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ORIGINAL ARTICLE

Expression and alterations of the PTEN/AKT/mTOR pathway in ameloblastomas

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OBJECTIVES: Recently, an allelic loss of phosphatase and tensin homologue (PTEN) was shown to occur in ameloblastomas. In carcinogenesis, loss of PTEN allows for overactivity of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway inducing an upregulation of mammalian-target of rapamycin (mTOR) and its downstream effector ribosomal-subunit-6 kinase (S6K); allowing for uncontrolled cell proliferation, apoptosis inhibition and cell cycle deregulation.

METHODS: Thirty ameloblastomas and five dental follicles were studied, looking at the immunohistochemical expression of total PTEN and AKT, as well as their phosphorylated (p) active forms, and the downstream effector and indicator of mTOR activity p70 ribosomal-subunit-6 kinase (pS6K). Also assessed was the expression of extracellular-signal-regulated kinase (ERK), which cross talks with AKT.

RESULTS: Total PTEN was absent in 33.3% of ameloblastomas, while its stabilized, phosphorylated$^{ser380/thr382/thr383}$ form was absent in 83.3% of tumors. In contrast, AKT was expressed in 83.3% of ameloblastomas, showing high expression of the p-thr^{308}AKT and p-ser^{473} AKT forms in 93.3% and 56.6% of cases, respectively. Further, the mTOR activated pS6K$^{ser240/244}$ was detected in 86.7% of ameloblastomas, while ERK was overexpressed in 70.0% of the cases.

CONCLUSION: Immunohistochemical analysis of aberrant signaling in the PI3K/AKT/mTOR pathway in ameloblastomas may represent a valuable tool for elucidating pathogenesis, aggressiveness and selecting optimal therapeutics.

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Introduction

Ameloblastoma is the most frequent odontogenic neoplasm derived from epithelial odontogenic elements. In the United States, ameloblastomas account for 11.7% of odontogenic neoplasms, while the corresponding percentage worldwide ranges from 9.4% to 88.2% (Buchner *et al*, 2006). It is considered a benign neoplasm, but has a tendency to locally invade the surrounding tissues with a relatively high risk of recurrence (Ghandhi *et al*, 2006). Ameloblastomas most frequently present as solid/multicystic intraosseous lesions, with a varied histologic appearance, however this is not reflective of their behavior (Ladeinde *et al*, 2006). Unicystic and peripheral ameloblastomas represent less common, but distinct clinicopathologic variants with differences in their biologic behavior (Reichart *et al*, 1995; Neville *et al*, 2002). The treatment of ameloblastoma ranges from conservative to radical resection of the jaw depending on the size and location. The recurrence rate after conservative treatment ranges from 36% to 93% for solid/multicystic tumors, which is significantly higher than the recurrence rate after more aggressive radical treatment, which ranges from 8.3–21% (Carlson and Marx, 2006).

Phosphatidylinositol-3-kinase/protein kinase B (PI3 K/AKT) pathway is activated through a variety of extracellular signals and in turn regulates various cellular processes including proliferation, apoptosis, cell cycle and cytoskeletal rearrangement (Vivanco and Sawyers, 2002; Dillon *et al*, 2007). Once activated, PI3K catalyzes the synthesis of the phosphatidylinositol (3,4,5)-triphosphate (PIP3), which serves to recruit AKT and to allow its activation by pyruvate dehydrogenase kinase (PDK) via phosphorylation at Threonine (thr) 308 (Vivanco and Sawyers, 2002; Song *et al*, 2005;

Dillon *et al*, 2007). Full activation of the AKT requires phosphorylation at the Serine (ser) 473 residue, mediated by a second kinase PDK2, the nature of which remains controversial (Vivanco and Sawyers, 2002; Song *et al*, 2005; Dillon *et al*, 2007). Activated AKT exerts its effects on several downstream molecules and pathways, including apoptosis prevention, through the phosphorylation of the pro-apoptotic molecules BAD, caspase 9 and FKHR (Vivanco and Sawyers, 2002; Song *et al*, 2005; Dillon *et al*, 2007), promotion of cell proliferation (via the regulation of cell cycle machinery by direct phosphorylation of cyclin D1 kinase glycogen synthase kinase-3β – GSK3-β), (Vivanco and Sawyers, 2002; Song *et al*, 2005; Dillon *et al*, 2007) and in cell growth regulation [by activating the mammalian target of rapamycin (mTOR), which regulates biogenesis by stimulating p70 ribosomal-subunit-6 kinase (pS6K), an enhancer of the mRNA translation, and by inhibiting eIF4E-Binding Protein 1 (4EBP1), a translational repressor of mRNAs] (Vivanco and Sawyers, 2002; Bjornsti and Houghton, 2004; Shaw and Cantley, 2006). Interestingly, the overactivation of the PI3K/AKT pathway has been found in a wide range of cancers, leading to uncontrolled cell proliferation and resistance to apoptosis (Vivanco and Sawyers, 2002; Dillon *et al*, 2007).

In addition to PI3K/AKT pathway, the mitogen-activated protein kinase/extracellular-signal-regulated kinase (Ras/Raf/MEK/ERK) signaling pathway is linked to tumorigenesis (Kolch, 2000; Shaw and Cantley, 2006). Activated ERK phosphorylates several downstream substrates involved in cell growth and cell cycle control (Kolch, 2000; Shaw and Cantley, 2006). Moreover, there is a complex interplay between PI3K/AKT and ERK signaling pathways, which converge at multiple points to promote cell proliferation (Chambard *et al*, 2006). In addition, both ERK and AKT can disrupt the tumor suppressor complex Tuberous sclerosis 1/Tuberous sclerosis 2 (TSC1/TSC2), thus unleashing mTOR activity and promoting downstream pS6K function, while Ras can activate both the ERK and PI3K/AKT pathways (Chambard *et al*, 2006). Therefore, mutations in this signaling pathway lead to loss of growth-control checkpoints and promotion of cell survival and may be linked through multiple points of interaction to aberrant PI3K/AKT signaling and its effects.

Phosphatase and tensin homologue (PTEN) is a tumor suppressor gene located on human chromosome 10q23.3, which is phosphorylated to a stable state, and regulates the PI3K/AKT pathway by dephosphorylating PIP3 and thus inhibiting the activation of AKT (Cantley and Neel, 1999). Inactivation of PTEN gene results in unconditional cell proliferation and a reduction in apoptosis through PI3K/AKT pathway (Cantley and Neel, 1999; Vivanco and Sawyers, 2002). The loss of PTEN has been seen in several types of human cancers including head and neck squamous cell carcinoma (Shin *et al*, 2002) and in multiple cancer-associated syndromes. (Inoki *et al*, 2005; Scheper *et al*, 2006).

In carcinogenesis, the upregulation of AKT has been proposed to occur via various mechanisms including the deletion or inactivation mutation of PTEN, PI3K activating mutation, AKT gene amplifications, RAS mutations, or an upregulation of receptor tyrosine kinases (Cantley and Neel, 1999; Squarize *et al*, 2002; Vivanco and Sawyers, 2002; Shaw and Cantley, 2006). Interestingly, a molecular analysis to detect DNA damage in ameloblastic tumors revealed an allelic loss of PTEN in 62% of total cases (Nodit *et al*, 2004). The purpose of this study was to examine the immunohistochemical expression profile of total and phosphorylated (active) PTEN and AKT, as well as the expression of the mTOR downstream effector S6K and the related molecule ERK in ameloblastomas.

Materials and methods

Patients and tumor samples

Thirty patients diagnosed with ameloblastoma of the maxilla, mandible, or gingiva and treated by primary tumor resection at the University of Maryland between 1997 and 2002 were identified. In addition, five dental follicles around impacted teeth were included as a control group. Formalin fixed, paraffin-embedded tissue samples from all patients were available and obtained from the pathology archive at the University of Maryland. Clinical records for the ameloblastoma patients were reviewed and information regarding histologic type, location, race, sex, and age, were obtained. Based on clinicopathologc criteria (4,5), the 30 ameloblastoma cases were classified as solid/multicystic (26), unicystic (1) and peripheral (3). The average age of the solid/multicystic ameloblastomas was 50.03 years (range 17–85), with a 3.33:1 male to female ratio and a 1.27:1 white to African American ratio. Most of these cases (21) were in the mandible (80.8%), with only five (19.2%) in the maxilla. Further, most of the solid/multicystic ameloblastomas presented histologically with a follicular pattern (10) followed by tumors exhibiting a mixed pattern (8) or a plexiform configuration (8). The single case of unicystic ameloblastoma affected a white male at the age of 63, and was located in the mandible. Finally, the three cases of peripheral ameloblastoma presented at an average age of 50.67 (range 44–60), with a 2:1 male to female ratio and a 1:1 white to African American ratio. Further, two of these cases presented histologically with a follicular pattern and one presented with a mixed pattern (Table 1). IRB exemption was received from the University of Maryland internal review board for the examination of tissues.

Immunohistochemistry

In order to assess the abnormalities in the PTEN/AKT pathway that possibly constitute the foundation of ameloblastoma proliferation, immunohistochemistry for total PTEN, phosphorylated PTEN (p-PTEN), total AKT, phosphorylated-theronine308 AKT (p-thr AKT), phosphorylated-serine 473 AKT (p-ser AKT), ERK and phosphorylated pS6K were performed on each specimen. The paraffin embedded tissue sections were deparaffinized, immersed in ethanol 100% and 95% and heated for antigen retrieval in 0.01 M citrate buffer for

Table 1 Demographic data

	Age		*Gender*		*Race*			*Location*			*Histologic typing*		
	Range	*Average*	*Male*	*Female*	*Black*	*White*	*NA*	*Mand*	*Max*	*Ging*	*Follicular*	*Plexiform*	*Mixed*
M/S (26)	17–85	50.03	20 (76.9%)	6 (23.1%)	11 (42.3%)	14 (53.8%)	1 (3.9%)	21 (80.8%)	5 (19.2%)	0	10 (38.4%)	8 (30.8%)	8 (30.8%)
U (1)	–	63	1 (100%)	0	0	1 (100%)	0	1 (100%)	0	0	–	–	–
P (3)	44–60	50.67	2 (66.7%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0	0	3 (100%)	2 (66.7%)	0	1 (33.3%)

M/S, multicystic/solid; U, unicystic; P, peripheral.

25 min in a pressure cooker inside a microwave oven. After dehydration in hydrogen peroxide, the sections were incubated with primary antibodies at room temperature for 1 h. The applied antibodies were mouse monoclonal anti-PTEN, and anti-p-PTEN (ser380/thr382/thr383); polyclonal rabbit anti-AKT, anti-ERK and anti-pS6K (phosphorylated ser 240/244) and monoclonal rabbit anti-p-AKT thr 308 and anti-p-AKT ser 473 antibodies (Cell Signaling Technologies, Beverly, MA, USA; all diluted at 1:50). The standard streptavidin–biotin–peroxidase complex method was employed to bind to the primary antibody along with Multilink concentrated biotinylated anti-IgG as a secondary antibody. The reaction products were visualized by counterstaining with the 3,3′-diaminobenzidine reagent set (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Sections were counterstained with hematoxylin. As a negative control, sections were treated with phosphate-buffered saline with omission of the primary antibody.

The immunostains were reviewed by three independent evaluators (MS, RC and NN). Immunohistochemical reactivity for all stains were graded according to the percentage of positive tumor cells: (0) 0%, (1) $<20\%$, (2) 20–50% and (3) $>50\%$, and the intensity of staining: (–) no staining, (w) weak, (m) moderate, or (s) strong, as compared with the negative control tissues. Known positive tissues for each marker were used as positive controls, while the vascular endothelium, which exhibits a consistently strong, predominantly nuclear, staining for PTEN, served as an internal positive control. The values for the quality ($w = 1$, $m = 2$, $s = 3$) and quantity (0–3) of staining were added to give a single number for each tumor, which was used in the final evaluation.

Statistical analysis

For all measurements as needed, a Student's *t*-test was employed to assess the statistical significance of treated groups *vs* control groups along with standard error. A statistically significant difference was considered to be present at $P < 0.05$.

Results

To evaluate potential molecular aberrations that may participate in the neoplastic growth of ameloblastomas, we evaluated 30 ameloblastomas and five dental follicles and characterized the quality and quantity of staining for PTEN, p-PTEN, AKT, p-thr AKT, p-ser AKT, ERK and pS6K. Further, we correlated the expression of these proteins with the clinical and histological subtypes of ameloblastoma and the demographics of age, gender, race, and location.

Immunohistochemical expression in ameloblastomas

In ameloblastomas, total PTEN was present in 20 of 30 (66.7%) cases; however, the active, stabilized, phosphorylated form of PTEN was present in only five of 30 (17.7%) cases (Figures 1 and 2). On the other hand, total AKT was upregulated in 25 of the 30 (83.3%)

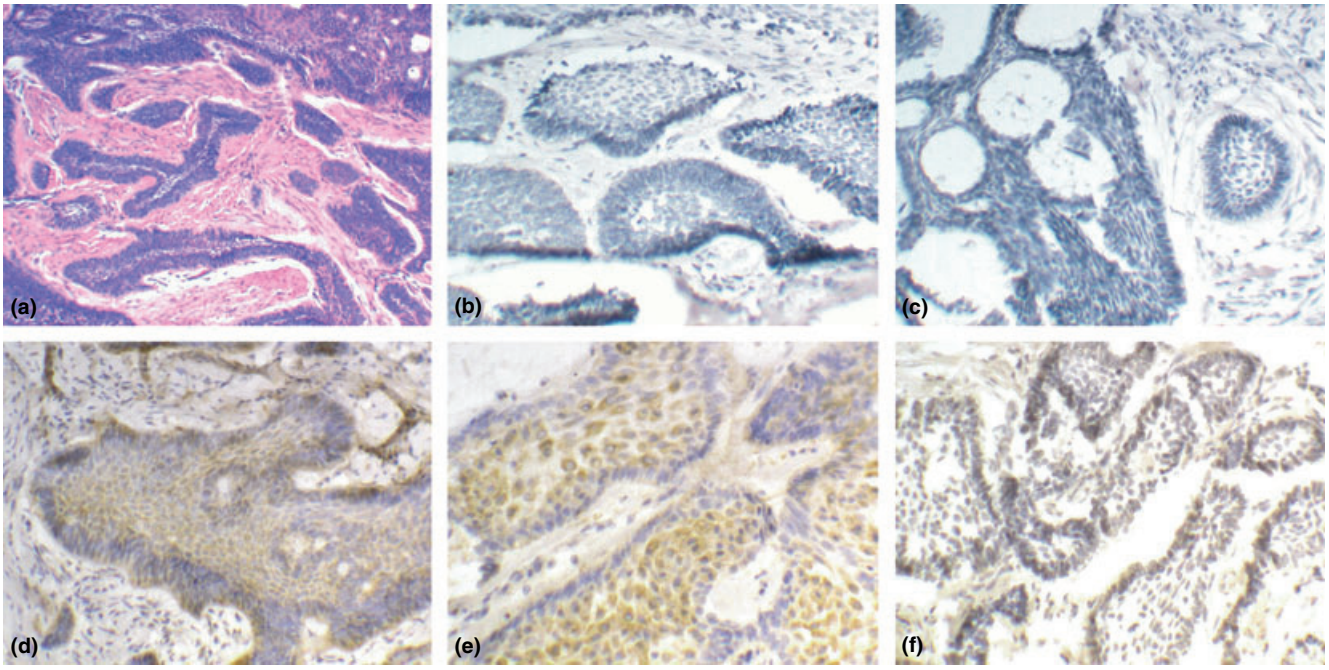

Figure 1 (**a**) Histological (hematoxylin and eosin) and immunohistochemical features of a case of ameloblastoma showing downregulation of phosphatase and tensin homologue (PTEN) signaling with lack of expression of PTEN (**b**) and p-PTEN (**c**) and activation of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway with upregulation of AKT (**d**), p-tyr AKT (**e**) and pS6K (**f**) (200×)

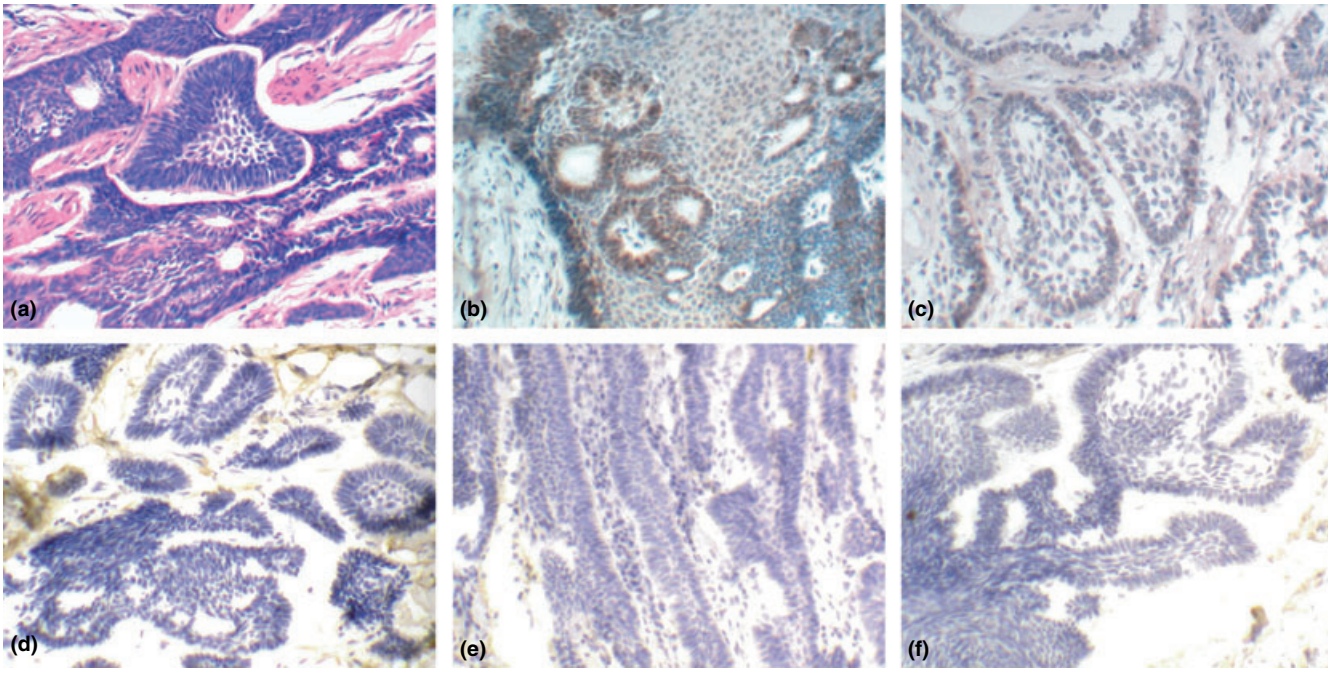

Figure 2 (**a**) Histological (hematoxylin and eosin) and immunohistochemical features of a case of ameloblastoma showing expression of phosphatase and tensin homologue (PTEN) (**b**) and p-PTEN (**c**) and inhibition of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway with lack of expression of AKT (**d**), p-tyr AKT (**e**) and pS6K (**f**) (200×)

cases of ameloblastoma (Figures 1 and 2). We also examined the expression of p-thr AKT and p-ser AKT, which were overexpressed in 28 (93.3%) cases and 17 (56.6%) cases, respectively (Figures 1 and 2). Moreover, there was a correlation between lack of PTEN expression or phosphorylation and upregulation of AKT signaling, in that only two cases showed PTEN/p-PTEN negativity along with AKT inactivity. Further, to confirm that AKT upregulation had an effect on its critical downstream effector mTOR, we looked at pS6K activity, the terminal effector of the mTOR pathway. Active pS6K expression was upregu-

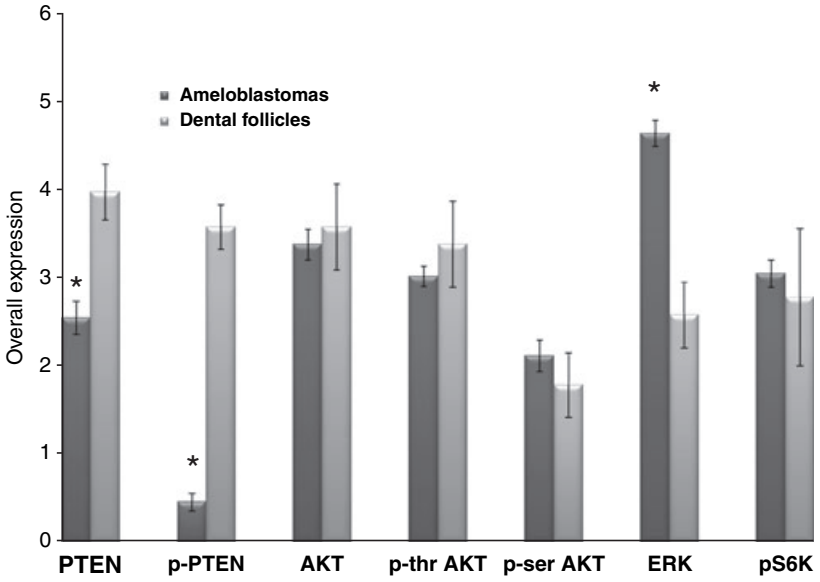

Figure 3 Average combined immunohistochemical scores (corresponding to the sum of the average scores for percentage of tumor cells and intensity of staining) for phosphatase and tensin homologue (PTEN), pPTEN, protein kinase B (AKT), p-thr AKT, p-ser AKT, extracellular-signal-regulated kinase and p70 ribosomal-subunit-6 kinase in ameloblastomas and dental follicles. The asterisks (*) indicate statistically significant differences between the two groups ($P < 0.0001$)

lated in 26 of 30 (86.67%) cases correlating well with the upregulation of AKT and its p-thr AKT form, with only three cases not showing pS6K activity in the face of AKT upregulation (Figures 1 and 2). Finally, ERK, a signaling molecule that cross talks with AKT and can also activate mTOR and pS6K, was also overexpressed in 21 of 30 (70.0%) cases. The overall mean expression scores for PTEN, p-PTEN, AKT, p-thr AKT, p-ser-AKT, ERK and pS6K staining in ameloblastomas were 2.56, 0.46, 3.4, 3.03, 2.13, 4.67 and 3.07, respectively (Figure 3).

Immunohistochemical expression in dental follicles

The dental follicles showed positive expression of PTEN/p-PTEN in all cases; in comparison with ameloblastomas, dental follicles showed significantly higher mean expression scores for PTEN (2.56 *vs* 4.0) and pPTEN (0.46 *vs* 3.6) ($P < 0.0001$) (Figure 3). AKT expression was detected in four of five dental follicles, while its phosphorylated forms p-thr AKT and p-ser AKT were expressed in four of five and three of five cases, respectively. When comparing ameloblastomas *vs* dental follicles, the levels of AKT (3.4 *vs* 3.6), p-thr AKT (3.03 *vs* 3.4), p-ser-AKT (2.13 *vs* 1.8), and pS6K (3.07 *vs* 2.8) did not differ significantly between the two entities (Figure 3). In contrast, ERK expression was higher in ameloblastomas compared with dental follicles (4.67 *vs* 2.6) ($P < 0.0001$) (Figure 3).

Correlation between immunohistochemical expression and ameloblastoma clinicopathological subtypes

Ameloblastomas can be separated into three distinct clinicopathologic subtypes, namely solid/multicystic, unicystic, and peripheral, which exhibit distinct behavior and risk of recurrence. As the solid/multicystic subtype is by far the most common, it corresponded to the majority (26 of 30) of our studied cases. Despite the limited number of unicystic (one of 30) and peripheral (three of 30) cases, there were not noticeable differences in their demographic data compared with the conventional (solid/multicystic) subtype.

When we compared the immunohistochemical profile among these three subtypes we did not see differences in PTEN, AKT or p-tyr AKT expression. pPTEN was expressed in a small percentage of solid/multicystic tumors (19%), while it was absent in the unicystic and peripheral cases. p-ser AKT, ERK and pS6K showed lower levels of expression in the peripheral tumors (0, 1.33 and 1.67, respectively) compared with both the solid/multicystic tumors (2.31, 2.52 and 3.2, respectively) and the unicystic variant (4.0, 3.0 and 4.0, respectively).

Correlation between immunohistochemical expression and ameloblastoma location

When we compared the mandibular to the maxillary lesions, the mandibular tumors had the least upregulation of AKT (2.86) and p-thr AKT (2.62), while the maxillary cases had the largest upregulation (4.0 and 4.2, respectively). These differences perhaps indicate the possibility of more aggressive behavior of ameloblastomas in the maxilla which may be related to greater cell proliferation and growth compared with the mandibular lesions.

Correlation between immunohistochemical expression and ameloblastoma histological subtypes

Looking at the histological subtypes of follicular *vs* plexiform *vs* mixed for solid/multicystic ameloblastomas, we saw that all forms showed equivalent

underexpression of p-PTEN and overexpression of p-thr AKT and pS6K regardless of histology. Nonetheless, the plexiform variant showed a decreased expression of total PTEN and AKT compared with the follicular and mixed variants. These results point toward the current principle that the histological subtypes of conventional ameloblastoma do not affect behavior or treatment as they act similarly.

Correlation between immunohistochemical expression and ameloblastoma patients' demographics

Finally, when we looked at the demographic data of age, sex, and race and correlated them with the immunohistochemical profile, only a few statistically significant differences were seen. Looking at different age groups of 0–24 years (5), 25–50 years (11) and > 50 years (14), the only significant difference seen was the higher expression of ERK in the youngest age group (4.0) *vs* the middle age (2.09, $P = 0.0471$) and oldest age groups (2.21, $P = 0.0474$). The oldest age group overexpressed p-thr AKT (3.5) and pS6K (3.38) to the largest extent, while the youngest group showed the lowest expression of PTEN (1.8). Interestingly, the expression levels in males (23) *vs* females (7) are slightly elevated across the board for males, with the only significant disparity being the expression of total AKT (3.78 *vs* 2.14, $P = 0.0418$). With respect to race, white (16) *vs* black (12) patients showed no significant differences in any expression profile. All other correlations were non-significant.

Discussion

A lack of accurate predictive markers of ameloblastoma behavior has lead many clinicians to treat all ameloblastomas with radical surgery to prevent local regional recurrence (Carlson and Marx, 2006). Hence, the elucidation of the molecular mechanisms underlying the pathogenesis of ameloblastomas would aid both in the identification of prognostic and predictive parameters and in the development of novel treatment applications for these lesions. Recent studies have looked at the value of integrins (Souza Andrade *et al*, 2007), growth factors (Kumamoto and Ooya, 2006b, 2007), telomerase and cell cycle regulators (Kumamoto and Ooya, 2006c; Zhong *et al*, 2006a,b), intracellular adhesion molecules (Bello *et al*, 2007; Usami *et al*, 2007), angiogenic factors (Kumamoto and Ooya, 2006b), matrix-degrading proteinase regulators (Kumamoto and Ooya, 2006a; Zhang *et al*, 2006), and apoptosis regulators (Kumamoto and Ooya, 2005; Sandra *et al*, 2005, 2006a; Luo *et al*, 2006) with regards to the occurrence, invasiveness, architectural characteristics, cell proliferation and survival of ameloblastomas.

A few studies have looked at the effects of different mediators of the MAPK and PI3K/AKT pathways in ameloblastomas. One study looked at the ability of TNFα to induce apoptosis in ameloblastoma cells, which was shown to be improved by inhibiting TNFα-induced AKT and p44/42 mitogen-activated protein kinase (MAPK) cell survival pathways (Sandra *et al*, 2006b). In a different study, TNFα was shown to be expressed in ameloblastomas and to induce AKT and p44/42 MAPK activation through PI3K, which may induce cell survival and proliferation (Hendarmin *et al*, 2005). Finally, another study supported the proliferative ability of the PI3K/AKT pathway showing midkine induced ameloblastoma growth via the MAPK and AKT pathways (Sandra *et al*, 2004). However, none of these studies have looked at other upstream regulators or at the downstream effectors of the AKT signaling. Only recently has the allelic loss of the tumor suppressor PTEN been studied in ameloblastic tumors, showing a 62% frequency of allelic loss in a group of ameloblastomas and ameloblastic carcinomas (Nodit *et al*, 2004). However, how this allelic loss correlates with aberrations in the PTEN/PI3K/AKT pathway has not been studied.

Here, we have shown that PTEN was completely absent in 33.3% of studied ameloblastomas, while its active, stabilized, phosphorylated form was absent in 83.3% of cases. Lack of PTEN activity may account for an increase in AKT activation. In our study, AKT was upregulated in the majority (83.3%) of studied ameloblastomas, with overexpression of the p-thr AKT and p-ser AKT seen in 93.3% and 56.6% of cases, respectively. Moreover, there was a correlation between PTEN inhibition and upregulation of AKT signaling. In contrast, dental follicles showed positive expression of PTEN and p-PTEN in all cases, although AKT expression and/or phosphorylation were noticed in 80% of studied cases. The lack of an inverse correlation between PTEN/pPTEN expression and AKT activation status in dental follicles may indicate the inability of PTEN to suppress AKT signaling during tooth development.

One of the most studied target factors of the PI3K/AKT pathway is mTOR which is critical for cancer cell proliferation, survival and tumorigenesis (Hay, 2005; Shaw and Cantley, 2006). The association of mTOR with Raptor (regulatory-associated protein of mTOR) results in regulation of mRNA translation via both S6K activation and 4E-BP1 inhibition. In our study, pS6K was upregulated in 86.7% of cases, generally showing a positive correlation with AKT upregulation. However, it should be kept in mind that mTOR-mediated pS6K activation may ensue from AKT-independent pathways, such as ERK activation. In addition, through a negative feedback loop, the prolonged activation of mTOR/S6K is shown to phosphorylate and inhibit IRS-1 and 2 (insulin receptor substrate) thus exerting an inhibitory effect on PI3-K/AKT (Shah *et al*, 2004). These mechanisms may be operative in cases showing pS6K overexpression in the face of AKT downregulation.

Considering the multiple points of convergence of the Ras/Raf/MEK/ERK and PI3K/AKT/mTOR pathways, we also looked at the levels of ERK in ameloblastomas and dental follicles. The overexpression of ERK in most ameloblastomas (70.0%), as opposed to its low expression in dental follicles, indicates that ERK activation may participate in the pathogenesis and growth of these tumors. Concomitant activation and coordinated function of ERK and AKT, as was observed in a number of ameloblasto-

mas, may be a critical factor for their neoplastic behavior through intricate regulation of molecular events such as cell proliferation and mTOR activation (Chambard *et al*, 2006).

The evaluation of patterns of aberrant expression of PTEN, AKT, pS6K and ERK markers in ameloblastomas may shed light to the pathogenesis of these tumors and may help provide important clues to the inherent aggressiveness of these tumors. Further investigation may determine other modalities able to upregulate the PI3K/AKT/mTOR pathway in ameloblastomas, focusing on growth factors, activation of receptor tyrosine kinases, RAS mutations, PI3K activating mutations, or AKT gene amplifications. Future studies with a larger population of patients with long follow-up will establish the potential prognostic significance of these markers in predicting the likelihood of aggressive and recurrent behavior. Also, *in vitro* and *in vivo* studies are needed to show that rapamycin treatment (mTOR inhibitor) or other interventions that specifically target components of the signaling machinery can inhibit ameloblast cell growth, similar to the effects seen in other neoplasms. In this case, a panel of markers may be used to determine sensitivity making the treatment of ameloblastoma individualized to the patient.

In conclusion, we have demonstrated that ameloblastomas exhibit alterations in the immunohistochemical protein expression of signaling molecules that play significant roles in the growth and survival of neoplastic cells. The immunohistochemical analysis of PTEN, AKT and pS6K may prove to be a valuable diagnostic tool in determining the aggressiveness of individual ameloblastomas and aid in treatment decisions.

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