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ORIGINAL ARTICLE

# Immunohistochemical expression of vascular endothelial growth factor (VEGF) in different types of odontogenic cysts

**Corrado Rubini · Luciano Artese · Antonio Zizzi · Massimiliano Fioroni · Giuliano Ascani · Gaia Goteri · Daniela Stramazzotti · Marcello Piccirilli · Giovanna Iezzi · Adriano Piattelli**

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**Abstract** The objective of the present study was to evaluate vascular endothelial growth factor (VEGF) expression in different types of odontogenic cysts. A total of 25 parakeratotic odontogenic keratocysts (POKCs), 16 orthokeratotic odontogenic keratocysts (OOKCs), and 28 follicular cysts (FCs) were evaluated semiquantitatively for immunohistochemical analysis of VEGF in epithelial cells, endothelial cells of blood vessels, inflammatory cells and focally stromal cells. A significant different expression of VEGF in all cell components was found in keratocysts compared to FCs. The POKCs (80%) and OOKCs (68%) showed more than 50% VEGF positive epithelial cells, whereas the majority of FCs (71%) were either negative in the epithelium or showed less than 10% positive cells. Similarly, the POKCs (88%) and OOKCs (68%) showed more than 50% positive endothelial cells, whereas the FCs (75%) were either negative or showed less than 10% VEGF positive endothelial cells. The highest percentage of cases with score 2 positivity in the stromal cells was observed in POKCs (68%); OOKCs showed a score 2 positivity in 44%, score 1 in 31% and score 0 in 25%, whereas 68% of FCs showed a score 0, 25% a score 1 and only 7% of cases showed a score 2. No statistically significant differences were observed between POKCs and OOKCs in VEGF expression in the epithelial and endothelial cells, whereas the positivity score in stromal cells was significantly higher in POKCs compared to OOKCs. The present results can support the hypothesis that angiogenesis is an active mechanism in the invasive growth of the OKC.

**Keywords** Odontogenic cysts · Angiogenesis · VEGF · Immunohistochemistry

C. Rubini · A. Zizzi · G. Goteri · D. Stramazzotti
Department of Neurosciences, Pathologic Anatomy and Histopathology, Polytechnic University of Marche, Ancona, Italy

L. Artese · G. Iezzi · A. Piattelli
Dental School, University of Chieti, Pescara, Italy

M. Fioroni
Dental School, Polytechnic University of Marche, Ancona, Italy

M. Piccirilli
Department of Stomatology and Oral Science, University of Chieti, Pescara, Italy

G. Ascani
Department of Maxillofacial Surgery, "Torrette" Regional Hospital, Ancona, Italy

C. Rubini (✉)
Department of Neurosciences, Pathologic Anatomy and Histopathology, Polytechnic University of Marche, Via Conca, 71 60121-Torrette, Ancona, Italy
e-mail: c.rubini@univpm.it

## Introduction

Odontogenic keratocysts (OKC) have a characteristic microscopic appearance [1, 2]. The epithelium of the OKC has been reported to show a higher rate of proliferation than other cyst types; p53, PCNA and Ki-67 are expressed more strongly in OKC [1, 3–5]. The invasive growth of OKC has been reported to be the result of an altered pattern of expression of collagens and fibronectin at the basement membrane [1, 6, 7]. OKC are clinically more aggressive and tend to recur with greater frequency than the

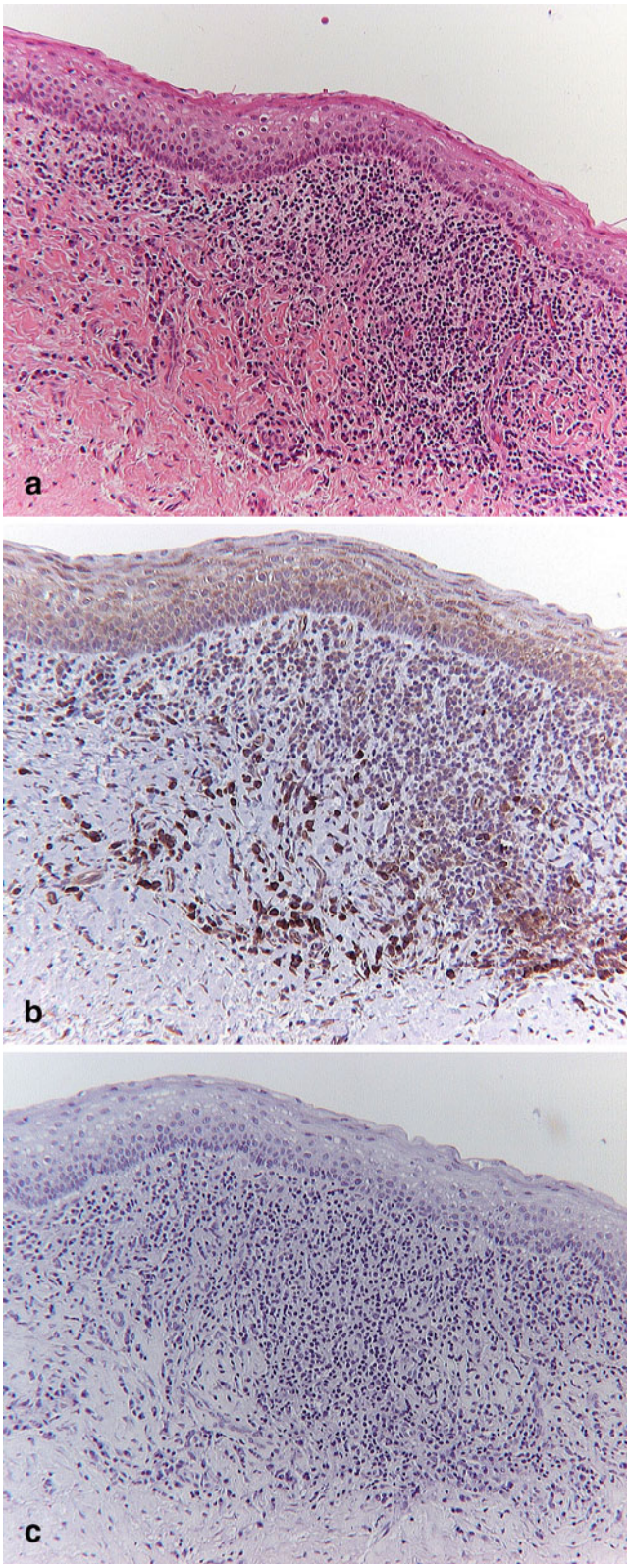


**Fig. 1** POKCs with inflammatory cells. **a** Hematoxylin and eosin staining. **b** VEGF expression in epithelial, endothelial, and inflammatory cells. **c** Negative control for anti-VEGF-165 staining. (Original magnification 200×)

other cyst types [8–12]. Orthokeratotic OKC (OOKC) have a different clinical behavior than parakeratotic OKC (POKC) and are not associated with basal cell nevus syndrome; thus, they probably represent a distinct clinicopathological entity [1, 3, 7, 13, 14]. Recently, WHO has classified OKC as a keratocystic odontogenic tumor, i.e., a benign uni- or multi-cystic, intraosseous tumor of odontogenic origin, with a characteristic lining of parakeratotic stratified squamous epithelium and potential aggressive, infiltrative behavior [15]. An evidence of the neoplastic rather than developmental nature of OKC has been supplied by the presence of allelic imbalance of tumor suppressor genes in the majority of OKC [2, 16, 17]. One of the mechanisms involved in the growth of jaw cysts could be the formation of new vessels. One key mediator of angiogenesis is vascular endothelial growth factor (VEGF) [18–22]. VEGF is a multifunctional cytokine. It is overexpressed in several conditions, which are characterized by vascular hyperpermeability and angiogenesis. VEGF is a secreted homo-dimeric glycoprotein that stimulates proliferation and migration of endothelial cells and enhances vascular permeability [23].

The known members of the human VEGF family include placental growth factor (PLGF), VEGF-A, VEGF-B, VEGF-C, VEGF-D [also known as c-Fos-induced growth factor, FIGF], and the viral VEGF-Es encoded by strains D1701, NZ2, and NZ7 of the parapoxvirus Orf (which causes pustular dermatitis) [24, 25]. Alternative splicing of the human VEGF-A gene gives rise to at least six different transcripts, encoding isoforms of the following lengths (in amino acids, excluding the signal peptide): 121, 145, 165, 183, 189, and 206 [26]. The larger isoforms, VEGF-189 and VEGF-206, remain cell-bound, whereas the smaller subtypes, VEGF-121 and VEGF-165, are efficiently secreted after production. VEGF is normally produced by various tissues [23].

VEGF seems to control the differentiation of the inner enamel epithelium to ameloblasts [27], seems to be involved in the healing processes around dental implants [28], is closely related to osteogenesis [29–38]. It seems to be involved in bone resorption in periprosthetic osteolysis of total hip replacement [39] and has been found to be important in the progression of oral dysplasia and oral squamous cell carcinoma [18] and of periodontitis [40].

The aim of the present study was an immunohistochemical characterization of the expression of VEGF in the various cell populations of different types of odontogenic cysts.

## Materials and methods

Tissue specimens were obtained from the archives of the Institute of Pathologic Anatomy and Histopathology,

**Table 1** Clinical features of the cysts

| Variables | FC | OOKC | POKC |
|---|---|---|---|
| No. of cases | 28 | 16 | 25 |
| Age (mean) | 40 (range 18–61) | 29 (range 21–43) | 23 (range 16–35) |
| Male | 16 | 12 | 20 |
| Female | 9 | 4 | 5 |
| Site | | | |
| Maxillary | 11 | 2 | 5 |
| Mandibular | 17 | 14 | 20 |
| Size (cm) | Range 1.5–2 | Range 1–2.5 | Range 1.5–3.8 |
| Recurrences | 0 | 1/16 | 4/25 |
| Multiple | 0 | 2/16 | 5/25 |

Polytechnic University of Marche, Ancona, Italy. All the biopsies were fixed in formalin (10% neutral buffered formalin) and paraffin-embedded. Pathologic description of cysts and histological slides were retrieved from the archives to evaluate morphologic parameters. The diagnosis for all cysts was made on hematoxylin- and eosin-stained sections and by comparing the histopathological data, clinical history, and radiographic appearance.

A total of 28 follicular cysts (FCs) and 41 (16 orthokeratotic, 25 parakeratotic) odontogenic keratocysts (OKCs) were evaluated. FCs were discovered during routine radiographic examinations or in cases where the tooth had failed to erupt; they were always associated with the crown of an unerupted tooth. Radiographically, all FCs presented as a well-defined multilocular radiolucency. All the FCs were single lesions. The OKCs were presented as well-circumscribed unilocular or multilocular radiolucencies with distinct margins. Most of the patients were asymptomatic, and only in a few cases pain and swelling were present. All OKCs were primary non-syndromic (not part of the nevoid basal cell carcinoma syndrome) and occurred as a single or multiple lesion in otherwise healthy patients.

**Table 2** Distribution of VEGF expression scores in the various cell populations

| | No. of Cases | | |
|---|---|---|---|
| | Score 0 | Score 1 | Score 2 |
| *POKC* | | | |
| Epithelial cells | 0 | 5 | 20 (4*) |
| Stromal cells | 0 | 8 (1*) | 17 (3*) |
| Endothelial cells | 0 | 3 | 22 (4*) |
| Inflammatory cells | 21 (3*) | 1 | 3 (1*) |
| *OOKC* | | | |
| Epithelial cells | 0 | 5 (1*) | 11 |
| Stromal cells | 4 | 5 (1*) | 7 |
| Endothelial cells | 2 | 3 (1*) | 11 |
| Inflammatory cells | 14 (1*) | 1 | 1 |
| *FC* | | | |
| Epithelial cells | 20 | 6 | 2 |
| Stromal cells | 19 | 7 | 2 |
| Endothelial cells | 21 | 3 | 4 |
| Inflammatory cells | 27 | 1 | 0 |

* Recurrent keratocysts

## Immunohistochemistry

Immunohistochemical analysis was performed on 5-μm paraffin-embedded tissue sections on poly-l-lysine-coated glass slides. After heat-drying, sections were deparaffinized in xylene and sequentially rehydrated in gradients of ethanol, treated with TUF solution (Histo-line Laboratories, Milan, Italy) at 90°C for 10 min better to unmask antigenic sites, and, after three washes with $H_2O$, they were incubated overnight at 4°C with anti-VEGF-165 monoclonal antibody (diluted at 1:200, Santa Cruz, CA). The reaction was revealed using the streptavidin–biotin–peroxidase technique; sections were incubated with 3,3'-diaminobenzidine (0.05 diaminobenzidine in 0.05 M Tris buffer, pH 7.6, and 0.01% hydrogen peroxide) and counterstained with Mayer's hematoxylin. Positive controls were paraffin-embedded sections from gastric carcinomas. Negative controls were performed by substituting the primary antibody with nonimmune serum (Fig. 1c).

VEGF expression was semiquantitatively evaluated, calculating the percentage of VEGF-positive vessels, epithelial cells, fibroblasts, and inflammatory cells in ten representative fields by using a microscope at 250× magnification. The cases were divided in the following categories: score 0 (≤ 10% positive cells); score 1 (> 10% and <50% positive cells); score 2 (≥ 50% positive cells).

Statistical analysis

Cases were analyzed based on the clinical and pathologic parameters by SPSS statistical package (SPSS Inc., Chicago). Differences in the distribution of VEGF expression among groups and cell populations were compared using the $\chi^2$ test. Significance was set at $P<0.05$.

## Results

The clinical features of the cysts are summarized in Table 1.

The POKCs are lined by a regular parakeratotic stratified squamous epithelium, usually about 5–8 cell layers thick and without rete ridges. There is a well-defined, often palisaded, basal layer of columnar or cuboidal cells. The nuclei of the columnar basal cells tend to be oriented away from the basement membrane and are often intensely basophilic. The parakeratotic layers often have a corrugated surface. Mitotic figures are found frequently in the suprabasal layers (Fig. 1a).

A significant different expression of VEGF in all cell components was found in OKCs compared to FCs (Table 2). Indeed, the majority of POKCs (80%) and OOKCs (68%) showed more than 50% VEGF-positive epithelial cells, whereas the majority of FCs (71%) were either negative in the epithelium or showed less than 10% positive cells ($\chi^2$, $p<0.0005$). Similarly, the majority of POKCs (88%) and OOKCs (68%) showed more than 50% positive endothelial cells, whereas the majority of FCs (75%) were either negative or showed less than 10% VEGF-positive endothelial cells ($\chi^2$, $p<0.005$). The highest percentage of cases with score 2 VEGF positivity in the stromal cells was observed in POKCs (68%) (Fig. 1b); OOKCs showed a score 2 positivity in 44%, score 1 in 31%, and score 0 in 25%, whereas 68% of FCs showed a score 0, 25% a score 1, and only 7% a score 2 ($\chi^2$, $p<0.0001$). No statistically significant differences were observed between POKCs and OOKCs in VEGF expression in the epithelial and endothelial cells, whereas the positivity score in stromal cells was significantly higher in POKCs compared to OOKCs ($\chi^2$, $p=0.0255$). Occasionally, a VEGF immunoreactivity was observed in inflammatory cells also. Recurrent cases showed VEGF positivity in >50% of epithelial and endothelial cells, but no statistically significant differences in VEGF expression was found between recurrent and nonrecurrent cases.

## Discussion

The present study shows that VEGF is expressed in the various types of odontogenic cysts. However, marked differences were found in OKC and OKC compared with FC. In all cases of OKC, there was a statistically significantly higher expression of VEGF positivity in epithelial, endothelial, and stromal cells, while, on the contrary, most of the FCs were negative for VEGF expression. Some researchers have reported an intense immunoreactivity in periapical and radicular cysts [41, 42], but this could be related to the frequent presence of an inflammatory infiltrate in this type of cysts. The present data show that angiogenesis can be important in the progression and enlargement of odontogenic cysts similarly to what occurs in neoplastic conditions [43]. Moreover, this higher positivity for VEGF of OKC could help to explain in part the infiltrative characteristic of this lesion. The present study was also directed at seeking correlations among VEGF cellular expression, clinical findings, and cellular composition of the odontogenic cysts. None of the clinical parameters evaluated correlated with VEGF expression. A correlation was observed between VEGF expression in epithelial cells and capsular fibroblasts and vessels, suggesting that these cell types might form a cellular network sharing regulation by the stimulatory signals promoting angiogenesis.

The present results are consistent with a mechanism that could combine the relationships of VEGF expression in epithelium, vessels, and stromal cells of the fibrous wall with the different clinical and biological behavior of various types of odontogenic cysts.

In conclusion, the present results can support the hypothesis that angiogenesis is an active mechanism in the invasive growth of the OKC.

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**Conflict of interest** We have no conflict of interest.

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