Immunohistochemical detection of p53 protein in ameloblastoma types

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رصد زيادة ظهور البروتين P53 في أنسجة الورم السني المينائي بأنواعه المختلفة

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خلاصة: يظهر البروتين P53 في حالة الورم السني المينائي وحيد التكيس على نحو أشد كثافـة عنـه في حالـة نظيره التقليدي، الأمر الذي يعني زيادة في النوع الأصلي من الـبروتين P53 وبالتـالي كبـت قـدرة النمـو في الـورم وحيـد التكيس. كذلك قد يكون هذا هو المؤشر المبكر للتحول الورمي في نشأة الورم السني المينائي من الجـدران الطلائيـة لبعض الأكياس السنية. إن زيادة كثافة البروتين P53 في الأكياس التقليدية وحيدة التكيس وفي الأنـواع السـرطانية، قد يعكس تراكم نوع طفري من هذا البروتين له فاعلية سرطانية تعزز النمو الورمي. وفـرط وجـود الـبروتين P53 يمكن أن يفيد كطريقة للتحري يمكن بها التكهن بالتغيرات السـرطانية في أنـواع الـورم السـني المينائي، مـن خـلال تواتر وجود الخلايا المنشطة مناعيا أو زيادة كثافة التلوين بالصبغات.

ABSTRACT Overexpression of p53 protein in unicystic ameloblastoma (uAB) is denser than in the conventional ameloblastoma (cAB) type, indicating increased wild type p53 — suppressing the growth potential of uAB and denoting the early event of neoplastic transformation, probably of a previous odontogenic cyst. Overexpression of p53 in borderline cAB and malignant ameloblastoma (mAB) types might reflect a mutational p53 protein playing an oncogenic role, promoting tumour growth. Overexpression of p53 protein could be a valid screening method for predicting underlying malignant genetic changes in AB types, through increased frequency of immunoreactive cells or increased staining density.

Détection immunohistochimique de la protéine p53 dans divers types d'améloblastome
RESUME La surexpression de la protéine p53 dans l'améloblastome de forme unikystique est plus dense que dans l'améloblastome de forme intraosseuse classique, ce qui indique une augmentation du taux de protéine p53 sauvage - supprimant le potentiel de croissance de l'améloblastome de forme unikystique et dénotant un phénomène précoce de transformation néoplasique, probablement d'un kyste odontogène antérieur. La surexpression de la protéine p53 dans l'améloblastome malin et l'améloblastome classique périphériques pourrait représenter une protéine p53 mutée jouant un rôle oncogène, favorisant la croissance tumorale. La surexpression de la protéine p53 pourrait être une méthode valable de dépistage pour prédire des transformations génétiques malignes sous-jacentes dans les diverses formes d'améloblastome, par une fréquence plus élevée des cellules immunoréactives ou une plus grande intensité de coloration.

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Introduction

Ameloblastoma (AB) is the most common significant odontogenic tumour. There are different types of the benign form, with different clinicopathologic variations, and a malignant counterpart. The conventional intraosseous form (cAB) is generally considered to be benign but locally invasive, demonstrating a considerable tendency to recur [1–3].

One fundamental concept that has a direct bearing on treatment is the need to differentiate benign AB by type: conventional (cAB) (whether solid or multicystic), unicystic form (uAB), or peripheral. The prognosis for the unicystic form is much better after limited surgical procedures, such as curettage, enucleation and simple excision, than for a conventional (solid or multicystic) AB [4,5].

Previous controversy over the terminology for AB cases with malignant features has been resolved by the definition cited by the World Health Organization (WHO) [6]. Malignant ameloblastoma (mAB) is defined as, "a neoplasm in which the pattern of an AB and cytological features of malignancy are shown by the primary growth in the jaws and/or by any metastatic growth". These malignant forms are often more aggressive lesions with poorer prognoses. Although radiological findings of mAB are essentially the same as those in typical non-metastasizing AB [4], little is known about the molecular genetic mechanism that contributes to the development of malignant ameloblastomatous changes.

It is now believed that neoplastic transformation, consisting of a multistep accumulation of adverse genetic events, occurs in a wide variety of human tumours over a large region of the genome [7]. Mutations in the p53 tumour suppressor gene are among the most common abnormalities in human cancer [8,9]. The p53 gene is a 53kD nuclear phosphoprotein encoded by a 16–20 kb gene on the short arm of chromosome 17 at position p13.1 [7,10]. It has been implicated in the control of the cell cycle, DNA repair and synthesis, cell differentiation, genomic plasticity and apoptosis [11].

The proto-oncogene product, p53, is a cellular protein expressed at low levels in non-transformed cells and acts as a negative regulator of cell division. In tumour-derived and transformed cell lines the levels of p53 are often elevated and several investigators have suggested that inactivation of the p53 gene confers a selective advantage for the development of the tumourigenic phenotype with its subsequent impact on changing cellular activity [12,13].

Currently, limited data exist on the occurrence of p53 in odontogenic lesions and a scientific explanation has yet to be presented for the different biological behaviour of the AB types. Assessment of p53 expression might give a better insight into the neoplastic transformation process ongoing in such lesions and help optimize their treatment regimens.

Materials and methods

Selection of cases
Study group
Eighteen cases of differing types of intraosseous AB lesions were selected from the files of the Department of Pathology, Faculty of Medicine of both Mansoura University and Alexandria University, and the Oral Pathology Department, Faculty of Dentistry, Mansoura University. The selected cases were nine cases of cAB, five of uAB and four of mAB. All diagnoses conformed with WHO classification of odontogenic tumours [6].

Control group

Six specimens of mandibles and maxillae from normal human fetuses (12–16 weeks gestation) were obtained from legal abortions conducted at the El-Shatby Hospital, Alexandria. They were fixed in 10% neutral buffered formalin and then decalcified and prepared as paraffin blocks.

Section preparation for light microscopical study

For immunohistochemical detection of p53 protein, 4µ-thick serial sections were cut from the paraffin blocks; all tissues having been previously fixed in 10% neutral buffered formalin.

The 4µ-thick serial sections were used for the following studies:

- Conventional haematoxylin and eosin staining for the re-evaluation and confirmation of the histological diagnosis;
- Immunohistochemical study, where the sections were placed on silanized glass slides for microwave antigen retrieval [14]. Following rehydration, the sections were immersed in a plastic jar of sodium citrate buffer (pH 6.0) and incubated for 5 minutes at 600 W. Sections were then left to cool to room temperature and rinsed in 0.05 M Tris-HCl buffer (pH 7.4).

Sections were immunostained for p53 using the avidin-biotin complex (ABC) technique [15], with a primary antibody clone DO-7 (Dako Patts, Copenhagen, Denmark). The marker used was a monoclonal antibody raised against the human wild type p53 protein expressed in *Escherichia coli* that recognizes both the wild and mutant forms of p53.

Sections were incubated overnight with the primary antibody at a dilution of 1:80 in Tris-HCl buffer at 4 °C. To observe a p53-positive reaction, DAB substrate solution

was used and counter-staining was performed with Meyer's haemotoxylin.

For the negative control sections for each batch, the primary antibody was omitted and replaced with non-immune rabbit serum to guard against any false positive results which might develop from a non-specific reaction. Sections of breast carcinoma immunohistochemically proven to be p53-positive were used as a positive control with each run.

Immunohistochemical assessment

The criterion for a positive reaction confirming the presence of p53 protein was a dark, brownish, intranuclear precipitate. An arbitrary, semiquantitative evaluation of the immunoreactivity to the p53 marker was assessed using the following:

- −   none of the cells revealed positivity for p53 marker
- +   mild: < 5% positive tumour cells
- ++   moderate: 5%–50% positive tumour cells
- +++ strong: > 50% positive tumour cells

The staining intensity in p53-positive cases was further categorized as dense or faint as follows:

- Dense: densely stained nuclei, easily seen at low magnification (objective 5×)
- Faint: faint nuclei staining that could only be detected by using higher magnification (objective 25× or 40×).

Results

The 18 AB cases in this study comprised 11 males and 7 females, with ages ranging from 17 years to 42 years. All AB cases were intraosseous, 14 located in the mandible and 4 affecting the maxilla.

Histological results

Light microscopic examination revealed that the nine cAB cases had well-known microscopic features. Six cases were of the follicular type and its variants, (classical pattern, granular cell and acanthomatous patterns) and three cases were of the plexiform pattern.

In the five uAB cases, the tumour was formed of a cyst lining of a relatively innocuous epithelium that in parts showed transformation to one with cuboidal or columnar basal cells with hyperchromatic nuclei, nuclear palisading and cytoplasmic vaculization with intercellular spacing. The wall of the cyst lining had been infiltrated by follicular and/or plexiform ameloblastoma patterns.

The four mAB cases had no reported metastasis. Diagnosis of malignancy was based on the detection of malignant cytological features, mainly hyperchromatism, pleomorphism and mitotic figures, and lesser differentiated neoplastic epithelial cells. Areas of squamous metaplasia were also evident.

Immunohistochemical results

None of the examined sections of the control group showed positive staining for the p53 protein marker used in this study.

Expression of positive p53 protein immunoreactivity was present in 11 of the 18 cases of AB types (61.1%). In uAB cases, positive cells were evident in 80% and the p53 positive cells showed mostly moderate immunoreactivity (Table 1). It was noticed that cells positive for p53 were found in groups of epithelial cells lining the cystic space, and were confined to the basal layer (Figures 1 and 2). The tumour tissue infiltrating the cyst wall also showed p53-positive cells arranged in strands (Figure 3), small collections (Figure 4) or evenly distributed among neoplastic cells (Figure 1).

Cases of cAB showed a high frequency (55.6%) of non-reactivity to the p53 marker (Table 1). The p53-positive cells among the reactive cases were mostly faintly stained (Table 2), and the distribution of immunoreactive cells was noticeably in the peripheral, ameloblast-like cells and occasionally in the central, stellate reticulum-like cells (Figures 5 and 6).

Table 1 Arbitrary semiquantitative evaluation of the frequency of p53 expression in 18 cases of ameloblastoma

| Ameloblastoma | Non-reactive | | | | Reactive | | | |
| | − | | + | | ++ | | +++ | |
	No.	%	No.	%	No.	%	No.	%
uAB (n = 5)	1	20.0	−	−	3	60.0	1	20.0
cAB (n = 9)	5	55.6	3	33.3	1	11.0	−	−
mAB (n = 4)	1	25.0	−	−	−	−	3	75.0
Total (n = 18)	7	38.9			11 (61.1%)			

− = none of the cells revealed positivity   + = mild: < 5% positive tumour cells
++ = moderate: 5%–50% positive tumour cells   +++ = strong: > 50% positive tumour cells
uAB = unicystic ameloblastoma
cAB = conventional ameloblastoma
mAB = malignant ameloblastoma

Table 2 **Variation in the staining intensity of p53 immunoreactive cells in positive ameloblastoma cases**

Ameloblastoma type	Faint		Dense	
	No.	%	No.	%
uAB (n = 4)	2	50	2	50
cAB (n = 4)	3	75	1	25
mAB (n = 3)	–	0	3	100
Total (n = 11)	5		6	

uAB = unicystic ameloblastoma
cAB = conventional ameloblastoma
mAB = malignant ameloblastoma

The highest frequency of p53-positive cells and dense staining was observed among mAB cases (Tables 1 and 2). These p53-positive cells were focally distributed in collections of neoplastic cells showing disorganized patterns (Figure 7), particularly surrounding areas of necrosis (Figure 8).

Discussion

The odontogenic epithelial cells of the control tissues investigated failed to reveal any immunoreactivity to the p53 marker used. These results match results from other normal tissue which have also proved negative for p53 [3,16]. This can be explained by the fact that the wild type protein of p53 does not normally accumulate to amounts detectable by immunohistochemical methods, probably because of its short half-life (6–20 minutes) [17].

In our study the percentage of p53-reactive cases is comparable to results from the only other available study of p53 expression in AB [18]. However, this earlier study did not verify p53 expression among the types encountered in our examination. Therefore, comparison with other previous reports of variation in p53 reactivity among the AB subtypes examined was not possible.

The positive staining for the p53 marker in the uAB cases examined might indicate the existence of certain stimuli due to disturbed growth regulation, which triggered neoplastic transformation. It has been suggested that detectable p53 proteins may reflect stabilization of the protein via interactions with other intracellular proteins [19], or transcriptional induction, rather than intragenetic mutations of the p53 locus [20]. These results might indicate that p53 abnormalities play a crucial role in early neoplastic transformation during the development of uAB. This concept has been previously suggested in the early development of other neoplasms [21].

The active and accumulating p53 wild type might contribute to the more benign course and low recurrence rate commonly reported for uAB in comparison with cAB [5], through the relative suppression of transforming tumour cells and limitation of their growth potential. This interpretation is in accordance with earlier results that revealed a significantly higher proliferative activity for the solid type of AB than the unicystic variant, as assessed by the proliferating cell nuclear antigen marker and Ki-67. The localization of the dense p53-positive cells among the basal layer of the epithelial cyst wall in the uAB cases might indicate p53 overexpression in these cells. Increased density, rather than increased number of p53-positive cells has been reported as related to proliferation in odontogenic epithelium [18]. These results support the idea that the origin of uAB is from a pre-existing odontogenic cyst (dentigerous cyst, odontogenic keratocyst or residual cyst) [22–24]. It is thought that in these basal cells, supposedly with high proliferative activity, the neoplastic transfor-
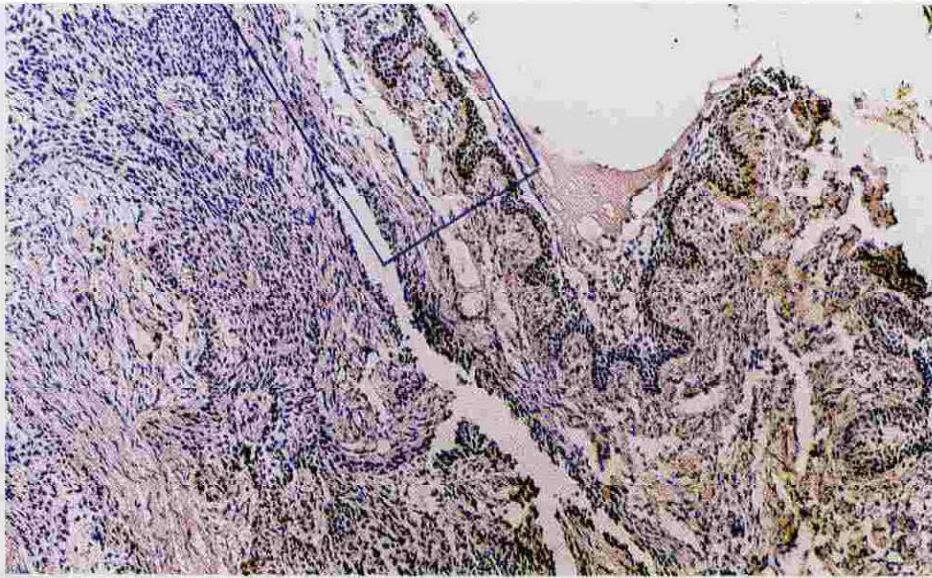
Figure 1 **uAB with p53 immunoreactive epithelium of the cyst lining as well as the neoplastic component in the cystic wall (p53 immune stain, ABC, counterstained with H&E × 100)**
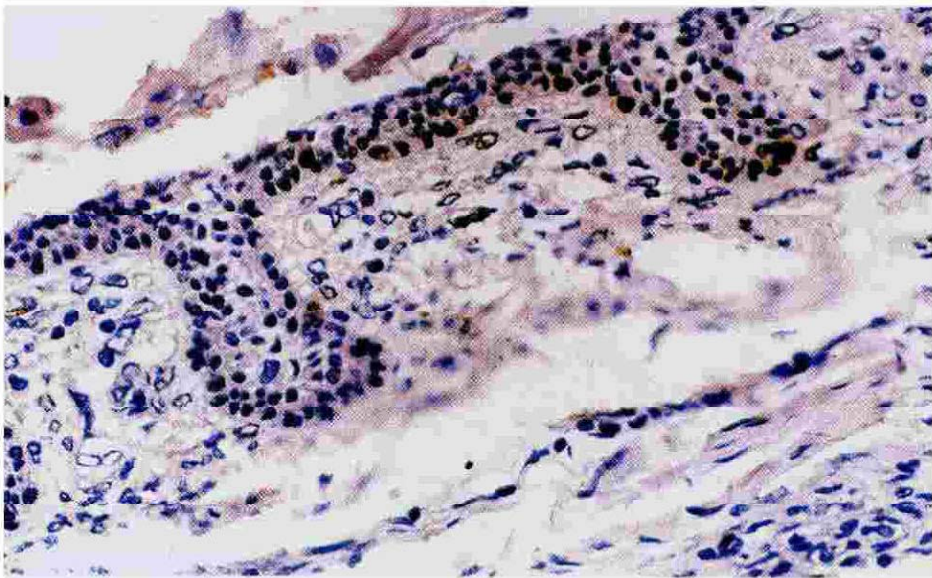

Figure 2 **Higher magnification showing intranuclear localization of the staining reaction among the reactive cells, mainly among the basal cell layer of the cystic lining of uAB (p53 immune stain, ABC, counterstained with H&E × 400)**
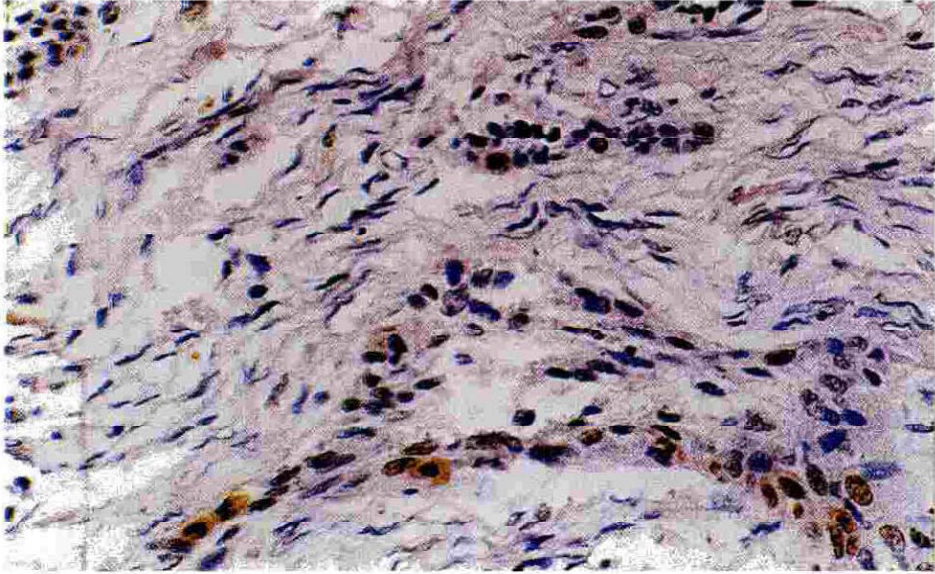
Figure 3 uAB with densely stained p53-positive neoplastic cells arranged in strands (p53 immune stain, ABC, counterstained with H&E × 400)
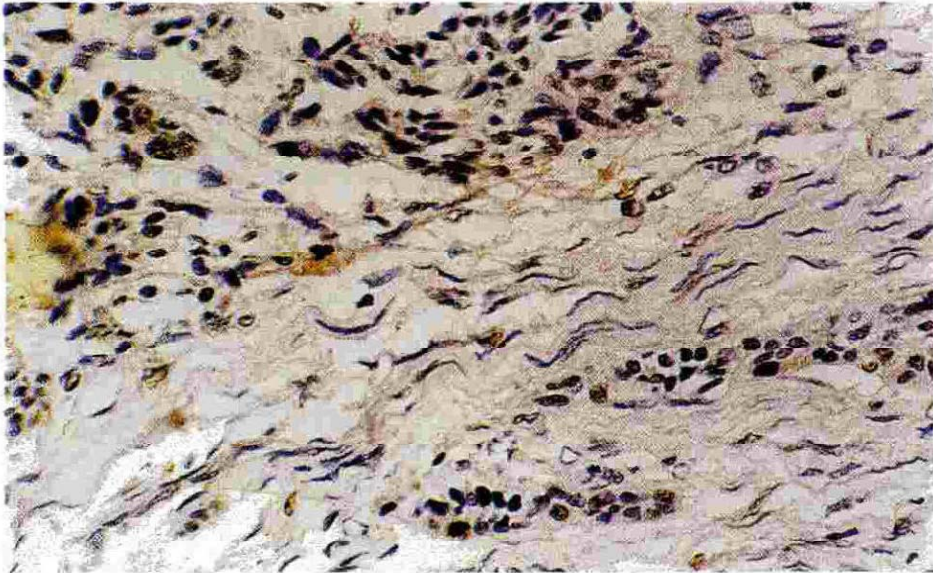
Figure 4 A nearby field of Figure 3 showing p53-positive cells in small collections while others are seen evenly distributed among tumour cells (p53 immune stain, ABC, counterstained with H&E × 400)

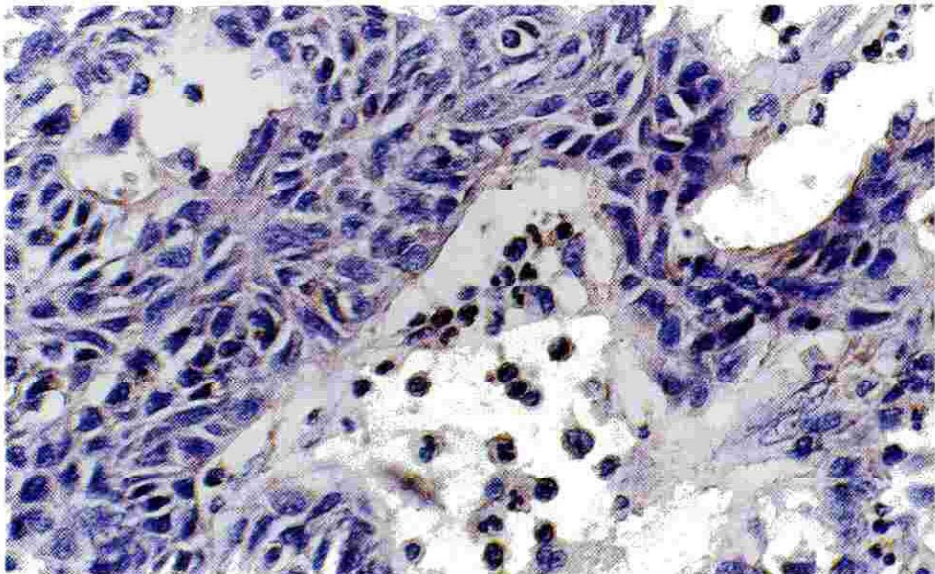Figure 5 **cAB (follicular) revealing faintly stained p53-positive cells among the peripheral columnar and few central stellate cells (p53 immune stain, ABC, counterstained with H&E × 100).**
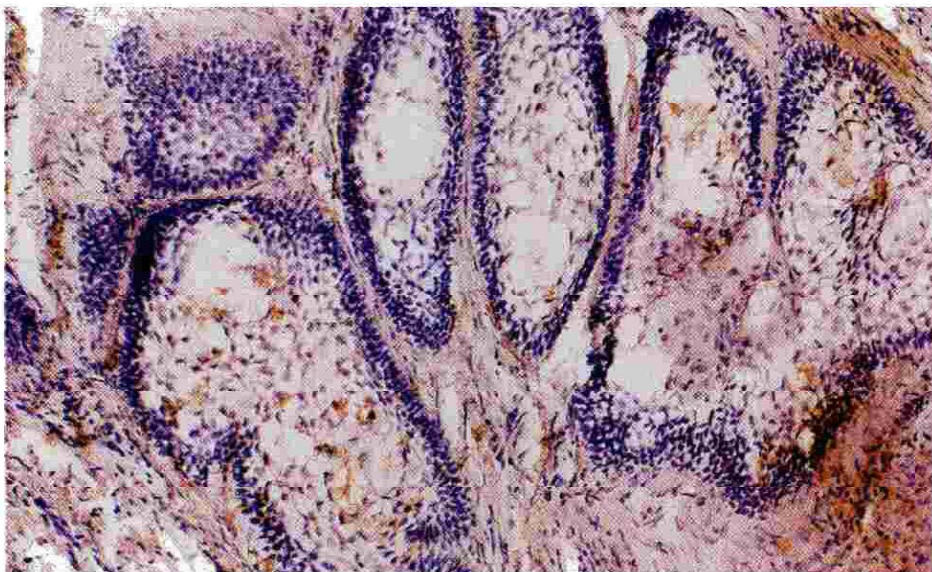

Figure 6 **cAB (plexiform) showing faint p53-positive reaction in the columnar neoplastic cells (p53 immune stain, ABC, counterstained with H&E × 100)**

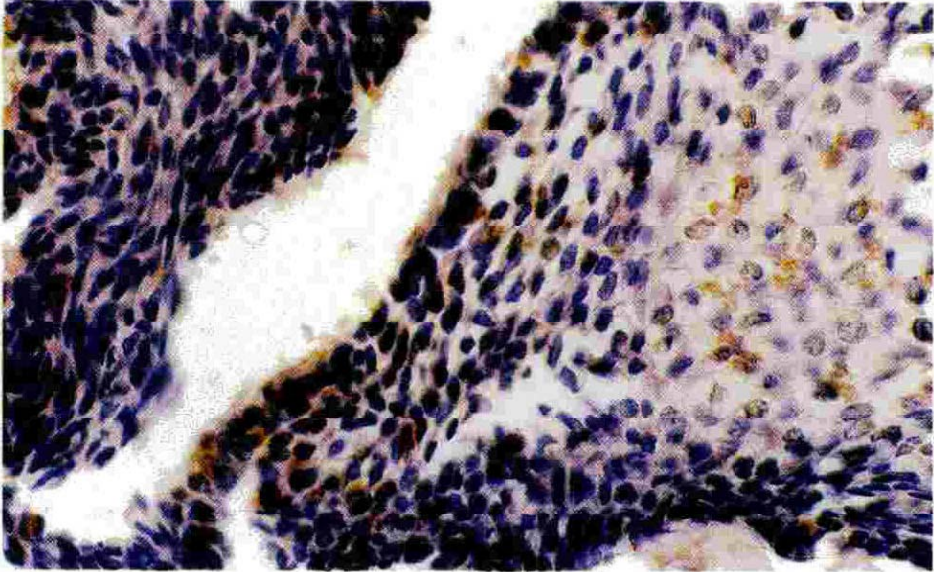Figure 7 **mAB with densely stained peripheral columnar cells for p53 marker (p53 immune stain, ABC, counterstained with H&E × 400)**
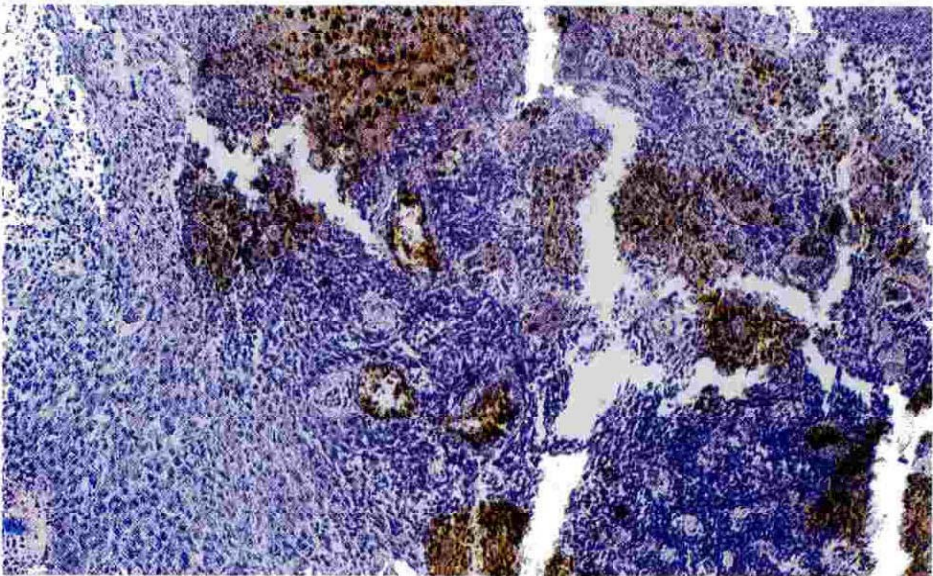

Figure 8 **mAB showing dense and intensely stained tumour cells, particularly in areas of necrosis and squamous metaplasia (p53 immune stain, ABC, counterstained with H&E × 100)**

mation may be triggered by p53 overexpression, possibly as a defence mechanism to perform its tumour suppressor function, resulting in the limited increased proliferation.

More than half of the cAB cases examined failed to react to the p53 marker and the positive cells were faintly stained in most of the reactive cases. Faint staining might point to these cells being responsible for slow tumour growth and expansion. In these cells there might be DNA changes or a perturbation effect causing the negative growth regulation of normal p53 to be suppressed, at least to some extent [13]. This could contribute to the benign nature of the neoplastic course resulting from the relatively quiescent tumour cells.

The strong immunoreaction to the p53 marker among the mAB concurs agreed with previous reports on p53 overexpression in various malignant tumours and in rapidly dividing cells [3,7,12]. It has been postulated that mutation of the p53 gene is associated with increased cellular proliferation [25]. Therefore, the dense staining seen in the mAB cases could possibly be related to accumulation of the mutant form of p53 protein rather than the wild type. The mutant p53 protein generally has different criteria for identification to the wild type p53; it is often more stable and can be detected immunohistochemically [19]. Meanwhile, the co-presence of a wild type cannot be excluded in malignant cases of ameloblastoma positive for p53 markers. The wild type p53 gene has characteristics of a recessive tumour suppressor gene, whereas mutant forms can act as a dominant oncogene [26]. Thus, positive staining might signal the presence of both proteins, where one is active (mutant) and the other is not (wild). The restricted distribution of p53-positive cells in mAB to those areas

with a less organized pattern and loosely arranged surrounding areas of necrosis, indicates areas of higher malignant transformation with evident DNA changes.

Conclusions

From these results it is possible to conclude the following.

- Normal odontogenic epithelium did not express immunohistochemically detectable levels of p53 protein.
- Immunohistochemistry is a valuable technique for the localization of p53 overexpression in the various forms of ameloblastoma.
- The altered p53 protein metabolism, whether due to mutation or to changed turnover of the wild type protein, occurs in both uAB and mAB and less frequently in cAB.
- Alterations in the p53 protein might be an early event in the pathogenesis of uAB.
- Immunohistochemistry for p53-protein expression has been proposed as a valid screening method for predicting malignancy in p53 protein in a variety of ameloblastomas, mainly through staining intensity as well as the frequency of p53-positive cells.

Recommendation

The immunohistochemical detection of p53 overexpression is recommended for cystic odontogenic conditions to disclose possible neoplastic transformation, and for conventional variants of AB to reveal malignant changes that the inexperienced eye might miss, particularly in borderline cases.

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The growing cancer burden, globally and in developing countries, calls for greater investment in health resources specific to cancer control and prevention strategies. The main risk factors involved are diet, tobacco, infection and hormones, all of which lend themselves to preventive action. Available evidence shows that cancer is attaining considerable proportions in many countries of the Region and is now reported as one of the leading causes of death.