KAI-1 Protein Expression in Odontogenic Cysts

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Abstract

The KAI-1 tumor suppressor gene is widely distributed in normal tissues and its down-regulation may be correlated with the invasive phenotype and metastases in several different epithelial tumors. The aim of the present study was an evaluation of KAI-1 expression in radicular cysts (RC), follicular cysts (FC), orthokeratinized keratocysts (OOKC), and parakeratinized keratocysts (POKC). Eighty-five odontogenic cysts, 28 RC, 22 FC, and 35 OKC (16 OOKC, 19 POKC) were selected. All the POKC were negative and only four of 16 of the OOKC were positive for KAI-1. On the contrary, all RC and FC cases were positive and immunoreactivity for KAI-1 was detected throughout all the layers of the cyst epithelium. The lack of KAI-1 expression in POKC could help to explain the differences in the clinical and pathologic behavior of OKC and, according to what has been reported for epithelial tumors, could be related to the increased aggressive behavior and invasiveness of OKC. *(J Endod 2007;33:235–238)*

Key Words

KAI-1, odontogenic cysts, odontogenic keratocysts, transmembrane signaling

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KAI-1 has been detected in all normal human tissues tested thus far (1, 2). It is an important regulator of cell behavior (3). It belongs to a separate family of membrane glycoproteins (the transmembrane-4 superfamily) (4). The transmembrane-4 superfamily of glycoproteins is a family of glycoproteins that presumably cross the cell membrane four times (5). These proteins may be involved in transmembrane signal transduction regulation of cell proliferation, differentiation, and motility (6, 7). It was first isolated from human chromosome 11p11.2 in prostate carcinoma cells (4). The KAI-1 gene product is identical to CD82, a surface glycoprotein of leukocytes encoding 267 amino acids (4). KAI-1 is a tumor suppressor gene whose down-regulation was previously shown to be associated with the development of metastases and a worse prognosis in overall survival (3, 8–17). Its overexpression, on the other hand, suppresses tumor metastases in various animals (18), and in non-small-cell lung carcinoma cells suppresses the tumor invasiveness and metastatic potential (19). A threshold level of KAI-1 protein expression may be necessary for suppression of the metastatic phenotype (10). KAI-1 significantly suppressed the metastasis in rat metastatic prostatic cancer cells, without affecting the tumor growth rate (1). Restoration of KAI-1 expression in a metastatic prostate cancer line inhibits integrin-mediated cell migration and invasion (20). KAI-1 expression is high in human normal prostate and benign prostatic hyperplasia, but decreases in a significant way in cancer cell lines derived from metastatic prostate tumors (1), and prostate carcinomas with a low expression level of KAI-1 have shown more aggressive features than those with high expression levels (4). These same characteristics were also reported for pancreatic carcinoma, bladder carcinoma, breast carcinoma, non-small-cell lung carcinoma, gastric carcinoma, esophageal carcinoma, and oral squamous cell carcinoma (2, 4, 21–24). The physiologic and pathologic functions of KAI-1 are largely unknown (4), and it has been proposed that association with other cell-surface proteins may be of utmost importance in directing its biological activities (2). Cancer cells expressing KAI-1 attach to vascular endothelial cells through direct interaction between KAI-1 and DARC (an endothelial cell surface protein) and this interaction leads to inhibition of tumor cell proliferation and induction of senescence (18); the tumor metastases seem to be suppressed mainly by a inhibition of cancer cell motility and invasiveness (24). Odontogenic keratocysts (OKCs) have an aggressive behavior and their frequency of recurrence has been reported to be as high as roughly 60% (25).

The aim of this study was to evaluate the KAI-1 expression in radicular cysts, follicular cysts, orthokeratotic keratocysts, and parakeratotic keratocysts.

Materials and Methods

A total of 85 consecutive odontogenic cysts, 28 radicular cysts (RC), 22 follicular cysts (FC), and 35 odontogenic keratocysts (OKC) were retrieved from the files of the Institute of Pathologic Anatomy and Histopathology of the Marche Polytechnic University, Ancona, Italy. The OKCs were separated into two histological categories: 16 were orthokeratotic (OOKC) and 19 were parakeratotic (POKC). No OKCs were associated with the nevoid basal cell carcinoma syndrome (NBCCS). Epithelial dysplasia was not observed. Biopsy specimens, obtained at surgery, were fixed in 10% buffered formalin (24 to 48 hours), dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. The diagnosis was based on clinical, radiographic, and histological examination. Using hematoxylin and eosin–stained sections, all histological slides were reviewed; the quality of the material was checked, and a selection of the slides for the immunohistochemical evaluation was performed.

Immunohistochemistry for KAI-1 Protein

Immunohistochemical staining was performed by using the standard avidin–biotin–peroxidase complex (ABC) method. Briefly, each 4-μm tissue section was deparaffinized, rehydrated, and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature. After rehydratation through a graded ethanol series, the sections for p53 were microwaved in zinc sulfate heptahydrate buffer at 90°C for 10 minutes and then cooled to 30°C. After incubation with normal goat serum for KAI-1 slides, the tissue sections were applied for 30 minutes and removed by blotting. The sections were then incubated with anti-KAI-1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:80 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin at 4°C overnight, washed in PBS, and incubated with secondary antibody for 30 minutes at room temperature. Immunohistochemistry was performed using the ABC system (Vectastain; Vector Laboratories, Burlingame, CA, USA). The chromogen was 3,3′-diaminobenzidine tetrahydrochloride applied as a 0.02% solution containing 0.0055% H_2O_2 in 50 mM ammonium acetate–citric acid buffer (pH 6.0). The sections were lightly counterstained with hematoxylin. A negative control was performed in all cases by omitting the primary antibody and no detectable staining was evident. In normal tissue immunostaining of KAI-1 was detected in the cell membrane of the leukocytes (positive control).

Evaluation of the Staining for KAI-1 Protein

The KAI-1–stained cells was evaluated from a minimum of 1,000 cells in each case in all the epithelial layers. The FC, RC, and OKC were subdivided into two groups: when >10% of the epithelial cells were positively stained, the specimen was considered to be KAI-1 positive; when the positivity of the epithelial cells was <10%, the specimen was classified as KAI-1 negative (4).

The specimens that were exactly 10% positive were included in the KAI-1 positive group. Descriptive statistical analysis was performed for each group of cysts and the χ^2 test was used to show statistically significant differences between the groups.

Results

Results are summarized in Table 1. An inverse pattern of immunoreactivity for KAI-1 was observed in the POKCs and OOKCs, which tended to be negative or focally positive, compared to RC and FC, which were instead strongly or moderately and diffusely positive. In particular, all the POKCs showed no KAI-1 immunoreactivity in the epithelial cells, whereas a focal positivity was observed in the intermediate and superficial layers of OOKCs. These differences between POKCs and OOKCs were not statistically significant (χ^2 test: $p > 0.05$). All FC cases showed KAI-1 immunoreactivity throughout all the epithelial layers, particularly in the intermediate and superficial ones (Fig. 1); the islands of odontogenic epithelium were also positive, whereas the areas with mucous metaplasia were negative. Statistically significant differences in the pattern of KAI-1 immunoreactivity were present between FC and POKC (χ^2 test: $p < 0.0005$) and between FC and OOKC (χ^2 test: $p < 0.0005$). The RC cases showed a moderate KAI-1 immunoreactivity throughout the epithelium (Fig. 2), and again statistically significant differences were observed compared with POKC cases (χ^2 test: $p < 0.0005$) and OOKC cases (χ^2 test: $p < 0.0005$).

TABLE 1. Correlation between KAI-1 protein expression and histopathologic features of odontogenic cysts*

Histologic Type	Number of Cases	Positive	Negative
Radicular cyst	28	28	0
Follicular cyst	22	22	0
Orthokeratinized keratocyst	16	4	12
Parakeratinized keratocyst	19	0	19

*Chi-square (χ^2) p value: OOKC and POKC vs. dentigerous cysts and radicular cysts ($p = 0.0125$).

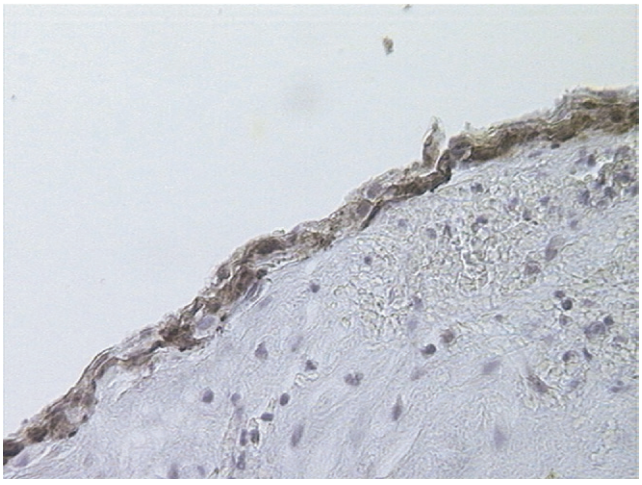

Figure 1. Follicular cyst. Positivity of the all epithelial layers. KAI-1: ×160.

Discussion

The KAI-1 gene consists of 11 exons, with the coding region starting at exon 3 and ending at exon 10[3]. Expression of this gene seems to be directly activated by p53 (4). There was a significant inverse corre-

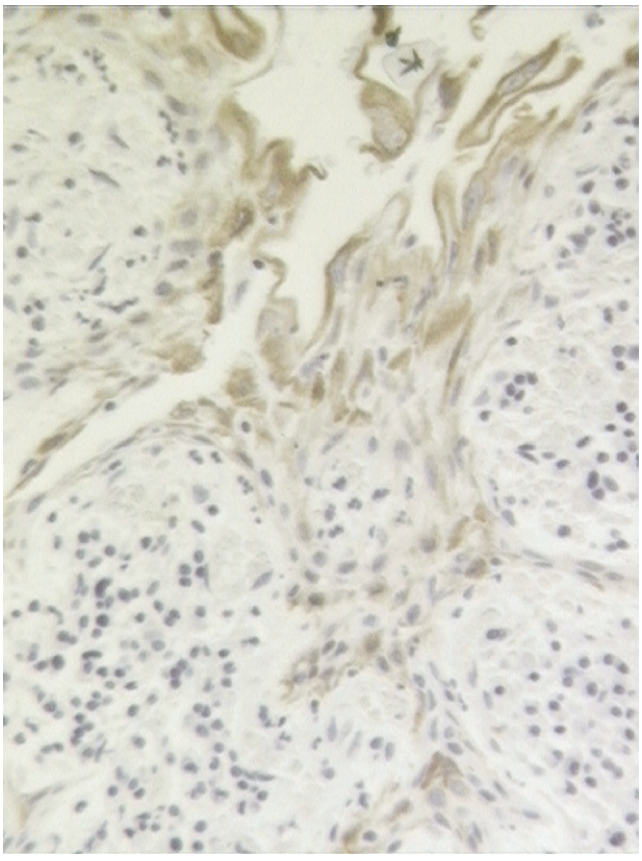

Figure 2. Radicular cyst. Positivity of the intermediate-superficial epithelial layers. KAI-1: ×160.