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Expression of p53, Ki-67, and EGFR in odontogenic keratocysts before and after decompression

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BACKGROUND: Sixteen odontogenic keratocysts were examined morphologically and immunohistochemically for changes in proliferative activity before and after decompression using p53, Ki-67, and expression of growth factor (EGFR).
METHODS: p53 and Ki-67 positivity was scored by counting 500 cells and then counting the number of brown staining nuclei out of these. EGFR was scored using guidelines for scoring Herceptest [Dako (Her-2)]. A Wilcoxon test was performed on the results.
RESULTS: The values of Ki-67 and p53 before and after decompression were without significant change. There was no significant change in EGFR expression either. No correlation was found between inflammation or decompression time and expression of EGFR, p53, and Ki-67. The degree of change of the epithelium was varying, yet the reduction of the cysts size was considerable (18–100% – average 47.6%).
CONCLUSION: The morphologic changes in the cysts could not be correlated with expression of Ki-67, p53 or EGFR, to the clinical reduction of the cysts or the time of decompression.
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Keywords: decompression; expression of growth factor; immunohistochemistry; keratocysts; Ki-67; p53

Introduction

Odontogenic keratocysts (OKC) are developmental epithelial cysts most often seen in the mandibular ramus and angle. It has a high frequency of recurrence – most often within the first 5 years after treatment (1). Various therapies have been advocated to decrease recurrence potential. Long-term follow up of OKC after decompression and irrigation showed a lower recurrence rate than treatment with surgery alone (1). The tendency of recurrence is probably due to increased activity of the epithelium as confirmed by studies that have compared OKC with other odontogenic cysts. These studies found an increased expression of growth factor (EGFR) and proliferation markers (Ki-67 and p53) to be associated with an increased proliferation in OKC (2, 3).

After decompression treatment, the phenotypic look is changed considerably (1, 4, 5) and the changes indicate growth and proliferative activity. It is therefore interesting to study whether the pronounced clinical shrinkage which occur in OKC after decompression also shows biologically.

The purpose of this study was to evaluate if the histologic changes that occur in OKC after decompression can be detected biologically as a difference in growth and proliferation activity, before and after decompression, using the immunohistochemical expression of EGFR, Ki-67, and p53 as markers.

Material and methods

Twenty-nine patients with OKC were found from the files of the Department of Pathology at Odense University Hospital from the period 1971 to 1993. Thirteen of these were excluded from the study because follow-up material was not available to us. In 16 cases we had histologic material from the cysts before and after decompression. The study was approved by the local ethics committee and was conducted in accordance with the Danish law for scientific ethical committees (VF 20030213). The diagnosis conformed to the WHO-classification of odontogenic cysts. The cysts were of different Forsell types (6).

Immunohistochemistry was performed on deparaffinized 4 μ sections using an antibody to EGFR (7, 8), Ki-67, and p53P respectively. One negative control was made.

The following procedure was used: EGFR, 113 (Novo Castra, Newcastle Upon Tyne, UK) with an antibody dilution at 1:50 and incubation for 60 min. Demasking was performed with T-EG for 15 min using microwave oven. Detection system used was Power Vision. For Ki-67 monoclonal antibody MIB-1 (Dako, Glostrup,

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Denmark) at 1:200 dilution was used. Antigen retrieval included microwave in T-EG for 15 min. Detection system for both Ki-67, p53 and negative control was EnVision (Dako). Immunostaining with mouse polyclonal antibody (Dako, dilution 1:50) for p53 detection was underwent for 60 min at room temperature and for demasking microwave heated in citrate for 15 min.

A negative control was made using antibody diluent and demasking with T-EG in microwave oven for 15 min. A random area was chosen in the HE-section. In this area brown staining nuclei were counted in sections stained for p53 and Ki-67. A spectrum of colorings was observed for both antigens from faint to dense. The total number of 500 epithelial cells was counted and the labeling index, defined as the number of brown staining nuclei divided by the total number of epithelial cells, was calculated. The sections stained for EGFR was scored using guidelines for scoring Herceptest [Dako (Her-2)]. Score varying from 0 to 3+ (0 and 1+ we define as being negative and 2+ and 3+ as being positive). The inflammatory density was graded using a 4-grade scoring system: no inflammation, light inflammation (≤15 cells/HPF), moderate inflammation (> 15 to 50 cells/HPF), and heavy inflammation (> 50 cells/HPF).

We applied a Wilcoxon matched pairs test (non-parametric test) because the material is considered not to be with a Gaussian distribution.

Results

The results for the investigated parameters are given in Table 1. Twelve of the examined OKCs were of Forsell Type I (Fig. 1), three were of Forsell Type II and one of Forsell Type IV.

Four cysts changed 100% from Forsell Type I to hyperplastic squamous epithelium, typical for the reaction of the epithelium after decompression (Fig. 2).

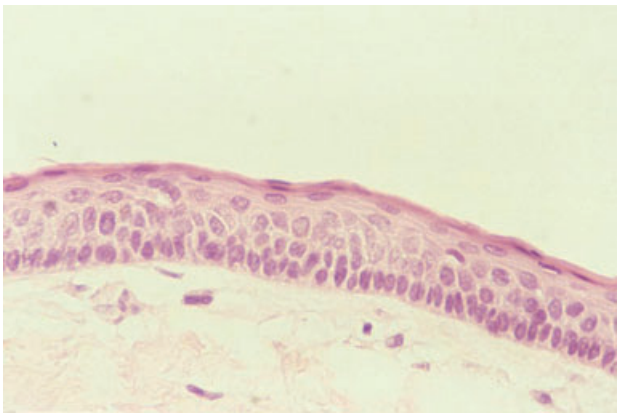

Figure 1 Epithelium in keratocyst (Forsell Type I) before decompression.

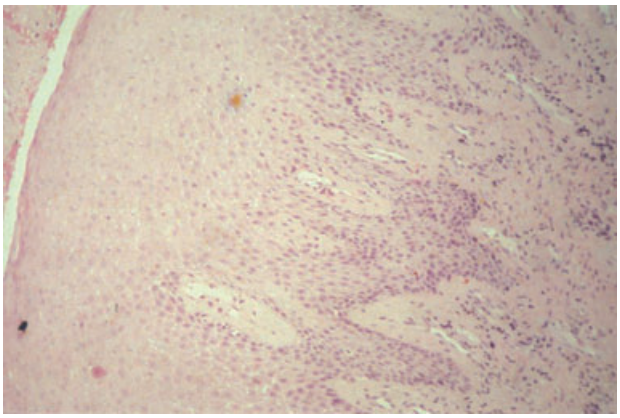

Figure 2 Epithelium in odontogenic keratocyst (OKC) after decompression. In this example there was 100% change into hyperplastic squamous epithelium.

Table 1 Results

Patient number	*Histologic diagnosis at:*		*Decompression time (months)*	*EGFR*		*Ki-67*[a]		*p-53*[a]	
	cystotomy	*cystectomy*		*Cystotomy*	*Cystectomy*	*Cystotomy*	*Cystectomy*	*Cystotomy*	*Cystectomy*
1	Forsell I	0% change	15	1+	1+	77	44	9	3
2	Forsell I	100% change	6	2+	2+	72	66	20	19
3	Forsell IV	0% change	12	2+	2+	133	133	16	17
4	Forsell I	100% change	12	1+	2+	27	92	0	8
5	Forsell I	90% change	9	2+	2+	123	67	52	7
6	Forsell II	75% change	17	2+	2+	58	52	25	84
7	Forsell II	100% change	5	3+	2+	123	164	31	98
8	Forsell I	100% change	10	2+	2+	133	111	9	74
9	Forsell I	0% change	6	2+	2+	98	99	16	13
10	Forsell I	0% change	9	2+	3+	31	102	55	56
11	Forsell I	100% change	5	1+	2+	131	97	5	1
12	Forsell II	0% change	10	2+	3+	94	300	87	16
13	Forsel I	100% change	8	2+	2+	106	140	142	46
14	Forsell I	100% change	11	2+	2+	130	72	27	26
15	Forsell I	25% change	13	2+	3+	187	192	46	0
16	Forsell I	0% change	10	2+	3+	70	78 ($P = 0.6387$ not significant)	43	40 ($P = 0.4637$ not significant)

Cystotomy, at time of biopsy; cystectomy, at time of removal of cyst after decompression.
[a]Number of positive cells out of 500 cells counted.

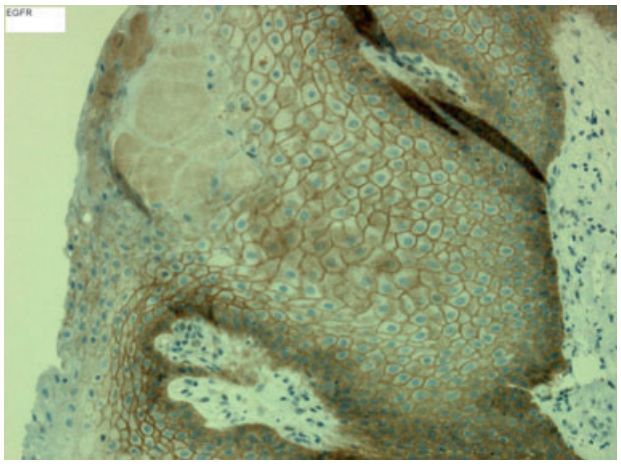

Figure 3 Positive expression of growth factor (EGFR; 3+). Staining located to the cell membrane.

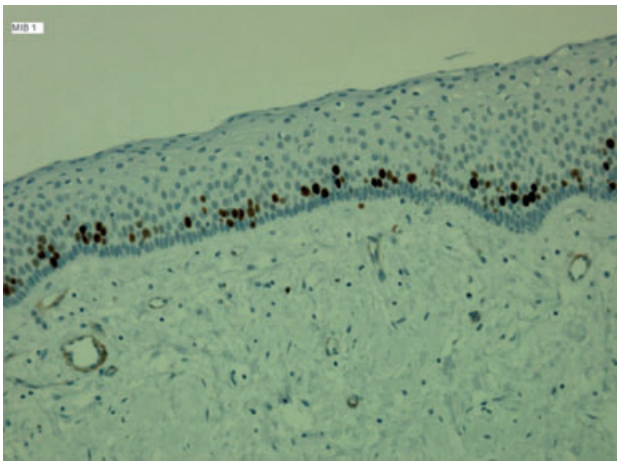

Figure 4 Ki-67. Staining mainly in the basal and parabasal layers.

Four cysts were unchanged (Forsell Type I). Four cysts changed from Forsell Type I to another Forsell Type. Four cysts were of another Forsell Type than I and of these one was unchanged. Two changed to another Forsell Type and one changed 100% to hyperplastic squamous epithelium.

Concerning EGFR, two (patient numbers 4 and 11) changed EGFR score from negative to positive after decompression. For one of these (patient number 4), this resulted in higher Ki-67 labeling and higher detectable p53, whereas, for patient number 11 it resulted in a lower proliferative rate and lower detectable p53 after decompression treatment. Fourteen patients remained unchanged with a positive EGFR score after decompression. Expression was located to the cell membrane in both the basal and suprabasal layer of the epithelium as well before and after decompression (Fig. 3). All but one – patient number 6 at time of cystectomy showed only EGFR positivity at the basal layer – showed positivity at the basal and suprabasal layer.

Seven cysts had a decrease in Ki-67 index after decompression. One was unchanged (133/133) and eight cysts had an increase in proliferation.

Ki-67-positive cells were mainly distributed in the basal and parabasal layers of the epithelium – both before and after decompression (Patient number 5 after decompression (Fig. 4)). All but one cyst expressed p53 at the time of cystotomy. Ten cysts had a decrease in p53 expression after decompression and six had an increase in p53 expression after decompression. p53-positive cells were seen throughout the epithelium but mainly in the parabasal layers (Patient number 14 after decompression (Fig. 5)). Inflammation and EGFR, Ki-67 and p53: no correlation was found between the degree of inflammation before and after decompression and the expression of EGFR, Ki-67, and p53. One cyst changed from light inflammation to heavy inflammation after decompression (patient number 5) without any effect on EGFR expression. The proliferative rate (Ki-67) and p53 expression both showed a decrease. The decompression time was relatively low – 9 months. One cyst changed from heavy inflammation to light inflammation (patient number 13) – likewise with no effect on EGFR expression, but in this case with a rise in Ki-67 and a decrease in p53 expression. Decompression time was 8 months.

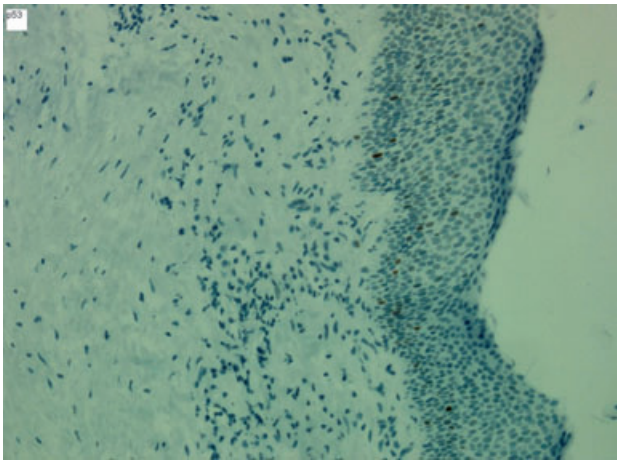

Figure 5 p53 staining throughout the epithelium but mainly parabasally.

The other cysts showed a rather inhomogenous pattern. Two cysts (patient numbers 4 and 11) showed an increase in inflammation from light to moderate after decompression and went from a negative EGFR score to a positive score. One of these cysts showed an increase in Ki-67 and p53 expression whereas the other cyst showed a decrease in expression of Ki-67 and p53. We applied statistics using a Wilcoxon matched pairs test (non-parametric test) on the values of Ki-67 before and after decompression resulting in a *P*-value of 0.6387, considered not significant. The same procedure was performed on values of p53 before and after decompression, resulting in a *P*-value of 0.4637, considered not significant.

Discussion

In the present material of 16 OKC, we found a histologically uniform epithelium showing great variation in Ki-67 labeling. There was considerable difference in the proliferation rate even in epithelium of same appearance. Counting positive nuclei from another area of the cyst may have given a totally different result. The Ki-67 expression found in the epithelium before and after decompression showed no significant difference ($P = 0.6387$). We found no correlation either between the proliferation rate and the decompression time. The decompression time was determined by the clinical response, was very varying and relatively short.

Looking at p53 expression in the cysts at cystotomy and at cystectomy – the amount of detectable p53 before and after decompression showed no significant difference ($P = 0.4637$). No correlation was found between expression of p53 and decompression time. Slootweg (2) has found the presence/absence of densely stained p53 positive cells to be broadly related to Ki-67 cell numbers (In other words that high numbers of densely stained p53 positive cells was broadly related to high Ki-67 cell numbers in highly proliferative areas and reverse). We did not find a connection between the number of p53-positive cells and Ki-67 expression in the proliferous cyst epithelium after decompression. In our investigation, however, a different way is used to count the p53-positive cells – all detectable coloring at 40× – which is of course less selective and will count positivity in less proliferative areas too. So the results cannot be compared.

We evaluated the effect of inflammation on EGFR, Ki-67, and p53 expression in the cysts in order to see if there was any difference in antigen expression before and after decompression. No significant difference was found between antigen expression in inflamed and non-inflamed cysts. Yet we found a tendency toward low decompression time and amount of inflammation at the time of cystectomy. Kaplan and Hirshberg (9) have found a focal increase in Ki-67 expression adjacent to moderate to severe inflammation with no significant effect on the overall proliferation activity of the cysts. Our results seem to confirm theirs. OKC showed a high degree of EGFR expression that could indicate that these cysts have a considerable growth potential. Thirteen of 16 (81.3%) of the cysts showed expression before decompression and the number increased to 15 of 16 (93.8%) after decompression. Shrestha et al. (7) in their study found expression of EGFR in 60% of OKC suggesting that the cystic epithelium is capable of EGFR-mediated proliferation. Most often, p53 is found in OKC than other odontogenic cysts (2) or only in OKC (3) suggesting that the increased epithelial activity may explain the tendency to recur. A recent study has even shown that OKC are monoclonal and should be seen as a neoplasia (10). In this study two of the 16 cysts (patient numbers 8 and 14) showed recurrence within 5 years from the time of cystectomy whereas patient number 2 presented a cyst 7 years after cystectomy – most likely a new cyst rather than a recurrence (1). The number of recurrences was neither correlated with the degree of histologic change nor to the expression of p53.

The presented material is old, small, and inhomogenous. It has been considerably reduced as almost half of it has to be excluded because either primary or follow-up material was not available to us. Six of 16 cysts (37.5%) showed no change at all of the cyst epithelium after decompression while seven of 16 (43.8%) showed a 100% change and the rest, three of 16 (18.8%) were from 25% to 90% changed. It seems that the same treatment results in a different morphology of the cysts and a different immunohistochemical expression of EGFR, Ki-67, and p53 and it does not seem to depend on the decompression time.

Clinically, after decompression treatment, shrinkage of the cysts can be observed, no matter what the histologic picture showed. It is interesting to note, however, that two cysts showed 100% change of the epithelium in as little as 5 months of decompression and one more after 6 months. This might indicate that longer time of decompression does not necessarily add further benefit to the treatment.

We found no study to which we can compare our results as only one study (4) has researched into decompression and epithelial changes and this study looked for loss of cytokeratin 10.

Our study has shown how complex the biology of OKC in conjunction with decompression really is, but it does not answer the question why decompression works.

So this small material has to be followed up by further research to improve our knowledge of the OKC and the mechanism of decompression. Maybe with a prospective study and maybe one has to extend the area of research from epithelial factors to stromal factors (integrines) as well.

Conclusion

The values of Ki-67, p53, and EGFR in OKC before and after decompression showed no significant change. There was no correlation between the expression of Ki-67 and p53.

Expression of EGFR was large and had a tendency to increase after decompression. No correlation between clinical shrinkage and morphologic change or expression of proliferation and growth markers could be found.

For those cysts that react positively on decompression optimal shrinkage seems to appear already after 5–6 months.

References

1. Marker P, Brøndum N, Clausen PP, Bastian HL. Treatment of large odontogenic keratocysts by decompression and later cystectomy: a long term follow up and a histologic study of 23 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; **82**: 122–31.
2. Slootweg P. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. *J Oral Pathol Med* 1995; **24**: 393–7.

3. Ogden GR, Chisholm DM, Kiddie RA, Lane DP. p53 protein in odontogenic cysts: increased expression in some odontogenic keratocysts. *J Clin Pathol* 1992; **45**: 1007–10.
4. August M, Faquin WC, Troulis MJ, Kaban LB. Dedifferentiation of odontogenic keratocyst epithelium after cyst decompression. *J Oral Maxillofac Surg* 2003; **61**: 678–83.
5. Nakamura N, Mitsuyasu T, Mitsuyasu Y, Taketomi T, Higuchi Y, Ohishi M. Marsupialization for odontogenic keratocysts: long-term follow-up analysis of the effects and changes in growth characteristics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **94**: 543–53.
6. Forsell K, Sainio P. Clinicopathological study of keratinized cysts of the jaws. *Proc Finn Dent Soc* 1979; **75**: 36–45.
7. Shrestha P, Yamada K, Higashiyama H, Takagi H, Mori M. Epidermal growth factor receptor in odontogenic cysts and tumors. *J Oral Pathol Med* 1992; **21**: 314–7.
8. Tiejun L, Browne RM, Matthews JB. Immunocytochemical expression of growth factors by odontogenic jaw cysts. *J Clin Pathol* 1997; **50**: 21–7.
9. Kaplan I, Hirschberg A. The correlation between epithelial cell proliferation and inflammation in odontogenic keratocyst. *Oral Oncol* 2004; **40**: 985–91.
10. Narasimhan PA, Bobby MC, Barnes L, et al. Molecular analysis to demonstrate that odontogenic keratocysts are neoplastic. *Arch Pathol Lab Med* 2004; **128**: 313–7.