

## Original Article

# mTOR activation is increased in pilocytic astrocytomas from older adults compared with children

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## Abstract

**Background:** Recent studies suggest that the behavior and biology of WHO grade I pilocytic astrocytomas (PAs) in adults is different than that associated with grade I PAs in children.

**Methods:** We evaluated Ki-67 labeling, BRAF abnormalities, isocitrate dehydrogenase R132 immunoreactivity phosphorylation (activation) of p44/42 mitogen activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR) in formalin-fixed tissue from 21 adult (18 years or older, mean age 37 years) and 10 children (mean age 9.4 years) WHO grade I PAs.

**Results:** The mean Ki-67 labeling was 4.8% in adults and 3.8% in children. There was no significant difference between Ki-67 labeling in children and adults or either subgroups of adults. No differences were found in phospho p44/42MAPK in adult subgroups (18–33 years and 34 and older) compared to children. Activation/phosphorylation of mTOR was biphasic in adults being significantly lower than children in young adults but significantly higher than children in older adults (age 34 and older).

**Conclusions:** Identifying mTOR phosphorylation/activation may represent a difference in biology and a new marker to guide chemotherapy with recently approved mTOR inhibitors.

**Key Words:** mTOR, pilocytic astrocytoma, p44/42MAPK

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## INTRODUCTION

A recent study suggests that the recurrence rate for pilocytic astrocytomas (PA) in adults may be significantly higher than that in children. Progression after gross total resection was found to be approximately 40% in adults compared to relatively infrequent in children.<sup>[2]</sup> The molecular differences promoting recurrence likely involve growth regulatory kinase cascades,<sup>[2,4,13]</sup> however, this has not been extensively evaluated in adult PAs.

Activation of the p44/42 mitogen activated protein kinase (MAPK) has been implicated in several

neoplasias<sup>[5,13,14]</sup> and is thought to be important in the pathogenesis of PAs in children.<sup>[2,10]</sup> Activation of

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the MAPK pathway appears to be, in some cases, by activation of upstream BRAF by the BRAF tandem duplication KIAA 1549 fusion or a mutation at BRAF V600E.<sup>[2,10,11]</sup> These result in the activation of the MAPK kinase (Raf-1)–MAP kinase/ERK kinase (MEK-1)–p44/42 MAPK cascade. BRAF activation also results in the activation of mammalian target of rapamycin (mTOR) by the phosphoinositide 3 kinase (PI3K)–protein kinase PKB/Akt–mTOR pathways in part by activation through the p44/42MAPK kinase.<sup>[7]</sup> Activation of the RAF1–MEK1 p44/42MAPK and PI3K–Akt–mTOR and other kinase cascades that promote cell proliferation and inhibit apoptosis<sup>[15,16,20,21]</sup> have been studied along with many types of gliomas, but have not been extensively evaluated in adult PAs.

## MATERIALS AND METHODS

### Pilocytic astrocytoma tissue

Thirty-one formalin-fixed paraffin embedded World Health Organization (WHO) grade I PAs from 21 adults (mean age 37 years, adults range 18–70, 10 females and 11 males) and 10 children, mean age 9.4 years (9 females and 1 male), were identified in the University of Rochester Medical Center archives and consultations with Institutional Review Board approval from 2008 to 2015 and reviewed following WHO criteria and recent findings.<sup>[3,4]</sup> In adult cases, the distribution of cases based on age shows a distinct clustering of cases in young and those in older adults. Consequently, adult cases were grouped as those 18 to 33 and those older than 33. Characteristics are listed in Table 1.

### Immunohistochemistry for phospho-p44/42MAPK and phospho- mTOR in pilocytic astrocytomas

Each case was analyzed with a polyclonal antibody to phospho p44/42 MAPK (Thr 202/Tyr 204, 1:400) and human phospho-mTOR (Ser 2448, 1:100 Cell Signaling, Beverly MA.) and MAC4 universal HRP-polymer (Biocare) with diaminobenzidine (DAB) chromagen and hematoxylin counterstain (Biocare), as described previously.<sup>[9]</sup> For antigen retrieval, tissue sections were incubated in a thermoresistant chamber with X Reveal Decloaker (Biocare Medical, Concord CA) at 120–123°C and pressure of 20–24 psi, according to manufacturer's specifications. Immunoreactivity in tumor cells (excluding blood vessels) was assessed by two of us as “0” for no distinct immunoreactivity, “1+” if 1–30% of cells were immunoreactive, “2+” if 30–50%, and “3+” if greater than 50% of cells were positive. Scores were analyzed by two-way *t*-tests comparing cumulative scores between young and adults, young and adult subgroups, and between 18 and 33 versus patients older than 33 years of age.

## RESULTS

### Comparison of pathological features of pilocytic astrocytomas in adults and children

The mean Ki-67 labeling index was 4.8% in adults and 3.8% in children. The Ki-67 labeling was not significantly different in either the 18–33 or 34 and older adults compared to children, and was not statistically different between the subgroups in adults. Neither adults nor children showed IDHR132 immunoreactivity. None of the 6 adults analyzed had a BRAFv600E mutation but one showed the BRAF-KIAA fusion. Two of the 4 tumors in children had BRAF mutations [Table 1].

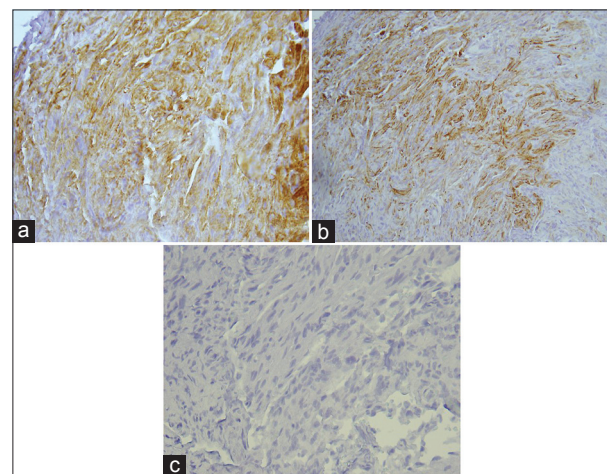
### Phospho-p44/42MAPK and phospho-mTOR immunoreactivity in pilocytic astrocytomas

As summarized in Table 1 the mean immunoreactivity for phospho p44/42 MAPK was  $1.1 \pm 0.93$  for children,  $0.8 \pm 1.03$  SD for 18–33-year olds and 1.11 for 34–70-year olds. This was not statistically different comparing younger and older adults and children.

Phospho-mTOR immunoreactivity had a mean of  $1.37 \pm 1.18$  SD in children (<18 years of age,  $0.33 \pm 0.487$  in young adults (18–33) and  $1.36 \pm 1.28$  in adults 34 or greater years of age. Phospho-mTOR was biphasic in adults being significantly lower than children in young adults ( $P = 0.05$ ) but significantly higher than children in older adults (age 34–70 years) ( $P = 0.05$ ) [Figure 1].

## DISCUSSION

The BRAF v600E mutation was found in only 1 of the 6 cases analyzed, which is consistent with previous reports that BRAF v600E mutations are rare in adults.<sup>[2,4]</sup> Similarly, BRAF fusion appears less common in adult PAs.<sup>[4,6]</sup>



**Figure 1: Phospho-MAPK 44/42 (a) phospho-mTOR (b), immunohistochemical control (c) in adult pilocytic astrocytoma (case 21) (Hematoxylin counterstain. Diaminobenzidine chromagen, original magnification ×400)**

**Table 1: Pilocytic astrocytomas characteristics and findings**

	Age	Location	Ki-67%	IDH Mutation	BRAF V600E	Phospho MAPK	Phospho mTOR
1	45M	Post. fossa	1	Neg	ND	1	2
2	33M	Post. fossa	6	Neg	ND	3	3
3	70F	4 <sup>th</sup> Vent	4	Neg	No v600E	0	0
4	35F	3rd Vent	3	Neg	No v600E	3	1
5	21F	Cerebellum	1	Neg	No v600E	1	0
6	44M	Septum	3	Neg	No v600E	1	3
7	46F	Left frontal	4	Neg	No	0	1
8	63F	Intra vent	2	Neg	Yes KIAA fusion	2	1
9	34F	Cerebellum	3	Neg	No v600E	3	1
10	29 F	Cerebellum		ND	ND	0	0
11	25M	Post. fossa	8	Neg	ND	1	1
12	64F	Cerebellum	3	ND	ND	2	0
13	26M	Cerebellum	7	ND	ND	0	0
14	28M	Optic nerve	2	Neg	ND	2	1
15	20M	Cerebellum	15	Neg	ND	0	0
16	57M	Cerebellum	1	ND	ND	0	0
17	20M	Thalamus	2	Neg	ND	0	0
18	18M	Hypothalamus	8	Neg	ND	1	0
19	20M	Cerebellum	11	Neg	ND	0	0
20	18F	Post. fossa	3	ND	ND	3	1
21	34F	Hypothalamus	8	ND	ND	3	3
22	10F	Cerebellum	Variable	Neg	ND	1	1
23	9F	Hypothalamus	3	Neg	ND	1	1
24	2F	Post. fossa	6	Neg	Abnl BRAF	ND	ND
25	14F	Parietal	5	Neg	ND	1	1
26	13F	Post. Fossa	4	Neg	Yes v600E	0	3
27	3F	Post. fossa	2	Neg	ND	1	3
28	15F	Cerebellum	2	Neg	No v600E	3	2
29	4M	Septum	1	Neg	No v600E	1	0
30	14F	Post. fossa	12	ND	ND	2	0
31	15F	Cerebellum	3	ND	ND	0	ND

M: Male, F: Female, Post: Posterior, Vent: Ventricle, ND: Not done, Neg: Negative, Abnl: Abnormal

Two studies suggest that the MEK-1-p44/42 MAPK pathway is involved in the pathogenesis of childhood PAs.<sup>[2,10]</sup> Our findings suggest that p44/42 MAPK phosphorylation/activation occurs in many Pas, however, this is not significantly different between adult and childhood WHO grade I PAs. In contrast, in children, this activation appears associated with anaplastic progression.<sup>[18]</sup> In addition, activation of p44/42 MAPK also influences oncogene-induced senescence in Pas.<sup>[8,17]</sup> The role of this pathway in tumor behavior warrants additional review after longer follow-up.

The PI3K- PKB/Akt-mTOR pathway influences several functions central to neoplasia including cell proliferation and metabolism.<sup>[1,21,24]</sup> mTOR is a serine/threonine kinase present in two compositionally distinct complexes with different functions. The mTOR complex 1 (mTORC1) contains PRAS40, and is activated

by numerous extracellular growth factors and cellular mitogens.<sup>[23]</sup> Activation of mTORC1 phosphorylates S6 kinase stimulating a number of growth-related functions including protein synthesis and cell proliferation. In contrast, mTORC2 is associated with cell metabolism and cytoskeletal organization. mTORC1 is strongly activated by rapamycin.<sup>[24]</sup> The effectiveness and side effect profiles of various mTOR inhibitors depends on their relative inhibition of the more growth regulatory mTORC1 compared to mTORC2.<sup>[1,21]</sup>

The PI3K- PKB/Akt-mTOR pathway is also activated in many WHO grade I adult PAs, and is significantly higher in older adults compared to children. This raises the possibility that activation of this pathway may participate in the pathogenesis of PAs in the older population. Activation of mTOR is also increased in anaplastic PAs.<sup>[18]</sup> Consequently, drugs inhibiting mTOR activation may

represent a viable chemotherapy for recurrent PAs in this population. Nonetheless, identifying the optimal therapy may require considerable study because several recently developed rapamycin analogues show different activities for mTORC1 and mTORC2.<sup>[1]</sup> Rapamycin (sirolimus) is a highly effective inhibitor of mTORC1 and associated components of the PI3K-PKB/Akt-mTOR pathway. Nonetheless, it has limited effectiveness in tuberous sclerosis, except possibly in some angiomyolipomas. This may reflect secondary activation of mTORC2 and Akt.<sup>[1]</sup> Everolimus has been approved for the treatment of tuberous sclerosis subependymal giant cell astrocytomas, neuroendocrine carcinomas of the pancreas, and advanced renal carcinomas.<sup>[1,12]</sup> More recently developed orally available analogues such as temsirolimus is currently approved for other malignancies such as mantle cell lymphoma and renal carcinoma.<sup>[1]</sup> Because of the secondary activation of other pathways, use of combination therapy with other kinase inhibitors may be more effective.<sup>[1]</sup>

Phosphorylation of p44/42MAPK and mTOR have been found to be less stable with cold ischemia after surgical removal than some other phosphoproteins.<sup>[23]</sup> Nonetheless, our tissues are rapidly placed in formalin (typically in less than 30 minutes). Moreover, in our recent studies on meningiomas, phospho p44/42 MAPK was consistently found in almost 10% of the cases by immunohistochemistry and western blot.<sup>[9]</sup>

In summary, phosphorylation/activation of mTOR appears increased, particularly in PAs in adults 34 years of age and older. Identifying mTOR phosphorylation/activation likely represents a new marker to guide chemotherapy because several rapamycin analogues that inhibit mTOR activation are now in clinical trials or FDA approved.<sup>[1,12,19]</sup>

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### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

1. Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: A new generation of mTOR inhibitors. *Nature* 2011;10:868-80.
2. Brokinkel B, Peetz-Dienhart S, Ligges S, Brentrup A, Stummer W, Paulus W, et al. A comparative analysis of MAPK pathway hallmark alterations in pilocytic astrocytomas: Age related and mutually exclusive. *Neuropathol Appl Neurobiol* 2015;41:258-61.
3. Collins VP, Tihan T, Tihan T, Vandenberg SR, Burger PC, Hawkins C, et al. Pilocytic astrocytoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, editors. *Tumours of the Nervous System*. Geneva, Switzerland: WHO Press; 2016 pp. 80-8.
4. Cykowki MD, Allen RA, Kabaly AC, Fung KM, Marshall R, Perry A, et al.

The differential diagnosis of pilocytic astrocytoma with atypical features and malignant glioma: An analysis of 16 cases with emphasis on distinguishing molecular features. *J Neurooncol* 2013;115:477-86.

5. Enralp Y, Derin D, Ozluk Y, Yavuz E, Guney E, Saip P, et al. MAPK overexpression is associated with anthracycline resistance and increased risk for recurrence in patients with triple-negative breast cancer. *Ann Oncol* 2008;19:669-74.
6. Hasselblatt M, Riesmeier B, Lechtape B, Brentrup A, Stummer W, Albert FK, et al. BRAF-KIAA 1549 fusion transcripts are less frequent in pilocytic astrocytomas diagnosed in adults. *Neuropathol Appl Neurobiol* 2011;37:803-6.
7. Gutmann DH, Chen YH. The molecular and cell biology of pediatric low grade gliomas. *Oncogene* 2014;33:2019-26.
8. Jacob K, Quang-Khuong DA, Jones DT, Witt H, Lambert S, Albrecht S, et al. Genetic alterations leading to MAPK pathway activation mediate oncogene-induced senescence in sporadic pilocytic astrocytomas. *Clin Cancer Res* 2011;17:4650-60.
9. Johnson MD, O'Connell M, Vito F, Bakos RS. Increased STAT-3 and synchronous activation of Raf-1-MEK-1-MAPK, and phosphatidylinositol 3-Kinase-Akt-mTOR pathways in atypical and anaplastic meningiomas. *J Neuro-Oncol* 2009;92:129-35.
10. Jones DTW, Gromuch J, Lichter P, Witt O, Pfister SM. MAPK pathway activation in pilocytic astrocytoma. *Cell Mol Life Sci* 2012;69:799-81.
11. Jones DTW, Hutter B, Jager N, Korshunov A, Kool M, Warnatz HJ, et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. *Nat Genet* 2013;45:927-32.
12. Kieran M, Yao X, Macy M. A prospective multi-institutional Phase II study of everolimus (Rad001), an mTOR inhibitor in pediatric patients with recurrent or progressive low-grade gliomas. *Ped Blood Cancer* 2013;60:300-10.
13. McCubrey JA, Steelman LS, Chappell WH, Sun L, Davis NM, Abrams SL, et al. Advances in targeting signal transduction pathways. *Oncotarget* 2012;3:123456-1521.
14. Nan Y, Yang S, Tian Y, Zhang W, Zhou B, Bu L, Huo S. Analysis of the expression protein profiles of lung squamous carcinoma cell using shot-gun proteomics strategy. *Med Oncol* 2009;26:215-21.
15. Nicholson KM, Anderson NG. The Akt/PKB signaling pathway in human malignancy. *Cell Signal* 2002;14:381-95.
16. Peti W, Page R. Molecular basis of MAP kinase regulation. *Protein Sci* 2013;12:1698-710.
17. Raabe EH, Lim KS, Kim JM, Meeker A, Mao XG, Nikkha G, et al. BRAF activation induces transformation and then senescence in human stem cells: A pilocytic astrocytoma model. *Clin Cancer Res* 2011;17:3590-9.
18. Rodriguez EF, Scheithauer BW, Giannini C, Rynearson A, Cen Ling, Hoesley B, et al. PI3K/AKT pathway alterations are associated with clinically aggressive and histologically anaplastic subsets of pilocytic astrocytoma. *Acta Neuropathol* 2011;121:407-20.
19. Rodriguez FJ, Raabe EH. mTOR: A new therapeutic target for pediatric low-grade glioma? *CNS Oncol* 2014;3:89-91.
20. Santarpia L, Lippman SL, El-Naggar A. Targeting the mitogen-activated protein kinase RAS-RAF signaling pathway in cancer therapy. *Expert Opin Ther Targets* 2012;16:103-19.
21. Sheppard K, Kinross KM, Soloman B, Pearson RB, Phillips WA. Targeting PI3 Kinase/AKT/mTOR signaling in cancer. *Crit Rev Oncol* 2012;17:69-95.
22. Theeler BJ, Ellezum B, Sadighi ZS, Mehta V, Tran MD, Adesina AM, et al. Adult pilocytic astrocytomas: Clinical features and molecular analysis. *Neuro-oncol* 2014;16:841-7.
23. Vassilakopoulou M, Parisi F, Siddiqui S, England AM, Zarell ER, Anagnostou V, et al. Preanalytic variables and phosphopeptide expression in FFPE tissue: A quantitative epitope assessment after variable cold ischemic time. *Lab Invest* 2015;95:334-41.
24. Yu JSL, Cui W. Proliferation, survival and metabolism: The role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. *Development* 2015;143:3050-60.