Diagnostic Pathology

RESEARCH

Open Access

Gene expression and immunohistochemistry analysis of ADAMTS-1 and its substrates in odontogenic keratocyst

Osvaldo Rodrigues de Souza Neto[1], Antonia Taiane Lopes de Moraes[2*], Hellen Thais Fuzii[3], Antonio Guilherme Maneschy Faria[4], Vanessa Morais Freitas[5], Maria Sueli da Silva Kataoka[1], Sérgio de Melo Alves Jr[1] and João de Jesus Viana Pinheiro[1]

Abstract

Background Considering the significant participation of the microenvironment in the local aggressiveness of odontogenic keratocysts, this study aims to evaluate the expression of ADAMTS-1 and its substrates, versican, aggrecan and brevican in this locally invasive odontogenic cyst.

Methods Immunohistochemistry and polymerase chain reaction (PCR) were conducted on 30 cases of odontogenic keratocysts (OKCs) and 20 dental follicles (DFs).

Results The immunohistochemical expression of these proteins was predominantly cytoplasmic and granular across all samples. In epithelial tissue, the immunoexpression of aggrecan and versican was higher in OKC ($p < 0.05$) compared to DF. Comparing the expression of proteins between the OKC epithelium and the cystic capsule, it was observed that all molecules were more expressed in the epithelium ($p < 0.001$). RT-PCR confirmed the expression of ADAMTS-1 and proteoglycans in all samples.

Conclusion ADAMTS-1, aggrecan, brevican, and versican were expressed in all samples with a granular and cytoplasmic pattern. RT-PCR confirmed their presence in both OKC and DF, but only aggrecan and versican exhibited significantly higher levels in OKC ($p < 0.05$). Protein expression was notably greater in the epithelial component of OKC. These findings underscore the potential role of these proteins in the biological behavior of OKC.

Keywords Odontogenic keratocyst, ADAMTS-1, Proteoglycans, Immunohistochemistry

This study is observational research that investigates the role of ADAM-TS1 and its substrates in the biological behavior of odontogenic keratocyst.

*Correspondence:
Antonia Taiane Lopes de Moraes
taiane_moraes12@hotmail.com
[1]Cell Culture Laboratory, School of Dentistry, Federal University of Para, Rua Augusto Correa, 01 Guama, Belem, PA 66075110, Brazil
[2]Oral Diagnosis Department, Piracicaba Dental School, University of Campinas (UNICAMP), Piracicaba, São Paulo 13414-903, Brazil
[3]Immunopathology Laboratory, Tropical Medicine Institute, Federal University of Pará, Av Generalíssimo Deodoro, 92 Umarizal, Belém, PA, Brazil
[4]School of Dentistry, Federal University of Para, Rua Augusto Correa, 01 Guama, Belem, PA 66075110, Brazil
[5]Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Introduction

The odontogenic keratocyst (OKC) is an intriguing odontogenic cyst characterized by a lining of keratinized stratified squamous epithelium. Initially misclassified as a primordial cyst, its diagnostic trajectory has been marked by ambiguity [1]. In 2005, due to factors such as a high recurrence risk, aggressive clinical behavior, mutations in the tumor suppressor gene (PTCH1), satellite cyst occurrence, and association with Gorlin–Goltz syndrome, the World Health Organization (WHO) reclassified it as a benign keratocystic odontogenic tumor (KCOT) [2]. However, the current WHO classification of head and neck tumors, insufficient evidence led to its reversion to the cyst category, retaining the term odontogenic keratocyst [3].

Histologically, OKCs display a distinctive profile, featuring a thin parakeratinized stratified squamous epithelium, approximately 5 to 8 cell layers thick [4]. This epithelium is capped by a thin, corrugated layer of parakeratin, displaying a characteristic palisaded pattern with uniform nuclei in the basal cell layer. A notable hallmark is the presence of daughter cysts formed by budding from the basal layer into the surrounding connective tissue. The fibrous cyst wall is typically thin and lacks significant inflammatory cell infiltrate [5].

In the tumor microenvironment, the extracellular matrix (ECM) plays a pivotal role beyond acting as a barrier to tumor invasion. It serves as a reservoir for ligand proteins and growth factors that regulate tumor cell behavior. Cell invasion is associated with the activity of metalloproteinases, including members of the ADAMTS family [6]. ADAMTS-1, the first identified protease of the ADAMTS family, is physiologically expressed in humans and plays a role in various biological processes. Dental pulp cells, including odontoblasts, cementoblasts, cementocytes, osteoblasts, osteocytes, and periodontal ligament cells, are implicated in the regulation of ADAMTS-1, ADAMTS-4, ADAMTS-5, and their versican substrate [7]. This intricate interplay within the molecular landscape highlights the dynamic mechanisms driving the pathogenesis and behavior of the odontogenic keratocyst [8–10].

The proteoglycans aggrecan, brevican and versican are fundamental components of ECM and represent the first initial barriers for tumor cells during invasion and metastasis [9]. Belonging to the aggrecanases subfamily, with which ADAMTS-1 is associated, these proteoglycans play a crucial role in modulating ECM. They have structural characteristics that confer a wide range of functions, including regulation of cell proliferation, adhesion, invasion and cell signaling [11, 12].

Given the significant role of the tumor environment, specifically the extracellular matrix, this study proposes an in-depth investigation of the expression of ADAMTS-1, along with as the proteoglycans aggrecan, versican and brevican, in OKC. Furthermore, the aim of this study is to explore the pathological and immunohistochemical findings that may contribute to a better understanding of the pathogenesis of this complex odontogenic entity. Detailed analysis of these molecular components may provide valuable insights for the development of more precise and targeted diagnostic and therapeutic strategies for this lesion. Additionally, to our knowledge, this is the first study to verify the expression of these proteins in OKC.

Methods

Aim, design and setting of the study

This study is observational research. The objective is to investigate the role of these proteins in the biological behavior of the odontogenic keratocyst. Thirty samples of keratocysts were obtained from the archives of the Department of Oral Pathology at the School of Dentistry of the University Center of Pará (CESUPA, Belém-PA, Brazil). Twenty cases of dental follicle (DF) were included as controls. This study was approved by the Ethics Committee of the Institute of Oncology Research Center of the Federal University of Pará, under approval number 2.371.646.

Immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) tissues were analyzed using immunohistochemistry. Five-micron sections were cut and mounted on poly-D-lysine-coated slides (Sigma Chemical Corp., St Louis, MO, USA). Sections were dewaxed in xylene and rehydrated in graded ethanol. Antigen retrieval was performed in the Pascal chamber (Dako, Carpinteria, CA, USA) for 30 s. Sections were immersed in 3% H2O2 in methanol for 20 min to inhibit of endogenous peroxidase activity and then blocked with 1% bovine serum albumin (BSA, Sigma®) in phosphate-buffered saline (PBS) for 1 h. The slides were then incubated with primary antibodies anti-ADAMTS-1 (1: 600, Abcam, Inc., Cambridge, MA, USA), anti-brevican (1: 50, Abcam, Inc., Cambridge, MA, USA), anti-aggrecan (1 :200, Abcam, Inc., Cambridge, MA, USA) and anti-versican (1: 50, Sigma Chemical Corp., St Louis MO, USA). All primary antibodies were diluted in antibody diluent solution (Dako®) and incubated for 1 h at room temperature. Subsequently, sections were incubated for 30 min with EnVision Plus detection system (Dako®). Diaminobenzidine (Sigma®) was used as a chromogen, and the sections were counterstained with Mayer's hematoxylin (Sigma®) and mounted with Permount (Fisher Scientific, Fair Lawn, NJ, USA). Invasive ductal breast carcinoma and ameloblastoma samples were used as a positive control. As a negative control, the primary antibody was replaced with non-immune serum.

Immunostaining evaluation

In each sample, 5 images were randomly acquired by a microscope (AxioScope, Carl Zeiss, Oberkochen, Germany) equipped with a color camera (AxioCam HRC, Carl Zeis) at 40x magnification. The areas stained with diaminobenzidine (DAB) were isolated and segmented using the Color Deconvolution plugin (developed by Gabriel Landini) in ImageJ, a public domain software created by Wayne Rasband (National Institutes of Health, Bethesda, MD, USA) and the fraction areas (%) were measured, and the data was used to determine the expression of ADAMTS-1, versican, aggrecan, and brevican in OKC and DF. This immunoscoring pattern was described in a previous study (Ribeiroet al., 2012).

RNA extraction

Total RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples using the ReliaPrep™ FFPE Total RNA Miniprep System kit, following the manufacturer's protocol (Promega, Madison, WI, USA). The isolated RNA was stored at -80 °C until further use.

Measuring quality and quantity of extracted RNA

RNA was quantified using the Invitrogen Qubit® Fluorometer and the Q32852 Quant-iT RNA Assay Kit (Invitrogen, Carlsbad, CA, USA), which allows for 100 assays of 5–100 ng (250 pg/µL to 100 ng/µL) according to the manufacturer's instructions. The integrity of the samples was then analyzed using the Agilent 2100 Bioanalyzer with the RNA 6000 Pico kit (Agilent, Santa Clara, CA, USA), following the manufacturer's guidelines.

Complementary DNA (cDNA) synthesis

Samples were subjected to reverse transcription to generate the cDNA necessary for real-time polymerase chain reaction (RT-PCR). This process utilized the Superscript® III Reverse Transcriptase kit (Invitrogen), following the manufacturer's protocol.

Table 1 Oligonucleotide primers used for this study

Gene	Initiator Oligonucleotide Pair
ADAMTS-1	F: 5′-TGTAGCCCAGATTCCACCTC-3′ R: 5′-CCCCGCAAACACCACATTTA-3′
ACAN	F: 5′-ATCAATTCCCTGGTGCGGTA-3′ R: 5′-GGGTCTGTGCAGGTGATCT-3′
BCAN	F: 5′-CTGGCTCAAGTGTTCGGC-3′ R: 5′-CGTTCTCTTGCATCGGATC-3′
VCAN	F: 5′-CCCCTGTTGTAGAAAATGCCA-3′ R: 5′-TCCATTTCCTAAGCACCGGA-3′
GAPDH	F: 5′-TCGGAGTCAACGGATTTGG-3′ R: 5′-GATGGCAACAATATCCACTTTACC-3′
β-ACTIN	F: 5′-TAATGTCACGCACGATTTCCC-3′ R: 5′-TCACCGAGCCCGGCT-3′

RT-PCR

Relative quantification was performed using the fluorescent agent SYBR Green for the detection of amplicons. Samples were prepared in duplicate. The oligonucleotides used for the qPCR reactions are listed in Table 1.

The qPCR reaction was performed on the StepOne Plus Real-Time PCR System (Applied Biosystems) using SYBR Green reagent (Applied Biosystems). The reaction mixture included cDNA from the reverse transcription, SYBR Green PCR master mix (2x), forward and reverse primers (18 µM), and 20 µL of autoclaved Milli-Q water. The reaction was initiated with 10 min at 50 °C for enzyme activation, followed by denaturation for 5 min at 95 °C. Subsequently, 45 cycles of 95 °C for 30 s and 60 °C for 1 min were conducted. At the end, a thermal dissociation protocol was performed to verify the specificity of the reaction.

Results were analyzed by StepOneTM Software v2.0. For relative quantification, the following calculation was performed: initially, the cycle threshold (CT) was determined, given by the number of the cycle in which the fluorescence signal reached threshold line, the line in which the emission of fluorescence is above background noise. The CT is invariably in the region corresponding to the exponential phase of amplification, which makes the estimate of quantification of the transcripts in the original sample more accurate. The CT values of the genes of interest were normalized in relation to the CT of the constitutive genes, GAPDH (Gylceraldehyde-3-phosphate dehydrogenase) e β-actin, resulting o CT, which CT gene – CT constitutive. Finally, 2-CT was calculated, which is the value worked as representative of the relative expression for each gene [13].

Statistical analysis

The data were analyzed using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA). The Shapiro-Wilk test was applied to assess the distribution of the samples. Differences in gene expression and immunohistochemical results of ADAMTS-1 and its substrates in odontogenic keratocysts and dental follicles were evaluated using the Student's t-test for parametric distributions and the Mann-Whitney test for non-parametric distributions. A p-value of less than 0.05 was considered statistically significant ($\alpha = 5\%$).

Results

ADAMTS-1, aggrecan, brevican and versican were expressed in all samples analyzed. ADAMTS-1 immunostaining was detected in both OKC and DF, with diffuse staining across the epithelial layers in OKC, predominantly localized in the cytoplasm. Similarly, DF exhibited cytoplasmic ADAMTS-1 expression. The staining area for ADAMTS-1 was comparable between OKC

and DF, though it was more pronounced in OKC's epithelial cells than in the capsule. ADAMTS-1 gene expression was lower in OKC than in DF, though this difference was not statistically significant (Fig. 1).

Aggrecan immunostaining in OKC was also primarily cytoplasmic, concentrated in the basal and parabasal epithelial layers. DF showed weak aggrecan expression, similarly localized in the cytoplasm. The staining area for

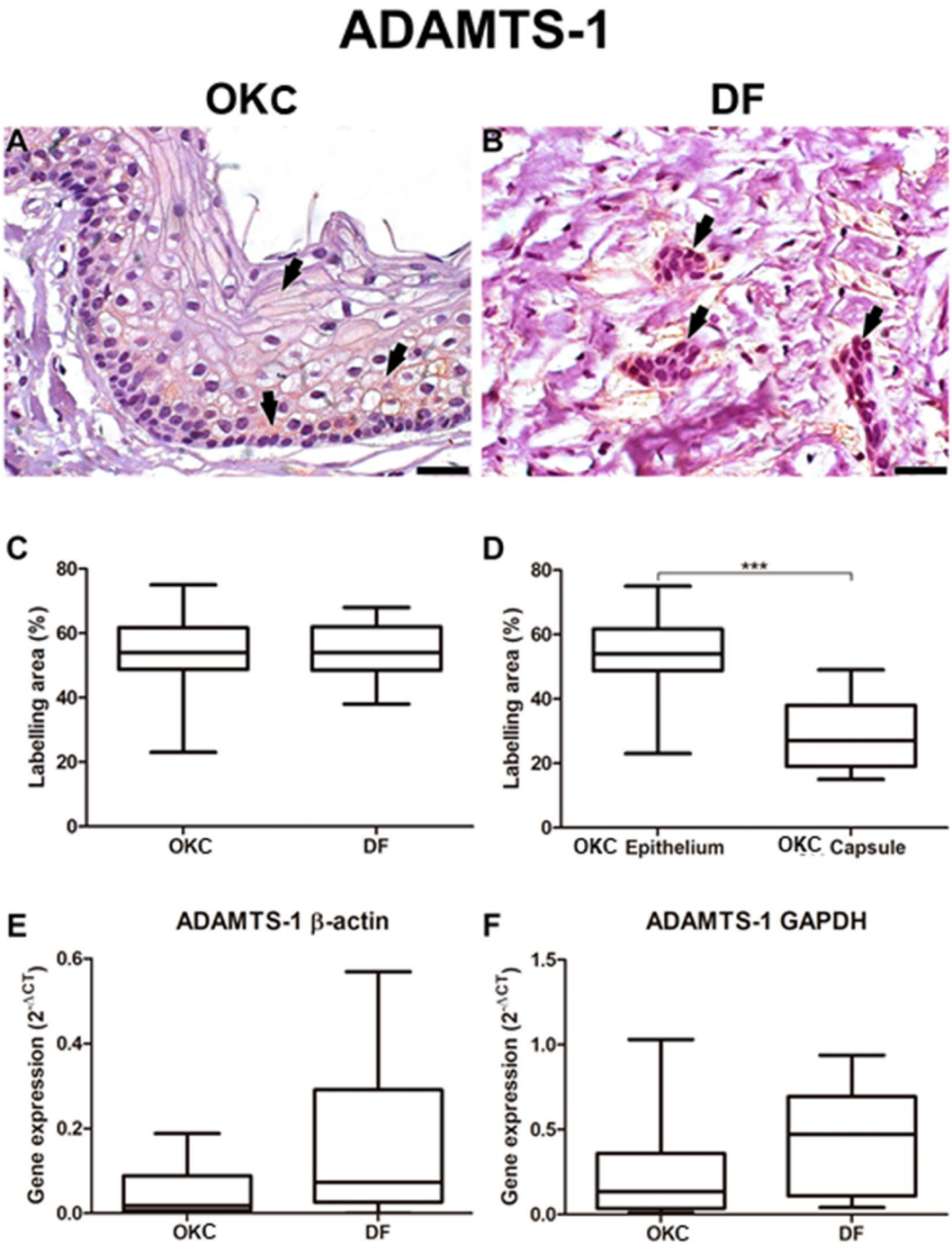

Fig. 1 ADAMTS-1 immunostaining in odontogenic keratocyst (OKC) and dental follicle (DF). ADAMTS-1 staining was diffuse in the epithelial layers of OKC, with a predominantly cytoplasmic localization (**A**, arrows). DF also expressed ADAMTS-1, with predominantly cytoplasmic staining (**B**, arrows). There was no significant difference in the area of ADAMTS-1 staining between OKC and DF (**C**). However, ADAMTS-1 immunostaining in OKC was more pronounced in epithelial cells than in the capsule (**D**). ADAMTS-1 gene expression was reduced in OKC compared with DF, although without a statistically significant difference. RT-PCR analysis was performed using the Pfaffl method to calculate relative mRNA levels normalized by β-actin (**E**) or GAPDH (**F**). Significance: ***$p < 0.001$. Scale bar: 20 µm

aggrecan was larger in OKC epithelial cells compared to DF. Additionally, aggrecan was more prominent in OKC epithelial cells than in the capsule. No statistically significant difference in aggrecan gene expression was found between OKC and DF based on RT-PCR results (Fig. 2).

Brevican immunostaining showed diffuse cytoplasmic expression in OKC's epithelial layers, while DF exhibited similar cytoplasmic staining. The labeling area for brevican was similar in both OKC and DF, though the epithelial cells of OKC displayed stronger staining than the capsule. Brevican gene expression levels between OKC and DF were not significantly different, according to RT-PCR analysis (Fig. 3).

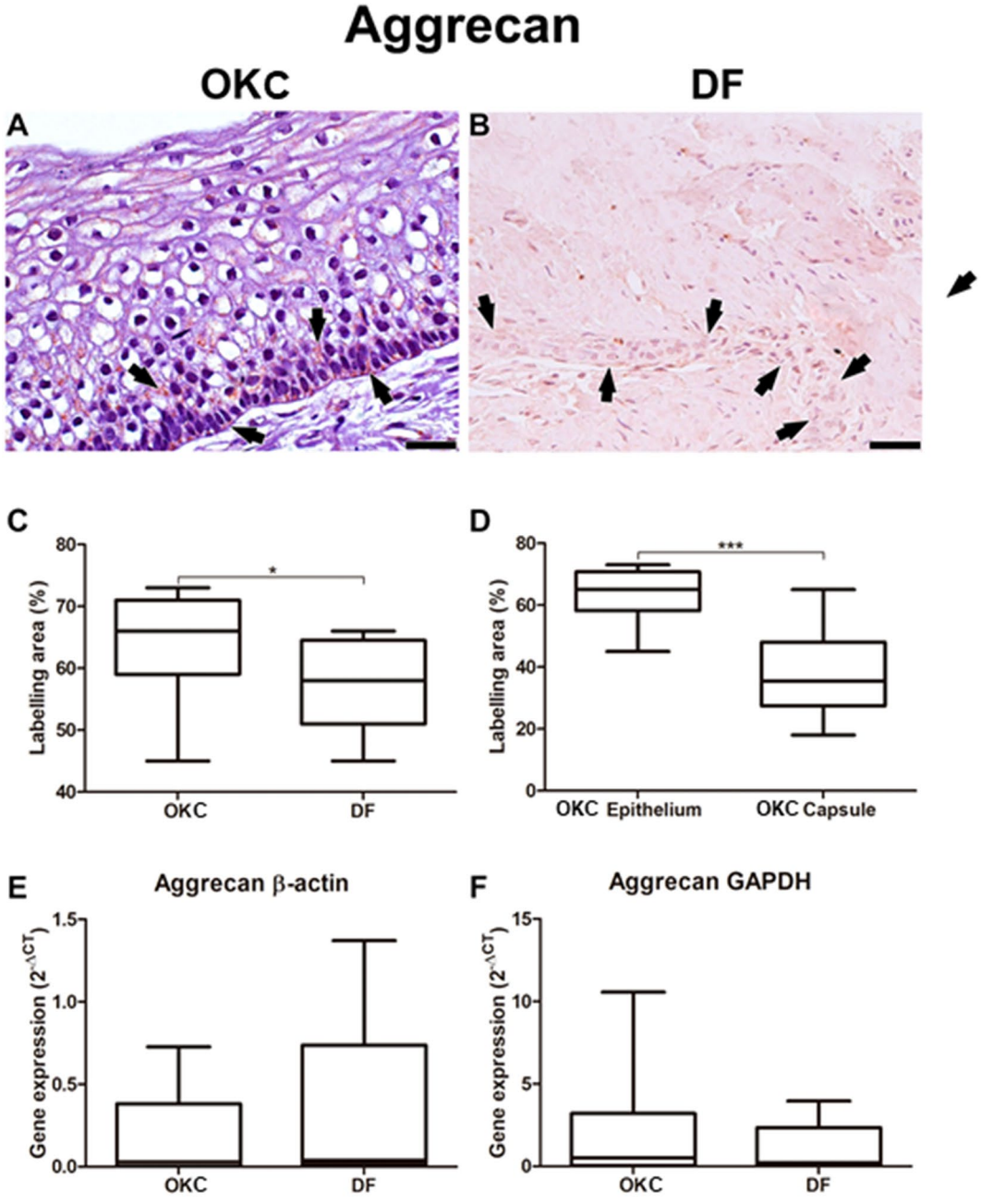

Fig. 2 Aggrecan immunostaining in odontogenic keratocyst (OKC) and dental follicle (DF). Aggrecan staining was predominantly cytoplasmic in the basal and parabasal layers of OKC epithelial cells (**A**, arrows). DF exhibited weak aggrecan labeling, primarily cytoplasmic (**B**, arrows). The labeling area of aggrecan showed higher expression in the epithelial cells of OKC compared to DF (**C**). Moreover, aggrecan immunostaining in OKC was more pronounced in the epithelial cells than in the capsule (**D**). There was no statistically significant difference in aggrecan gene expression between OKC and DF, as determined by RT-PCR analysis using the Pfaffl method to calculate relative mRNA levels normalized by β-actin (**E**) or GAPDH (**F**). Significance: $*p < 0.05$, $***p < 0.001$. Scale bar: 20 µm

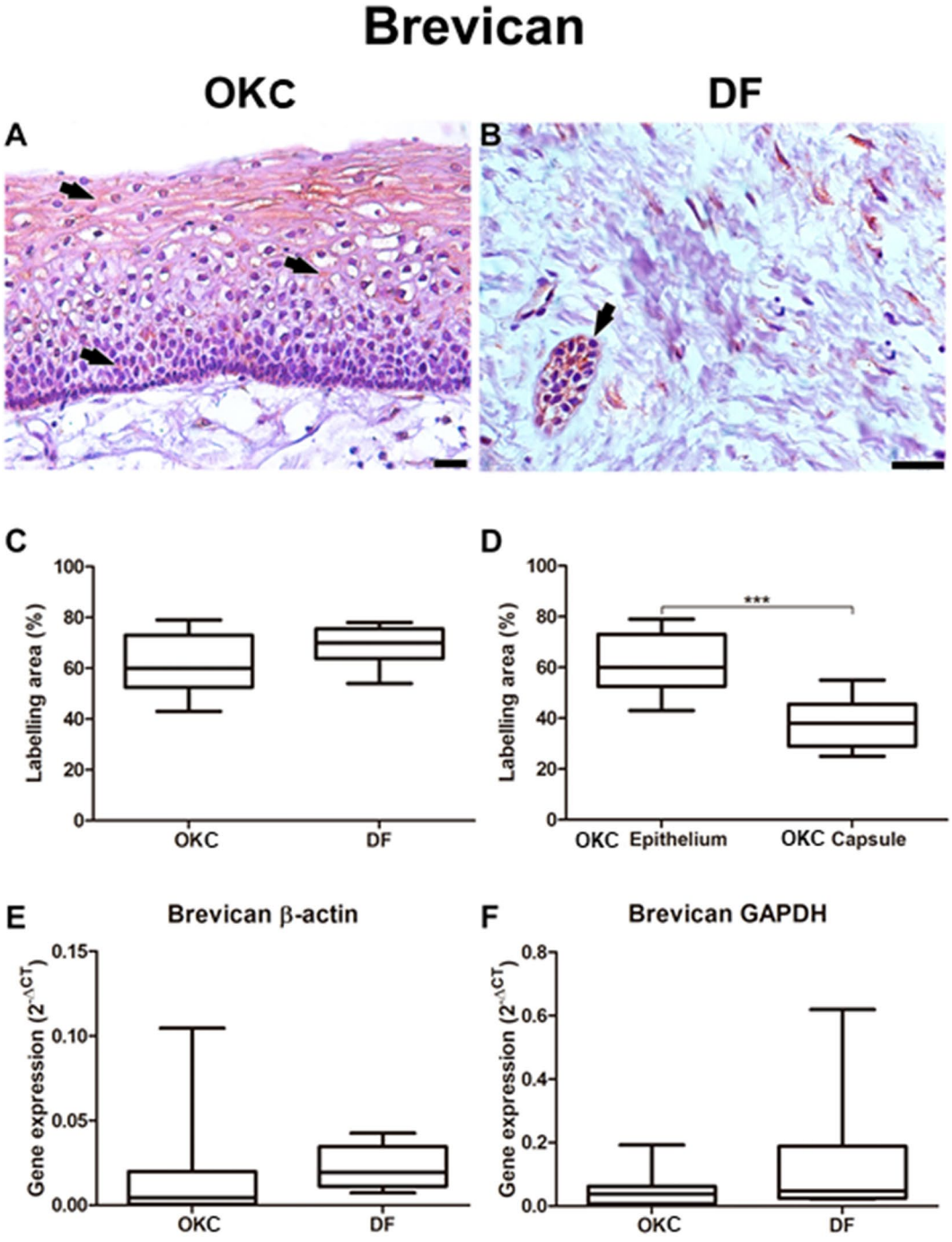

Fig. 3 Brevican immunostaining in odontogenic keratocyst (OKC) and dental follicle (DF). Brevican staining was diffuse in the epithelial layers of OKC, with predominantly cytoplasmic localization (**A**, arrows). DF also expressed brevican, with predominantly cytoplasmic staining (**B**, arrows). There was no significant difference in brevican labeling area between OKC and DF (**C**). However, brevican immunostaining in OKC was more pronounced in the epithelial cells than in the capsule (**D**). There was no statistically significant difference in brevican gene expression between OKC and DF, as determined by RT-PCR analysis using the Pfaffl method to calculate relative mRNA levels normalized by β-actin (**E**) or GAPDH (**F**). Significance: $***p < 0.001$. Scale bars: 20 µm and 50 µm

Versican staining was primarily cytoplasmic in the basal and parabasal layers of OKC epithelial cells. DF showed weaker cytoplasmic versican labeling. The labeling area was greater in OKC's epithelial cells compared to DF, with more prominent staining in the epithelial cells than in the capsule. RT-PCR analysis revealed no statistically significant difference in versican gene expression between OKC and DF (Fig. 4).

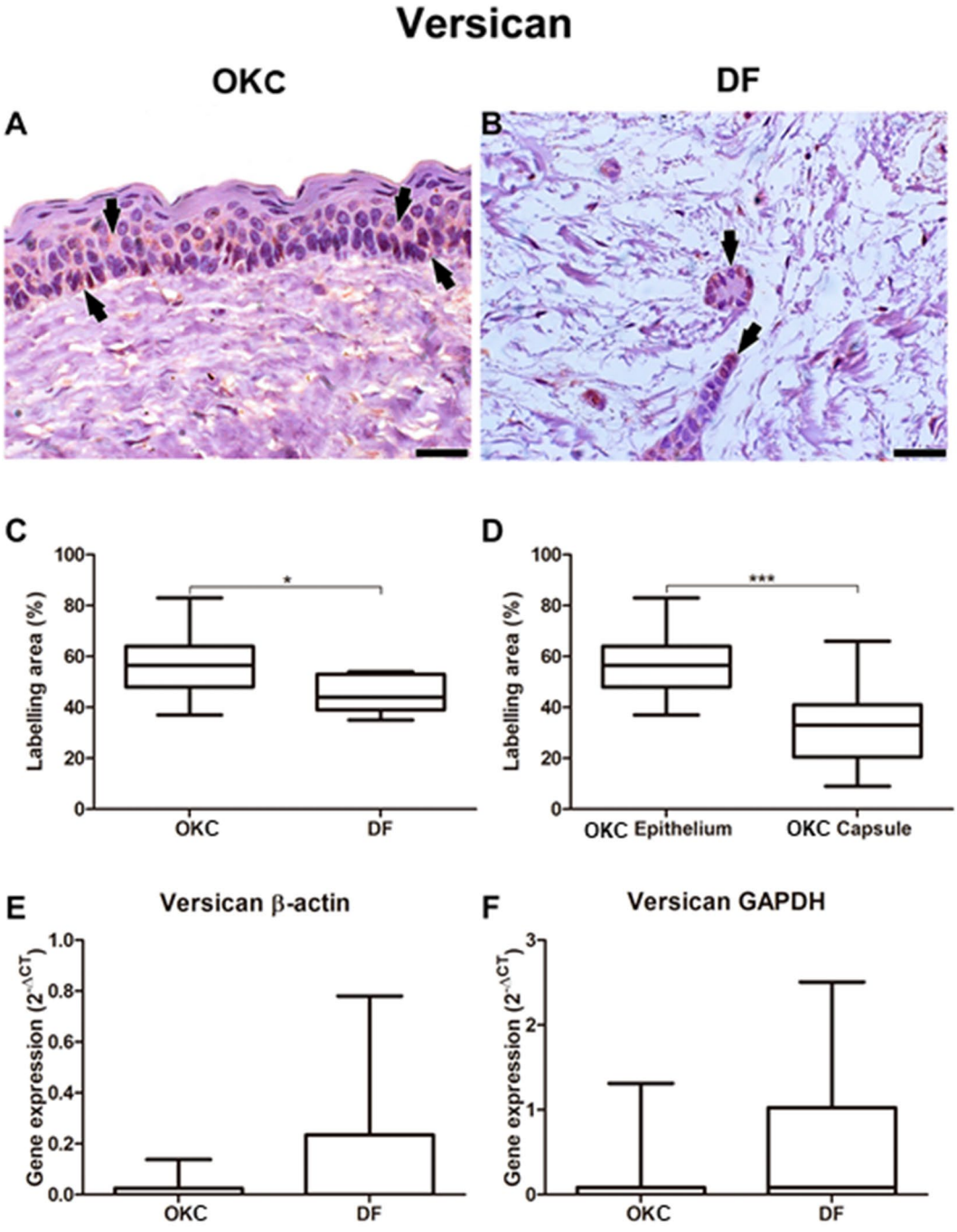

Fig. 4 Versican immunostaining in odontogenic keratocyst (OKC) and dental follicle (DF). Versican staining was predominantly cytoplasmic in the basal and parabasal layers of OKC epithelial cells (**A**, arrows). DF showed weak versican labeling, with predominantly cytoplasmic localization (**B**, arrows). The versican labeling area indicated higher expression in the epithelial cells of OKC compared to DF (**C**). Moreover, versican immunostaining in OKC was more prominent in the epithelial cells than in the capsule (**D**). There was no statistically significant difference in versican gene expression between OKC and DF, as determined by RT-PCR analysis using the Pfaffl method to calculate relative mRNA levels normalized by β-actin (**E**) or GAPDH (**F**). Significance: $*p < 0.05$, $***p < 0.001$. Scale bar: 20 µm

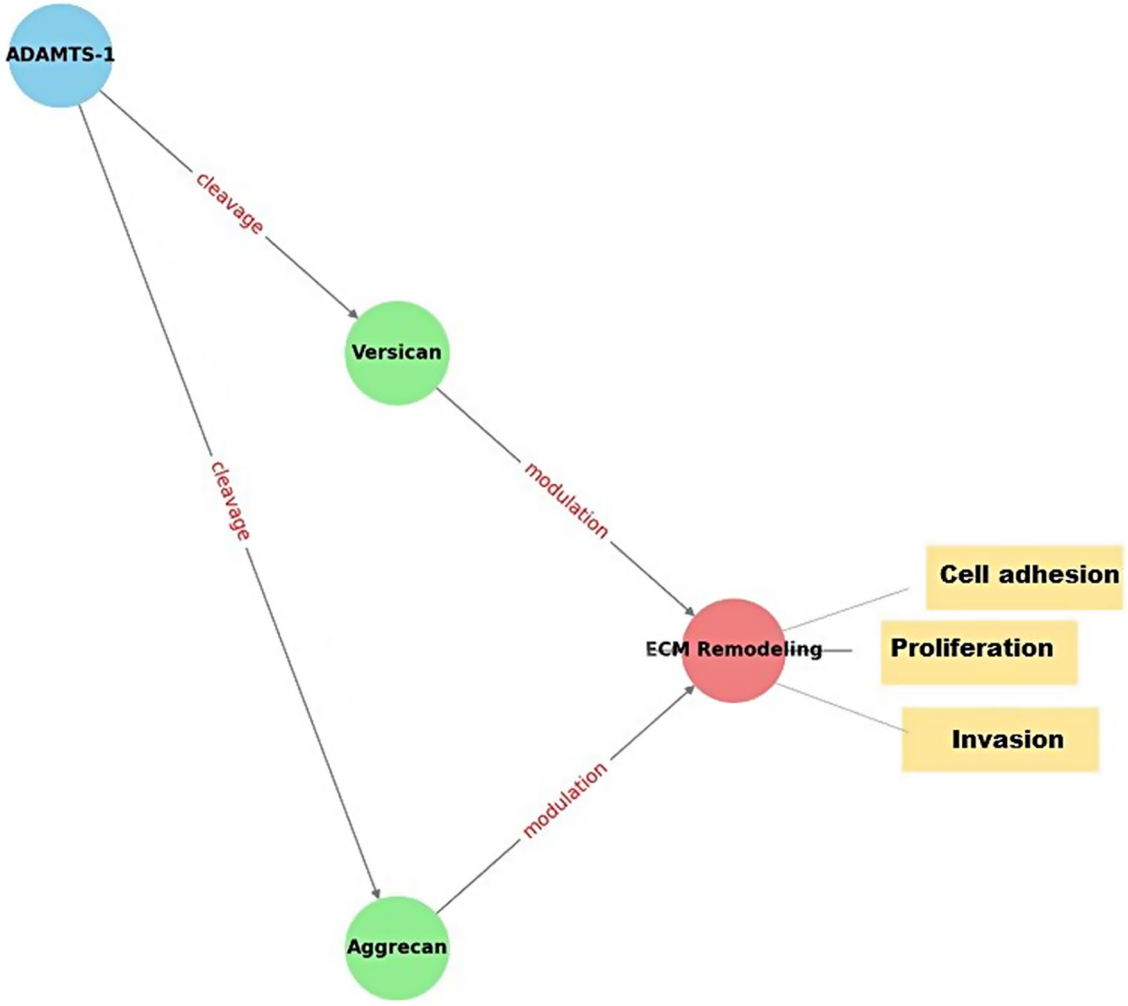

Fig. 5 Signaling pathway between ADAMTS-1, Versican, Aggrecan in the context of keratocysts

When contrasting the two samples under study, the OKC and the dental follicle, it was observed that, statistically, only the aggrecan and versican expression presented significantly higher values in the OKC samples compared to those from the DF ($p<0.05$) (Figs. 2 and 4).

When comparing all samples between epithelium and stroma, it was observed that all proteins showed greater labeling in the epithelium of OKC (Figs. 1, 2, 3 and 4).

Discussion

In our study, all samples of OKC and DF demonstrated immunohistochemical and genomic expression for ADAMTS-1 and its proteoglycan substrates, aggrecan, brevican, and versican. The broad expression of these molecules in both pathological and normal tissues highlights their essential roles in maintaining extracellular matrix integrity. However, the immunohistochemical analysis revealed a notably higher expression of these proteins in the OKC epithelium compared to the stroma, with predominantly granular and cytoplasmic localization. This increased epithelial expression, particularly of aggrecan and brevican, suggests that these proteoglycans may be contributing to the more aggressive and invasive behavior observed in OKC compared to other odontogenic lesions.

Although no statistically significant difference was observed between OKC and DF in the gene expression analysis, the differential immunohistochemical expression, especially in the epithelial component of OKC, could indicate a post-transcriptional regulatory mechanism that enhances protein accumulation at the site of tumor invasion. This points to a potential role for ADAMTS-1 and its substrates in modifying the extracellular matrix to facilitate cellular proliferation, invasion, and migration in OKC, similar to what has been observed in other aggressive neoplasms [14–16]. These findings warrant further investigation into how these proteoglycans contribute to the invasive phenotype of OKC and how they may differ from less aggressive or non-inflammatory odontogenic cysts [17–19].

Although the odontogenic keratocyst is a lesion originating from the odontogenic epithelium, it stands out for its locally invasive behavior and high propensity for recurrence [14].

The growth of odontogenic cysts and tumors may be related to the secretion and regulation of MMPs [15, 16]. Odontogenic keratocysts grow and develop through the bone medullary spaces in an anteroposterior direction, reaching large extents without causing significant bone expansion [14]. This behavior, unusual among cysts, has been attributed to the proteolytic activity of this cyst [3, 17].

In Freitas et al. (2013), ADAMTS-1 is described as a molecule with broad catalytic activity, capable of degrading several key proteoglycans, including aggrecan, brevican, and versican, which are integral components of the extracellular matrix in nearly all eukaryotes. This enzyme has a multifaceted role, interacting with various molecules such as growth factors and cytokines that influence cell proliferation, migration, and angiogenesis. The disintegrin and cysteine-rich domains of ADAMTS-1 are known to regulate cell adhesion and migration, underscoring its multifunctional nature and suggesting its involvement in both proteolytic and non-proteolytic events during tumor progression [12, 18]. ADAMTS-1 has garnered attention as a prognostic marker in various tumors due to its significant role in extracellular matrix remodeling and tumor invasion [18]. Our previous research identified elevated ADAMTS-1 expression in ameloblastoma epithelium, with intense staining in epithelial islands and dispersion in the connective tissue, a pattern similar to that observed in the present study [19]. Sone et al. [20] also demonstrated ADAMTS-1 expression in alveolar bone and periodontal ligament formation, where it plays a critical role in extracellular matrix remodeling. These findings suggest that ADAMTS-1 may contribute to the invasive behavior of OKC by degrading its ECM substrates, thus facilitating cystic groth.

The notable expression of aggrecan in the keratocyst samples suggests its significant role in the aggressive profile of the lesion. Aggrecan, composed of 2,316 amino acids and encoded by the ACAN gene, is the primary proteoglycan found in cartilage tissue [21].

Although aggrecan is generally considered a cartilage-specific proteoglycan, its expression has been documented in various odontogenic and non-odontogenic cystic lesions, as well as in certain tumors, including ameloblastoma and radicular cysts [19, 22–25]. A study by Batista et al. [26] demonstrated the presence of ADAMTS-1 and aggrecan in odontogenic cysts, specifically radicular cysts and periapical granulomas, which supports the involvement of these proteins in cystic pathology. These findings underscore the significance of proteoglycan degradation and extracellular matrix remodeling not only in neoplastic conditions but also in the invasive potential of cystic lesions such as OKC. Furthermore, our results are consistent with observations in non-inflammatory cystic lesions, supporting the hypothesis that ADAMTS-1 and aggrecan play a crucial role in the invasive behavior of OKC by regulating extracellular matrix components.

In the present study, aggrecan expression was higher in the keratocyst compared to the dental follicle group. Furthermore, its expression was more intense in the keratocyst epithelium than in the connective tissue, results similar to those found in our previous study with ameloblastoma [19]. In this regard, Lima et al. [27] demonstrated that during tumorigenesis, aggrecan expression is increased in several cartilage tumors, such as chondroblastoma, chondroma, and chondrosarcoma.

Although some studies indicate that brevican expression is primarily restricted to the central nervous system and absent in extracranial organs [28], our study revealed brevican labeling in both the epithelium and stroma of OKC, with more prominent expression in the epithelium. Predominantly, a cytoplasmic staining pattern was observed in the cystic epithelial cells. Binder et al. [29] highlight the role of brevican as a key activator of EGFR, stimulating the expression of cell adhesion molecules and inducing the secretion of fibronectin, as well as the accumulation of fibronectin microfibrils on the cell surface. Additionally, when cleaved, brevican can bind to EGFR due to the presence of an EGF-like domain, promoting cell proliferation [24]. In this study, the expression of this proteoglycan in odontogenic keratocysts suggests its potential contribution to the growth of this lesion.

In the immunohistochemical analysis of this study, versican immunoexpression was observed in both the epithelium and tumor stroma of OKC, with more intense expression in the epithelium compared to dental follicles (DF). Witt et al. [30] reported versican expression in colon cancer epithelial cells, particularly in the tumor's peripheral neoplastic cells. Similarly, Abiko et al. [31] found strong immunoexpression of versican in the peripheral epithelium of pleomorphic adenoma of the salivary gland. In contrast, Zhao et al. [32] observed intense immunohistochemical expression of versican in all species of odontogenic myxoma. Additionally, Ito et al. [33] reported positive versican expression in the stroma of various odontogenic tumors. These findings highlight the potential role of versican in OKC, suggesting its involvement in cyst progression and in the interaction between cystic cells and the microenvironment.

The nearly equivalent expression of ADAMTS-1 in keratocyst and dental follicle samples underscores the complexity of protein interactions in this context. ADAMTS-1 plays a crucial role in extracellular matrix remodeling, and its consistent levels may suggest specific

regulatory mechanisms in the keratocyst. This regulatory activity could influence proteoglycan degradation and potentially contribute to the invasiveness of the keratocyst. Analyzing epithelial tissue, particularly in OKC samples, reinforces the connection between these molecules and cellular differentiation. In this regard, the increased expression of aggrecan and versican in OKC may be associated with alterations in epithelial development that could contribute to the locally aggressive profile of the keratocyst (Fig. 5).

Given the limitations of the methods employed in this immunohistochemistry and qPCR study, the results should be validated against findings from other studies exploring the biological mechanisms involved. Additionally, future research with larger sample sizes of OKC cases is necessary to confirm these results.

In summary, the interaction of these proteins within the odontogenic keratocyst likely contributes to its aggressive behavior, impacting the regulation of the extracellular matrix and cellular processes associated with this cystic lesion. A deeper understanding of these mechanisms could pave the way for developing targeted therapeutic approaches and identifying prognostic biomarkers to enhance the clinical management of keratocysts.

Conclusion

In the present study, gene expression and immunohistochemical analysis of ADAMTS-1 and its substrates (aggrecan, brevican and versican) in odontogenic keratocyst were demonstrated. Our results suggest that the interaction of these proteins may play a significant role in the biological behavior of this lesion.

Abbreviations

OKC	Odontogenic Keratocyst
KCOT	Keratocystic Odontogenic Tumor
WHO	World Health Organization
ECM	Extracellular Matrix
ADAMTS	A Disintegrin And Metalloproteinase with Thrombospondin motifs
DF	Dental Follicle
BSA	Bovine Serum Albumin
PBS	Phosphate-buffered Saline
FFPE	Formalin Fixation Paraffin Embedding
RNA	Ribonucleic Acid
cDNA	Complementary DNA
RT-PCR	Real-Time Polymerase Chain Reaction
CT	Cycle Threshold
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase
DAB	Diaminobenzidine
MMPs	Matrix Metalloproteinases

Acknowledgements

We gratefully acknowledge Higher Education Personnel Improvement Coordination (CAPES) for their financial support.

Author contributions

ORSN: Conceptualization; Data curation; Formal analysis, Methodology; Writing - original draft; and Writing - review & editing ATLM: Data curation; Formal analysis and Writing - review & editing. HTF: Methodology; Writing - review & editing. AGMF: Writing - review & editing. VMF: Writing - review & editing. MSSK: Writing - review & editing SMAJ: Writing - review & editing JJVP: Conceptualization; Data curation; Writing - review & editing.

Funding

this work was supported in part by the Higher Education Personnel Improvement Coordination (CAPES), Ministry of Education, Brazil (Finance code 001 - Grant number 88887.640723/2021-00).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

this study was approved by the Ethics Committee of the Institute of Oncology Research Center of the Federal University of Pará (n°2.371.646). Consent was obtained from patients for use of their samples.

Consent for publication

not applicable.

Competing interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (version GPT-4) only to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Received: 24 August 2024 / Accepted: 6 November 2024
Published online: 07 January 2025

References

1. Pogrel MA. (2013). The keratocystic odontogenic tumor. Oral and maxillofacial surgery clinics of North America, 25(1), 21–30, v. https://doi.org/10.1016/j.coms.2012.11.003
2. Kaczmarzyk T, Stypułkowska J, Tomaszewska R. Update of the WHO classification of odontogenic and maxillofacial bone tumors. J Stomatology. 2017;70(5):484–506.
3. WHO Classification of Tumors Editorial Board. Head and Neck Tumours. 5th ed. Lyon: IARC; 2024.
4. Stoelinga PJW. Keratocystic odontogenic tumour (KCOT) has again been renamed odontogenic keratocyst (OKC). Int J Oral Maxillofac Surg. 2019;48(3):415–6.
5. Mendes RA, Carvalho JF, van der Waal I. Characterization and management of the keratocystic odontogenic tumor in relation to its histopathological and biological features. Oral Oncol. 2010;46(4):219–25. https://doi.org/10.1016/j.oraloncology.2010.01.012.
6. Ribeiro AL, Nobre RM, Alves-Junior SM, Kataoka MS, Barroso RF, Jaeger RG, Pinheiro JJ. Matrix metalloproteinases, tissue inhibitors of metalloproteinases, and growth factors regulate the aggressiveness and proliferative activity of keratocystic odontogenic tumors. Oral surgery, oral medicine, oral Pathology. Oral Radiol. 2012;114(4):487–96. https://doi.org/10.1016/j.oooo.2012.06.011.
7. Amorim MT, Ferreira JFL, Neto WFF, de Melo KFL, Araújo APS, de Luna FCF, et al. Theoretical study of the origin of odontogenic tumors and correlation of the protein expression of ADAMTS-1 in aggrecan, brevican and versican (ACAN, BCAN AND VCAN). Brazilian Archives Biology Technol. 2020;4(4):2597–605.
8. Kaczmarzyk T, Mojsa I, Stypułkowska J. A systematic review of the recurrence rate for keratocystic odontogenic tumour in relation to treatment modalities. Int J Oral Maxillofac Surg. 2012;41(6):756–67.
9. Gómez Z, Molina-Frechero N, Damián-Matsumura P, Bologna-Molina R. Proteoglycans as potential biomarkers in odontogenic tumors. J Oral Maxillofacial Pathol. 2018;22(1):98–102.
10. Freitas VM, Amaral do, Silva JB, Santos TA, Mangone ES, Pinheiro FR, Jaeger JJV, Nagai RG, M. A., Machado-Santelli GM. Decreased expression of

ADAMTS-1 in human breast tumors stimulates migration and invasion. Mol Cancer. 2013;12:2. https://doi.org/10.1186/1476-4598-12-2.
11. Souza RS, Pinhal MAS. Interactions in physiological processes: the importance of the dynamics between extracellular matrix and proteoglycans. Archives Health Sci. 2011;36(1):48–54.
12. Rocks N, Paulissen G, Calvo FQ, Munauf CM, Gonzalez MLA, Gueders M, Hacha J, Gilles C, Foidart JM, Noel A, Cataldo DD. ADAMTS-1 metalloproteinase promotes tumor development through the induction of a stromal reaction in vivo. J Cancer Res. 2008;68(22):9541–50. https://doi.org/10.1158/0008-5472.CAN-08-1323.
13. Pfaffl MW. (2011). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res, 29(9), e45.
14. Schuch LF, de Arruda JAA, Mosconi C, et al. A Brazilian multicentre study of 2,497 isolated cases of odontogenic keratocysts. Oral Dis. 2020;26(3):711–5. https://doi.org/10.1111/odi.13278.
15. Heocharis AD, Skandalis SS, Tzanakakis GN, Karamanos NK. Proteoglycans in health and disease: novel roles for proteoglycans in malignancy and their pharmacological targeting. FEBS J. 2010;277(19):3904–23.
16. Turner SL, Blair-Zajdel ME, Bunning RA. ADAMs and ADAMTSs in cancer. Br J Biomed Sci. 2009;66(2):117–28. https://doi.org/10.1080/09674845.2009.11730257.
17. de Vicente JC, Torre-Iturraspe A, Gutiérrez AM, Lequerica-Fernández P. Immunohistochemical comparative study of the odontogenic keratocysts and other odontogenic lesions. Medicina Oral. Patologia Oral y Cir Bucal. 2010;15(5):e709–15. https://doi.org/10.4317/medoral.15.e709.
18. Porter S, Clark IM, Kevorkian L, Edwards DR. The ADAMTS metalloproteinase. Biochem J. 2005;386(pt1):15–27.
19. de Souza Neto OR, Thais Fuzii H, Vieira Da Silva S, Morais Freitas V, Viana Pinheiro J J. Gene expression and immunohistochemistry analysis of ADAMTS-1 and Versican in Ameloblastoma. Int J Dent. 2022;2022:5235376. https://doi.org/10.1155/2022/5235376.
20. Sone S, Nakamura N, Maruya Y, Takahashi I, Mizoguchi I, Mayanagi H, Sasano Y. Expression of versican and ADAMTS during rat tooth eruption. J Mol Histol. 2005;36(4):281–88.
21. Kiani C, Chen L, Wu YJJ, Yee A, Yang BB. Structure and function of aggrecan. Cell Res. 2002;12(1):19–32.
22. Jia XL, Li SY, Dang SS, Cheng YA, Zhang X, Wang WJ, et al. Increased expression of chondroitin sulphate proteoglycans in rat hepatocellular carcinoma tissues. World J Gastroenterol. 2012;18(30):3962–76.
23. Theocharis AD, Gialeti C, Bouris P, Giannopoulou E, Skandalis SS, Aletras AJ, et al. Cell-matrix interactions: focus in proteoglycan-proteinase interplay and pharmacological targeting in cancer. FEBS J. 2014;281(22):5023–42.
24. Kusafuka K, Muramatsu K, Kasami M, Kuriki K, Hirobe K, Hayashi I, et al. Cartilaginous features in matrix-producing carcinoma of breast: four cases report with histochemical and immunohistochemical analysis of matrix molecules. Mod Pathol. 2008;21(10):1282–92.
25. Suhovskih AV, Mostovich LA, Kunin IS, Boboev MM, Nepomnyashchikh GI, Aidagulova SV, Grigorjeva EV. (2013). Proteoglycans expression in normal human prostate tissue and prostate cancer. ISRN Oncol, 10680136.
26. Batista NMG, de Moraes ATL, Balbinot KM, de Souza Neto OR, da Silva Brandão JM, da Silva Kataoka MS. Melo Alves Júnior S, de Jesus Viana Pinheiro J. Immunohistochemical analysis of ADAMTS-1, versican and pEGFR expressions in periapical granuloma and radicular cyst. BMC Oral Health. 2021;21(1):102. https://doi.org/10.1186/s12903-021-01462-x.
27. Lima MA, Santos D, Turri L, Nonogaki JA, Buim S, Lima M, J. F., et al. Prognostic value of ADAMTS proteases and their substrates in epithelial ovarian cancer. Pathobiology. 2016;83(6):316–26.
28. Lu R, Wu C, Guo L, Liu Y, Mo W, Wang H, et al. The role of brevican in glioma: promoting tumor cell motility in vitro and in vivo. BMC Cancer. 2012;12:607.
29. Binder MJ, McCoombe S, Williams ED, McCulloch DR, Ward AC. (2016). The extracellular matrix in cancer progression: role of hyalectan proteoglycans and ADAMTS enzymes. Cancer letters. 2017; 28;(385):55-64. doi: 10.1016/j.canlet.2016.11.001.
30. Wit M, Belt EJ, Delis-van Diemen PM, Carvalho B, Coupe VM, Stockmann HB, et al. Lumican and versican are associated with good outcome in stage II and III colon cancer. Ann Surg Oncol. 2013;Supl 3:S348–59.
31. Abiko Y, Nishimura M, Mitamura J, Rahemtulla F, Sasano Y, Kuwabara M, et al. An immunohistochemical localization of biglycan, decorin and large chondroitin sulphate proteoglycan in pleomorphic adenoma. Acta Histochem Cytochem. 1999;32(6):459–63.
32. Zhao M, Takata T, Kudo Y, Sato S, Ogawa I, Wakida T, Nikai H. Biosynthesis of glycosaminoglycans and aggrecan by tumor cells in salivary pleomorphic adenoma: ultrastructural evidence. J Oral Pathol Med. 1999;28(10):442–50.
33. Ito Y, Abiko Y, Tanaka Y, Kaku T. Immunohistochemical localization of large chondroitin sulfate proteoglycan in odontogenic tumour. Med Electron Microscopy. 2002;35:173–77.

Publisher's note