CASE REPORT

PTCH expression in odontogenic cysts, a cause of pathogenesis or reason for clinical complication

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Summary  PTCH gene is well-known to be responsible for the development of Gorlin syndrome. It is also believed that PTCH mutation is responsible for the developmental of odontogenic keratocysts both in the Gorlin syndrome related and the sporadic cases. There is a conflict opinion in the literature regarding its role in odontogenic cysts. Furthermore, it is not known whether PTCH expression has any relevant clinical role in the outcome of the odontogenic cysts. In this study 8 odontogenic keratocysts, 16 dentigerous cysts and 23 radicular cysts treated in the academic hospital of the Free University of Brussels were subjected to an immunohistochemistry study for their expression of PTCH. The data obtained were linked to the clinical behaviour of the cysts for a follow-up of up to 20 years. Eighty eight percent of the odontogenic keratocysts (OKC), 67% of dentigerous cysts, (DC) and only 25% of radicular cysts (RC) expressed PTCH. This difference was statistically significant. Seventeen patients could be called back for clinical evaluation, the follow-up ranged from 10 to 20 years. Four patients suffering from OKC had clinical complications, 3 in the DC group and 2 in the RC group. Complications ranged from (multiple) recurrence to one case of transformation into an ameloblastoma. Only one patient suffered from clinical complication of a RC without being PTCH positive. One patient who had a PTCH negative DC and seven patients with PTCH negative RC had no clinical complications. The results indicate that PTCH expression in odontogenic cysts is more likely to be the reason of clinical complication rather than a cause of pathogenesis.

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KEYWORDS
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Odontogenic cysts;
Clinical complication;
Follow-up

Introduction
In 1996 the gene responsible for Gorlin syndrome (an inherited condition that causes unusual facial...
features and disorders of the skin, bone, nervous system, eyes, and endocrine glands. Also called basal cell nevus syndrome) was discovered\textsuperscript{1} namely PTCH gene. PTCH is a trans-membrane receptor which binds to Sonic hedgehog (SHH) protein to form a complex compound.\textsuperscript{2} PTCH expression is intended to down-regulate and stop the signaling triggered by the arrival of the SHH secreted protein at the cell membrane.\textsuperscript{3,4} This auto-regulating PTCH role, which ensures limited programmed proliferation, fails if the gene is altered by mutations or deletions. The result is uncontrolled cell proliferation, as well as a continuous and useless synthesis of the non-functional PTCH protein.\textsuperscript{1–5}

Several studies focused on this gene function in odontogenic keratocysts both sporadic and syndrome related.\textsuperscript{1,6–8} The result of these studies was that in Gorlin syndrome a predisposing mutation is already present in the germ line and only a single mutational event is required in the somatic cell to cause homozygous inactivation and neoplastic progression, whereas in sporadic cysts two independent mutational events are required in the somatic cell.\textsuperscript{7,8}

Levam et al.\textsuperscript{6} thought it possible that the OKC represented one of the first clinical manifestations of homozygous inactivation of the PTCH gene,\textsuperscript{12} at that time named NBCCS.

After this discovery some authors investigated whether PTCH was responsible for the developing of sporadic odontogenic keratocysts.

Levam et al.\textsuperscript{6} studied 6 syndrome cysts and 14 sporadic cysts. They found that each syndrome cyst retained the mutant allele and lost the allele from the unaffected parent. In sporadic cysts two mutational hits were observed in PTCH gene in somatic cells. They were confident that there was a clear relationship between the observed molecular change (PTCH mutation) and the developmental defect.\textsuperscript{6}

In 2000 Barreto et al.\textsuperscript{7} studied PTCH mutation in 6 odontogenic keratocysts, three of them were sporadic cases. They concluded that PTCH has a major role in the formation of sporadic and Gorlin syndrome associated odontogenic keratocysts.\textsuperscript{7}

To that stage of the research, the above mentioned authors believed that PTCH mutation is responsible for the developmental of odontogenic keratocysts both in the Gorlin syndrome related and the sporadic cases. By 2001 an antibody for PTCH was commercially available allowing its routine application in immunohistochemical studies on odontogenic cysts.

PTCH is only expressed during the embryonic development,\textsuperscript{3–4} later on it is downregulated and its expression is not detected. As all the known mutations in PTCH affect the function but not the stability of its protein;\textsuperscript{4,5} we believe that this antibody detects mainly the mutant PTCH in adult tissue.

Few years ago the theory of PTCH and its relation to odontogenic keratocysts was extended to another developmental cyst type: the dentigerous cysts.

Barreto et al.\textsuperscript{8} applied this antibody to 15 radicular cysts, 29 odontogenic keratocysts, and 6 dentigerous cysts. It is interesting that their study revealed the presence of PTCH protein in virtually all cysts.\textsuperscript{8} Pavelic et al.\textsuperscript{9} investigated the expression of PTCH in 10 odontogenic keratocysts, 10 dentigerous cysts and 10 radicular cysts. By studying PTCH–RNA and protein expression they found no activity for PTCH in radicular cysts while this expression was strongly observed in odontogenic keratocysts and dentigerous cysts. In the contrast to the previous authors the main conclusion was that PTCH is responsible for the developing of OKS and DC, and that it is not related to radicular cysts.\textsuperscript{9}

To summarize: Up-to-date the literature gives a conflicting view regarding the role of PTCH expression in odontogenic cysts, as some authors believe that PTCH is the main pathogenesis of all odontogenic cysts while others believe that it is involved in the pathogenesis of developmental cysts only and not related to radicular cysts.

Trying to solve this conflict and uncover the role of PTCH in the pathogenesis of odontogenic cysts we applied PTCH antibody to samples of the three major types of odontogenic cysts. Furthermore, we tried to investigate the relation (if exists) between PTCH expression and the clinical outcome.

Material and methods

All samples of odontogenic keratocysts and dentigerous cysts treated in the VUB university hospital until 1995 were included in this study. The group consisted of 8 odontogenic keratocysts and 16 dentigerous cysts. Twenty-three radicular cysts were selected to match for gender, age and anatomical location of the developmental odontogenic cysts. Paraffin blocks were retrieved from the pathology department archive. New H\&E slides were made to confirm the diagnosis. After re-evaluating the cases there was no single new diagnosis for any of the examined cases, and the original histopathological diagnosis was kept.
Five gingival samples obtained from periodontal surgery patients were included to the study sample as negative control. All samples were formalin or boin fixed tissue embedded in paraffin. All the cysts were treated in the head and neck surgery department, academic hospital of the Free University of Brussels during the period 1982–1995. The gingival samples were obtained from periodontal surgery patients treated in the clinics of dentistry institute of the Free University of Brussels. This sample (cysts and gingivas) was subjected to PTCH antibody through immunohistochemistry.

Cysts patients were called back for clinical evaluation, each patient we could reach was asked to answer a questionnaire regarding his/her general and dental health, whether or not he/she had had complications in the cyst site.

On the same appointment date the patient underwent clinical evaluation in the dentistry institute clinic or in the stomatology department’s outpatient clinic. This evaluation focused on the area of the previous lesion and mainly whether or not there was any fistula, chronic swelling, resistant pain or uncomfortable sensation in the jaw or the covering mucosa.

Lastly, a panoramic radiograph was taken for each patient to see the eventual recovery of the operation site, or to detect any recurrence or other suspicious lesion in the same area.

Immunohistochemistry procedure: Slides of 5 μm were sectioned, washed in xylol two times for 5 min followed by dehydration in an alcohol series. Antigen unmasking was done by immersing the slides in 6 M urea in a microwave oven till boiling followed by cooling down to room temperature for 20 min. Blocking peroxidase was achieved by 3% H₂O₂ in PBS for 10 min. The primary antibody (PTCH antibody, Santa Cruz, sc-6149) of 1:200 was applied over night at 4°C. Negative controls incubated in BSA 1% under similar conditions. The secondary antibody was applied for 30 min in room temperature in a dilution of 1:500. Then slides were incubated in DAB for 10 min. Between each step the slides were rinsed in PBS two times for 3 min. Slides were then alcohol dried and mounted.

As negative control each sample was subjected to the identical procedure with the exception of the addition of the primary antibody.

Microscopic evaluation methods: The black spots in the cytoplasm of the epithelial lining cells of the cysts were detected as the positive sign of the antibody. All cysts were studied under low magnification (40×) using standard light microscopy (Zeiss, Germany).

Results

No positivity was detected in all negative control. When positivity was detected the intensity differed from cysts to another and ranged from moderate intensity (Fig. 1) to high intensity (Fig. 2), but it was neglected and all positive cases were judged as positive cases regardless from the positivity intensity. In positive cases positivity was shown in all the cell layers of the epithelial lining of odontogenic cysts. All gingival samples were completely negative for PTCH antibody. Seven out of the odontogenic keratocysts expressed PTCH (88%), 11 dentigerous cysts (67%) and only 6 radicular cysts (25%) (Table 1). This difference was statistically significant with a p value less than 0.05% according to the χ²-test.
Only 17 patients could be called back for clinical evaluation of their dental health, they represent 4 OKC, 4 DC and 10 RC. The follow-up ranged from 10 to 20 years. Follow-up study relieved some cases that suffered from (multiple) recurrence or neoplastic transformation; these cases were scored as the clinical complication cases (Table 2).

Four patients suffering from OKC had clinical complications, 3 in the DC group and 2 in the RC group. Complications ranged from (multiple) recurrence to one case of transformation into an ameloblastoma. The neoplastic transformation case occurred in a radicular cyst that was treated 15 years ago. Only one patient suffered from clinical complication of a RC without being PTCH positive. One patient had a PTCH negative DC without clinical complication, in fact this dentigerous cyst was attached to an impacted fourth molar. Seven patients with PTCH negative RC had no clinical complications.

Although the small sample of follow-up study did not allow a thorough statistical evaluation, still it is clear that only two patients suffered from clinical complication without being PTCH positive.

### Discussion and conclusion

Since Johnson et al.\(^1\) reported that PTCH gene is a candidate gene for the basal cell nevus syndrome (BCNS), also called Gorlin syndrome, other abnormalities were linked to mutation and/or over-expression of PTCH in conditions such as colonic neoplasia,\(^10\) odontogenic keratocysts\(^11\) and ameloblastomas.\(^4,5\)

Later on some authors suggested that expression of the tumour suppressor PTCH and the oncogene Shh may cause loss of PTCH function and so lead to OKC development\(^6–8\) by stimulating continuous proliferation of dental lamina cells instead of their normal degeneration.

Moreover, some authors suggested that PTCH plays a major role in odontogenic cysts formation. They stated that PTCH is responsible for the formation of dentigerous cysts and not only of odontogenic keratocysts. In the same article the authors found no expression of PTCH in their radicular cysts sample and stated that this subtype of cysts may have a different pathogenesis that is not related to PTCH.\(^11\)

In contrast to this, another study revealed that PTCH expression is detectable in all odontogenic cysts including radicular cysts,\(^10\) and so PTCH might be the major cause for the pathogenesis of odontogenic cysts.

Our results show that all major subtypes of odontogenic cysts might express PTCH. Still 88% of odontogenic keratocysts are PTCH positive, the percentage drops to 67% of dentigerous cysts and reach only 25% of radicular cysts. Factors as the fixation method and duration, of historical pathological samples should be taken into account for differences in staining pattern. Other lab procedures such as antigen retrieval technique may also interfere with the results and quality of immunohistochemistry. Finally, and on top small sample size that is generally used to study gene expression in odontogenic cysts might raise the risk of over or down estimating any observation. Still we believe that our results indicate that PTCH is not the only pathogenesis factor in odontogenic cysts; although it plays a major role in their pathogenesis especially the developmental odontogenic cysts. As not all the odontogenic cysts express PTCH, it is an over-conclusion to state that PTCH is the main reason for the origin of odontogenic cysts or any of its subtypes as proposed by Barreto et al.\(^8\) and Pavelic et al.\(^9\).

Due to the fact that only small number of patients answered our call back, which is a limitation of any retrospective study, still almost all the cases (90%) that expressed PTCH had a sort of clinical complication in a follow-up of 10–20 years. In the light of the clinical follow-up this might indicate that PTCH expression in odontogenic cysts is more likely to be the reason of clinical complication rather than a cause of pathogenesis.

### Table 1  Immunohistochemistry study results

<table>
<thead>
<tr>
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<th>PTCH negative</th>
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<td>Odontogenic keratocysts</td>
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<td>1</td>
</tr>
<tr>
<td>Dentigerous cysts</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Radicular cysts</td>
<td>6</td>
<td>17</td>
</tr>
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### Table 2  Follow-up study results

<table>
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<th>Clinical complication</th>
<th>No clinical complication</th>
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<tbody>
<tr>
<td></td>
<td>PTCH +</td>
<td>PTCH –</td>
</tr>
<tr>
<td>OKC</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>DC</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>RC</td>
<td>2</td>
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* Case of a fourth molar.
References