grade astrocytomas, which is a unique feature of PAs compared with other LGA, and for the better survival of patients with this tumor. Further studies are warranted to determine whether the nature of the genetic alteration, the cell of origin, or the microenvironment play an additional role in the maintenance of OIS and in the striking lack of potential for transformation seen in PAs. Finally, artificial acceleration of this OIS program could also present an interesting therapeutic possibility in this tumor type.

References


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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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