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ORIGINAL ARTICLE

Damage-regulated autophagy modulator 1 in oral inflammation and infection

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Abstract

Objectives

Damage-regulated autophagy modulator (DRAM) 1 is a p53 target gene with possible involvement in oral inflammation and infection. This study sought to examine the presence and regulation of DRAM1 in periodontal diseases.

Material and methods

In vitro, human periodontal ligament fibroblasts were exposed to interleukin (IL)-1β and *Fusobacterium nucleatum* for up to 2 days. The DRAM1 synthesis and its regulation were analyzed by real-time PCR, immunocytochemistry, and ELISA. Expressions of other autophagy-associated genes were also studied by real-time PCR. In vivo, synthesis of DRAM1 in gingival biopsies from rats and patients with and without periodontal disease was examined by real-time PCR and immunohistochemistry. For statistics, ANOVA and post-hoc tests were applied ($p < 0.05$).

Results

In vitro, DRAM1 was significantly upregulated by IL-1β and *F. nucleatum* over 2 days and a wide range of concentrations. Additionally, increased DRAM1 protein levels in response to both stimulants were observed. Autophagy-associated genes ATG3, BAK1, HDAC6, and IRGM were also upregulated under inflammatory or infectious conditions. In vivo, the DRAM1 gene expression was significantly enhanced in rat gingival biopsies with induced periodontitis as compared to control. Significantly increased DRAM1 levels were also detected in human gingival biopsies from sites of periodontitis as compared to healthy sites.

Conclusion

Our data provide novel evidence that DRAM1 is increased under inflammatory and infectious conditions in periodontal cells and tissues, suggesting a pivotal role of DRAM1 in oral inflammation and infection.

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Clinical relevance
DRAM1 might be a promising target in future diagnostic and treatment strategies for periodontitis.

Keywords Damage-regulated autophagy modulator · Autophagy · *Fusobacterium nucleatum* · Interleukin-1β · Periodontal ligament · Periodontitis

Introduction

Periodontitis is a highly prevalent chronic inflammatory disease, which is characterized by the progressive destruction of the tooth-supporting tissues, i.e., gingiva, periodontal ligament (PDL), root cementum, and alveolar bone [1]. Initiation and progression of the disease is characterized by a complex interplay of various microorganisms, such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Treponema denticola*, with the host immune and inflammatory response, resulting in the release of inflammatory mediators, such as interleukin (IL)-1β, matrix-degrading proteases, and osteoclast-activating factors [2, 3].

Autophagy (Greek, meaning "self-devouring") is an evolutionary conserved process of the cell that under normal conditions enables degradation of unnecessary or dysfunctional cytosolic proteins and organelles. This process promotes survival during cellular stress. However, when the level of stress exceeds a critical duration or intensity threshold, autophagy can also be engaged in promotion of cell death [4–7]. Recent evidence emphasizes the importance of autophagy in the maintenance of the inflammatory balance as well as its pivotal role in the innate and adaptive immune system. Deficiency in autophagy may have pathological consequences and lead to multiple diseases, such as diabetes mellitus and inflammatory bowel disease [8, 9]. One of the first direct links between periodontitis and autophagy has been reported by Bullon and colleagues, who found increased expression levels of autophagy-related genes in peripheral blood mononuclear cells from patients with periodontitis, suggesting a critical role of autophagy in periodontal diseases [10]. However, the significance of autophagy in PDL fibroblasts exposed to an inflammatory and microbial environment has yet to be studied.

A large number of autophagy-related genes (ATGs) and additional regulators, such as damage-regulated autophagy modulator 1 (DRAM1), ATG3, BCL2 antagonist killer 1 (BAK1), B-cell lymphoma 2 (BCL2), chemokine, CXC motif, receptor 4 (CXCR4), death-associated protein kinase 1 (DAPK1), histone deacetylase 6 (HDAC6), immunity-related GTPase family M (IRGM), and transmembrane protein 74 (TMEM74) are involved in autophagy [11–13]. DRAM1 is a lysosomal membrane protein regulated as part of the p53 signal transduction pathway and promotes the induction of autophagy in a p53-dependent manner [12, 14, 15]. Interestingly, DRAM1 is not only required for the induction of autophagy but also for programmed cell death. DRAM1 controls apoptosis by inhibition of BAX degradation [16]. Therefore, DRAM1 represents a crucial element in the control system of p53-mediated apoptosis and autophagy [17].

A better understanding of DRAM1 and its regulation in periodontal cells and tissues will help to further unravel the inflammatory and destructive processes in periodontitis and may even lead to new treatment strategies. Therefore, this study sought to examine the presence and regulation of DRAM1 in periodontal diseases. It was hypothesized that inflammatory and microbial challenges would have regulatory effects on DRAM1 in periodontal cells and tissues.

Material and methods

Isolation and characterization of PDL fibroblasts

After the approval of the Ethics Committee of the University of Bonn (#117/15) and the informed consent by the patients or their parents, PDL fibroblasts were obtained from healthy subjects (mean age 14.1 years, min/max 11/19 years; gender four males/three females). Teeth that had to be extracted for space management in orthodontic treatment, but were otherwise caries free and periodontally healthy, were used to harvest the cells, as previously described [18, 19]. In brief, PDL fibroblasts were taken from the medial part of the tooth root, grown in Dulbecco's minimal essential medium (DMEM, Invitrogen, Karlsruhe, Germany), supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2, and phenotyped according to Basdra and Komposch [19]. For the experiments, cells were used between passages 3 and 5 at 80% confluence after reduction of FBS concentration to 1% 1 day prior to stimulation.

Cell treatment

The pro-inflammatory cytokine IL-1β (PromoKine, Heidelberg, Germany; 0.1–10 ng/ml) was used to simulate an inflammatory environment in vitro. Microbial conditions were mimicked in vitro by the periodontopathogen *F. nucleatum* (ATCC 25586; optical density at wave length of 660 nm: 0.0125–0.050). Inactivation of the bacteria was performed by suspension in PBS (OD_{660} nm = 1, equivalent

to 1.2×10^9 bacterial cells/ml) and ultrasonication (160 W two times for 15 min). Afterwards it was checked by subcultivation on Schaedler agar plates (Oxoid, Basingstoke, UK) in anaerobic conditions. To achieve comparability of results, we used the same physiological concentrations of IL-1β and *F. nucleatum* as in our previous studies [20–25]. PDL fibroblasts were exposed to IL-1β and *F. nucleatum* for up to 2 days. Untreated PDL fibroblasts served as control.

Analysis of gene expression

Gene expressions of DRAM1 and other autophagy-associated molecules (ATG3, BAK1, BCL2, CXCR4, DAPK1, HDAC6, IRGM, and TMEM74) found to be present in PDL fibroblasts by an array-based approach in a previous study [26] were analyzed by real-time polymerase chain reaction (PCR). PDL cells were exposed to IL-1β or *F. nucleatum* for 24 h or left untreated. After RNA extraction with a commercially available kit (RNeasy Protect Minikit, Qiagen, Hilden, Germany) and transcription to cDNA with an iScript Select cDNA Synthesis Kit (Bio-Rad Laboratories, Munich, Germany), 1 μl of cDNA was used in a 25-μl reaction mixture containing 2.5 μl of respective QuantiTect Primer Assay (Qiagen), 12.5 μl of QuantiTect SYBR Green Master Mix (Qiagen) and 9 μl of nuclease free water, for real-time PCR in an iCycler iQ™ real-time PCR detection system (Bio-Rad Laboratories). The protocol of a heating phase at 95 °C for 5 min to activate the enzyme, 40 cycles including a denaturation step at 95 °C for 10 s, and a combined annealing/extension step at 60 °C for 30 s per cycle was followed. Melting point analysis was carried out after each run. GAPDH served as a housekeeping gene for normalization. Further analysis of gene expressions in rat and human gingival tissues were performed likewise.

Immunocytochemistry

Immunocytochemistry was performed on PDL fibroblasts cultured on glass coverslips (Carl Roth, Karlsruhe, Germany) and treated with IL-1β (1 ng/ml) or *F. nucleatum* (OD 0.025) for 1 day. Untreated cells served as control. First, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich, Munich, Germany) at pH 7.4 and room temperature, and then treated with 0.1% Triton X-100 (Sigma-Aldrich) for permeabilization. Each incubation step was followed by two washing steps with PBS (Sigma-Aldrich). Serum block (Dako, Hamburg, Germany) was applied for 20 min to prevent background staining. The cells were then incubated with a rabbit polyclonal anti-DRAM1 antibody (Abcam, Cambridge, MA, USA; 1:500) at 4 °C overnight. As a secondary antibody goat anti-rabbit IgG-HRP (Dako) was applied for 45 min. Antibody binding was visualized by DAB chromogen (Thermo Fisher Scientific, Waltham, MA, USA) staining for 10 min at room temperature. Cells were counterstained with Mayer's Haematoxylin (Merck, Darmstadt, Germany) for 1 min and coverslipped with DePex mounting medium (Serva Electrophoresis, Heidelberg, Germany). Standardized images were captured with an Axioskop 2 microscope (Carl Zeiss, Jena, Germany) and an AxioCam MRc camera (Carl Zeiss), and analyzed with AxioVision 4.7 software (Carl Zeiss).

ELISA

The protein level of DRAM1 in cell-free supernatants of cells exposed to IL-1β or *F. nucleatum* and of control fibroblasts at 1 and 2 day was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (LSBio, Seattle, WA, USA) according to the manufacturer's protocol. Final absorbance was determined with a microplate reader (PowerWave x, BioTek Instruments, Winooski, VT, USA) at 450 nm. Cells were collected, counted with an automatic cell counter (Moelab, Hilden, Germany) and used for data normalization.

Experimental periodontitis model

To study DRAM1 gene expression in gingival biopsies during the development of periodontal disease, a rat model was applied [27]. After approval by the Ethical Committee on Animal Experimentation (protocol number 23/2012) from the School of Dentistry at Araraquara, São Paulo State University—UNESP, animal experiments were carried out following the recommendations of the ARRIVE guidelines. For the experiment, male adult Holtzman rats (average weight 300 g) were kept under controlled conditions (22–25 °C, 12-h light/dark cycle, standard laboratory diet, and water ad libitum) in plastic cages in the animal facilities of the School of Dentistry at Araraquara. Twenty-four rats were used and assigned randomly to two experimental groups: control (sham-operated) and ligature-induced periodontal disease. Prior to intervention, both groups of animals were anesthetized with intramuscular injections of 10% ketamine chlorhydrate (0.08 ml/100 g body weight) and 2% xylazine chlorhydrate (0.04 ml/100 g body weight). In the test group, periodontitis was induced through cotton ligatures located at the cervical area of both maxillary first molars and fixed with a mesial knot. The ligatures remained in place for 6, 8, and 12 days. At the end of each of these periods, four animals from each group were sacrificed by an anesthetic overdose and the maxilla was collected for analysis. The gingival tissues around the maxillary first molars were dissected for the extraction of total RNA and real-time PCR (see above).

Human biopsies

After the approval of the Ethics Committee of the University of Bonn (#043/11) and patients' informed consent, gingival tissue biopsies were collected from 14 systemically healthy non-smokers in the Dept. of Oral Surgery of the University of Bonn during wisdom tooth extractions or extractions of teeth for orthodontic or periodontal reasons [23]. Seven subjects were periodontally healthy (mean age 22.1 years, min–max 18–26 years; gender two males/five females) and seven were diagnosed with periodontitis (mean age 58.4 years, min–max 29–81 years; gender five males/two females). Prior to the extractions, the periodontal status of the teeth was assessed according to the periodontal diagnoses and classification system of the American Academy of Periodontology (AAP) [28]. GI = 0 (no clinical inflammation), PD ≤ 3 mm and neither clinical nor radiographic attachment/bone loss was classified as healthy. Sites with GI > 1 and PD ≥ 5 mm and clinical as well as radiographic attachment/bone loss ≥ 3 mm were diagnosed with periodontitis.

RNA extraction and real-time PCR were performed as described above. Furthermore, tissues were fixed with 4% phosphate-buffered paraformaldehyde (Sigma-Aldrich) for 2 days and embedded in paraffin (McCormick Scientific, Richmond, IL, USA). Tissue samples were cut in 3 μm thick serial slices and left to dry on glass slides (Carl Roth). Selected tissue sections were de-waxed, rehydrated, and rinsed in TBS (components: TRIS, MP Biomedicals, Illkirch, France, and NaCl, Merck) for 10 min prior to immunoreaction. A solution of 0.3% methanol (AppliChem, Darmstadt, Germany)/H_2O_2 (Merck) was applied for 10 min to block endogenous peroxidase. Subsequently, another blocking step with serum block (Dako) for 20 min was carried out, before the tissue sections were incubated with rabbit polyclonal anti-DRAM1 antibody (Santa Cruz Biotechnology, Dallas, TX, USA, 1: 200) in a humid chamber at 4 °C overnight. Antibody binding was revealed by incubation with goat anti-rabbit IgG-HRP secondary antibody (Dako) at room temperature for 30 min and subsequent application of DAB chromogen (Thermo Fisher Scientific) for 10 min. Slides were rinsed, counterstained with Mayer's hematoxylin (Merck), and coverslipped for analysis. Standardized imaging was carried out as described above.

Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics software (Version 22, IBM SPSS, Chicago, IL, USA). Mean values and standard errors of the mean (SEM) were calculated. Experiments were carried out in triplicate and repeated at least twice. For statistical comparisons between the groups, the *t*-test, ANOVA followed by the post-hoc Dunnett's and Tukey tests, and the Mann-Whitney-U test were applied. Differences between groups were considered significant at $p < 0.05$.

Results

Regulation of autophagy-associated molecules by IL-1β and F. nucleatum

In the presence of IL-1β and *F. nucleatum*, several autophagy-associated genes were significantly upregulated in PDL fibroblasts. Notably, DRAM1 was increased most strongly by 2.8-fold and 5.2-fold through IL-1β and *F. nucleatum*, respectively (Fig. 1a). Like DRAM1, other autophagy-associated genes were also regulated by both stimulants at 1 day. IL-1β and *F. nucleatum* caused a significant upregulation of BAK1 gene expression by 1.7-fold and 1.9-fold, respectively (Fig. 1b). Furthermore, IL-1β increased significantly the mRNA levels of ATG3 and HDAC6, whereas stimulation of cells with *F. nucleatum* resulted in an elevated IGRM gene expression (Fig. 1b). Although the gene expressions of BCL2, CXCR4, DAPK1, and TMEM74 were also altered under inflammatory and/or infectious conditions, these changes were not significant (Fig. 1b). DRAM1 was chosen for further analyses including time and dose responses. Our experiments revealed that the *F. nucleatum*-induced stimulation was significant over 2 days and a wide range of concentrations (Fig. 1c). Similar gene expression changes were also observed after stimulation with different doses of IL-1β at both time points (data not shown).

Regulation of DRAM1 protein levels in PDL fibroblasts by IL-1β and F. nucleatum

The enhanced transcriptional levels of DRAM1 under inflammatory or infectious conditions were paralleled at protein level. Exposure of cells to IL-1β or *F. nucleatum* resulted in elevated DRAM1 protein at 1 day, as analyzed by immunocytochemistry (Fig. 2a). DRAM1 protein was equally distributed throughout the cell cytoplasm. Interestingly, incubation of PDL fibroblasts with IL-1β or *F. nucleatum* for 1 day did not lead to higher DRAM1 protein levels in the cell supernatants, as analyzed by ELISA (Fig. 2b). However, after 2 days, the supernatants of the stimulated cells also showed increased DRAM1 protein levels, thereby supporting the findings from the PCR and immunocytochemistry analyses (Fig. 2b).

DRAM1 expression in rat and human gingival biopsies

To study the DRAM1 expression in inflammation and infection in a more complex environment and over time,

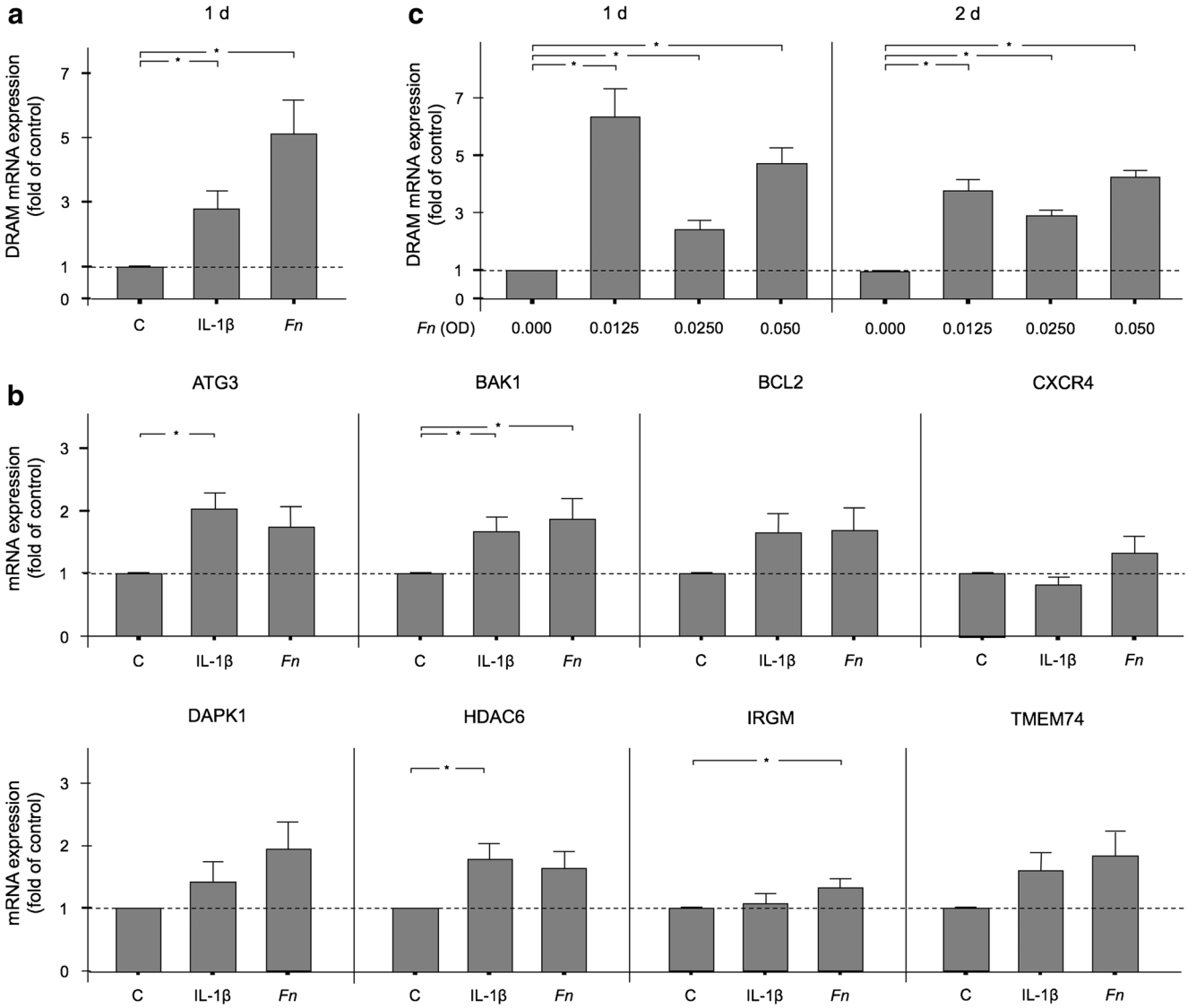

Fig. 1 Effects of IL-1β (1 ng/ml) or *F. nucleatum* (ATCC 25586, OD 0.025) on the gene expressions of DRAM1 (**a**) and ATG3, BAK1, BCL2, CXCR4, DAPK1, HDAC6, IRGM and TMEM74 (**b**) in PDL fibroblasts at 1 day. DRAM1 gene expression in PDL fibroblasts exposed to various doses of *F. nucleatum* (ATCC 25586, OD 0.0125-0.050) for up to 2 days (**c**). Untreated cells served as control. Mean ± SEM ($n = 9$); * significant ($p < 0.05$) difference between groups

an established rat model with ligature-induced periodontitis was used. As analyzed by real-time PCR, the DRAM1 gene expression in gingival biopsies was significantly upregulated in rats with periodontitis as compared to gingival tissue samples from control animals at 6, 8, and 12 days (Fig. 3a). Furthermore, the DRAM1 levels were examined in human gingiva by RT-PCR and immunohistochemistry. As expected, biopsies from sites of periodontitis showed significantly higher DRAM1 gene expression levels and more pronounced protein levels than biopsies from periodontally healthy sites (Fig. 3b, c). As revealed by immunostaining, DRAM1 protein was mainly observed in the *stratum basale* of the epithelium and in macrophages of the inflammatory infiltrate (Fig. 3c).

Discussion

The present study provides novel evidence that DRAM1 is significantly increased under inflammatory and microbial conditions in periodontal cells and tissues, suggesting that DRAM1 may play a critical role in the aetiopathogenesis of periodontal diseases. Since DRAM1 is an important regulator of autophagy and cell death, our findings also emphasize the possible involvement of autophagic processes in the initiation and progression of periodontitis.

The DRAM1 gene encodes a protein localized in the lysosomal membrane [12, 14]. DRAM1 influences autophagic flux by promoting lysosomal activation, fusion of lysosomes with autophagosomes and degradation of their contents [15].

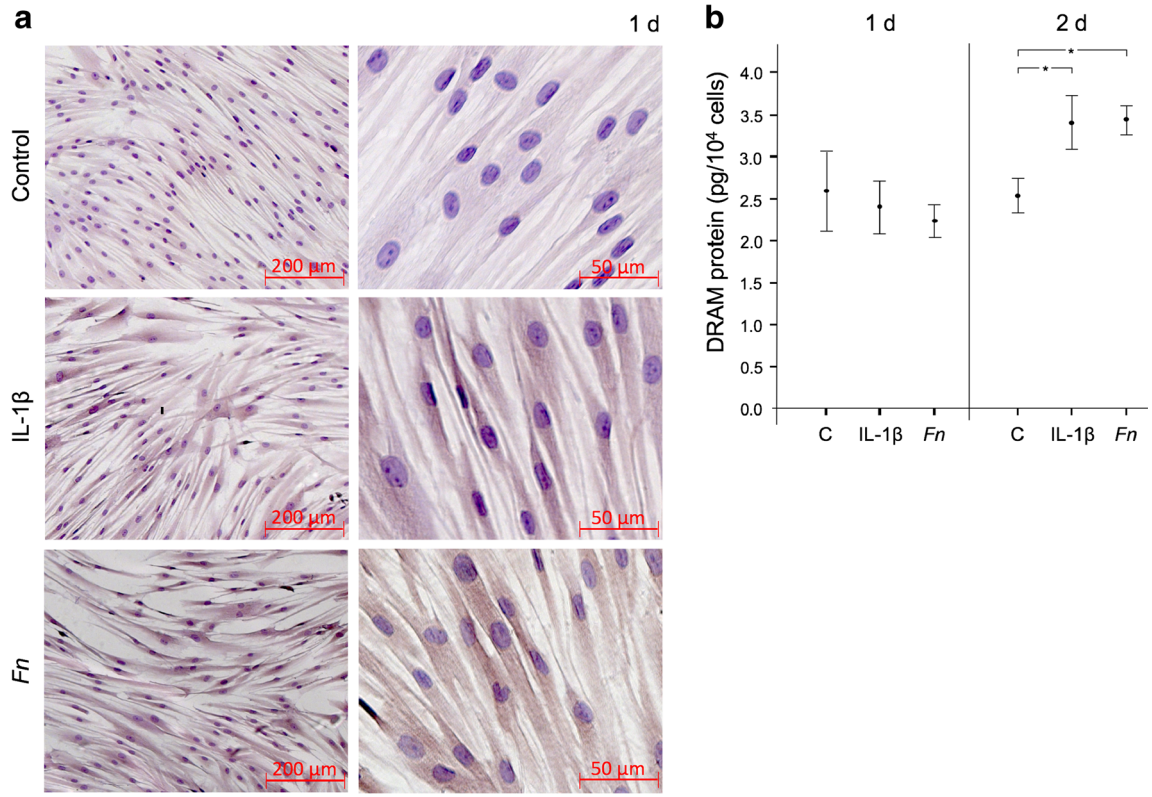

Fig. 2 DRAM1 protein levels in PDL fibroblasts stimulated with IL-1β (1 ng/ml) or *F. nucleatum* (ATCC 25586, OD 0.025) for 1 day, as analyzed by immunocytochemistry (**a**). Untreated cells served as control. Images of one representative donor are shown. Effect of IL-1β (1 ng/ml) or *F. nucleatum* (ATCC 25586, OD: 0.025) on DRAM1 protein levels in supernatants of PDL fibroblasts at 1 and 2 days (**b**). Untreated cells served as control. Mean ± SEM ($n = 12$); * significant ($p < 0.05$) difference between groups

Moreover, DRAM1 represents a p53 target gene, mediating programmed cell death in a BAX-dependent manner. By protein interaction of DRAM1 and BAX, the degradation of this pro-apoptotic protein is inhibited. BAX induces the release of cytochrome c from mitochondria, thus activating caspase-3 and initiating apoptotic cell death [16]. Interestingly, our previous study on periodontal cells demonstrated increased p53 levels and reduced cell viability after stimulation of the cells with lipopolysaccharide from the periodontopathogen *P. gingivalis* [29], thus further emphasizing the crucial crosstalk between pro-inflammatory stimuli, p53 signaling, and manifestation of periodontitis.

In another study, we found that inflammatory and microbial insults led to an altered expression of several autophagy-associated molecules [26]. Since DRAM1 was strongly upregulated under these conditions, we focused especially on this p53 target gene in the present study. DRAM1 gene expression increased significantly in response to IL-1β and *F. nucleatum* over a period of 2 days. The transcriptional results were also found at protein level, as analyzed by ELISA and immunocytochemistry, confirming and expanding our gene expression findings.

By using an established in vivo model, we could follow up the DRAM1 gene expression over time during the initiation and development of periodontitis. Moreover, we could study the DRAM1 expression under a more complex and realistic environment, as compared to the in vitro setting. In the rat model, the ligature-induced plaque accumulation led to significant periodontal inflammation and bone loss, as previously described [27]. Interestingly, our analyses revealed that the induction of periodontitis resulted in a significant upregulation of DRAM1 mRNA levels over 12 days. Although the observation period was rather short, the findings from this animal study concurred with our in vitro results. It would be very interesting to correlate the DRAM1 regulation with that of other cytokines in this experimental periodontitis model. In order to extrapolate our findings obtained by experimental periodontitis in rats to humans, gingival biopsies from periodontally healthy subjects and periodontitis patients were examined for DRAM1 [23]. Our analyses revealed that both mRNA and protein levels of DRAM1 were higher in gingival biopsies from periodontitis patients than in gingiva samples from healthy individuals. These data further confirmed and expanded our in vitro and rat in vivo findings by identifying periodontal cells capable of producing DRAM1. Our studies demonstrated that PDL fibroblasts but also epithelial cells and macrophages can contribute to DRAM1 levels in periodontium. Further studies should examine if DRAM1 is also

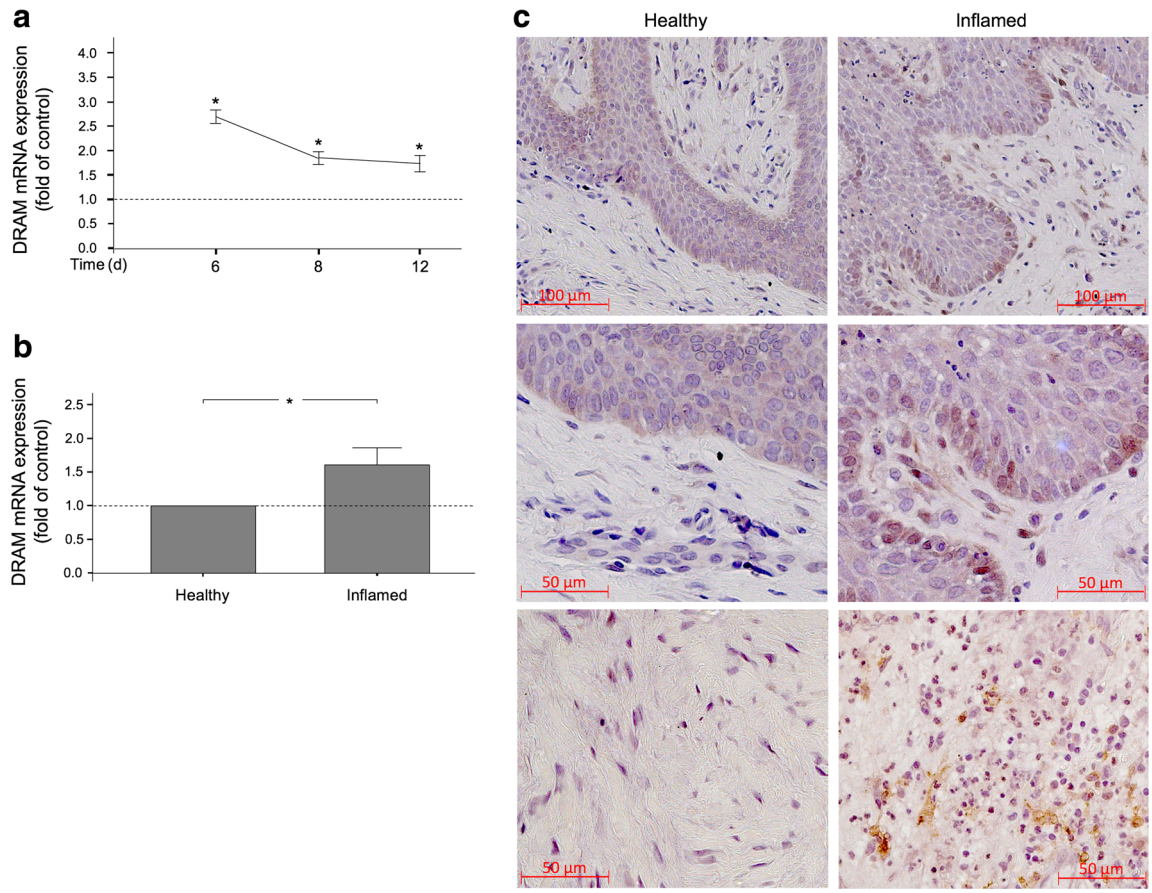

Fig. 3 DRAM1 gene expression in gingival biopsies of rats with and without experimental ligature-induced periodontitis at 6, 8, and 12 days (**a**). Mean ± SEM ($n = 4$ animals/group and time point); * significantly ($p < 0.05$) different from control. DRAM1 gene expression in gingival biopsies of periodontally healthy and periodontitis subjects (**b**). Mean ± SEM ($n = 7$ patients/group); * significant ($p < 0.05$) difference between groups. DRAM1 protein levels in gingival biopsies of periodontally healthy and periodontitis subjects, as analyzed by immunocytochemistry (**c**). Representative images of one donor per group are shown

produced by other resident or infiltrating cells of periodontium. Additionally, future studies should also focus on the regulation of the other autophagy-related molecules identified in the present investigation, in rat and human tissues. Periodontitis is a complex inflammatory disease induced by periodontopathogens located in the subgingival biofilm [30–33]. In our in vitro experiments, *F. nucleatum* (ATCC 25586) was chosen to simulate a microbial environment, because this gram-negative anaerobic microorganism is one of the first gram-negative anaerobic microorganisms involved in the establishment of the dental biofilm and significantly associated with periodontitis. Moreover, it is considered a bridge bacterium between early and late colonizers [34–36]. In our experiments, *F. nucleatum* was used as a suspension comprising multiple bacterial fragments. Lysates of dead bacteria were used, which ensured a strong stimulatory effect on cells. In subgingival biofilm, dead bacteria are also present and provide an important source of lipopolysaccharides at sites of periodontitis. Further investigations should also involve live cells in similar experiments. Although lipopolysaccharide was a main constituent of this bacterial suspension, other cellular components might also have contributed to the stimulatory effects observed in our experiments. Further studies should clarify the role of these components (e.g., bacterial DNA) in the regulation of DRAM1. In the present investigation, the reference strain of *F. nucleatum* was used. If clinical isolates obtained from periodontally healthy and diseased individuals have the same effects should be examined in further research. Moreover, periodontitis is caused by a complex biofilm with a special role of keystone pathogens [37]. Therefore, further studies should investigate if other microorganisms of the subgingival biofilm can also regulate DRAM1 synthesis in periodontal cells, as observed for *F. nucleatum* in the present study.

Notably, like DRAM1, other autophagy-associated genes were also regulated in the present study. IL-1β and *F. nucleatum* caused a significant upregulation of BAK1 gene expression. Moreover, IL-1β caused an increase in the ATG3 and HDAC6 gene expressions, whereas stimulation of cells with *F. nucleatum* resulted in an increased IGRM gene expression. These findings further underline the critical role of autophagy in periodontal diseases.

Peridontitis is characterized by increased levels of inflammatory molecules in gingival crevicular fluid and gingival tissues [38–40]. IL-1β is a pro-inflammatory cytokine, which stimulates recruitment of immunoinflammatory cells, production and release of proteases, and development and activation of osteoclasts, thereby promoting periodontal inflammation and destruction [41–45]. Therefore, IL-1β was used to mimic inflammatory conditions in vitro, as shown in our previous studies [20, 22, 23, 25]. Nevertheless, in this chronic inflammatory disease, a great number of inflammatory mediators synergize to elicit soft tissue destruction and bone loss. Further studies should therefore clarify whether other inflammatory mediators cause similar responses by periodontal cells.

The role of DRAM1 and autophagy in pathogenesis of periodontitis has yet to be unraveled. Recently, it was shown that DRAM1 is involved in the autophagic host defense against bacteria. As shown by van der Vaart and colleagues, mycobacterial infection of human macrophages and zebrafish embryos induced DRAM1 expression [46]. Therefore, DRAM1 might also be a part of the human host defense against microbial challenge in periodontium. Interestingly Bullon et al. have recently shown that autophagy-related genes are upregulated in mononuclear cells isolated from peripheral blood of patients with periodontitis [10]. Furthermore, An et al. reported higher levels of LC3, Beclin-1, ATG7, and ATG12 in human PDL stem cells after stimulation with TNF-α, and a higher autophagosome production in PDL tissues harvested from patients with periodontitis [47]. These studies imply that autophagy and autophagy-related molecules are enhanced by periodontal infection, which is in line with our results.

In conclusion, our study provides original evidence that DRAM1, an important regulator of autophagy as well as programmed cell death, is significantly upregulated under inflammatory and microbial conditions in periodontal cells and tissues. These findings imply a pivotal role of DRAM1 in the aetiopathogenesis of periodontal diseases. The specific actions of DRAM1 on autophagic processes and apoptosis in periodontal tissues have to be explored in further studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Approval of the Ethics Committee of the University of Bonn was obtained (#117/15 #043/11) and of the Ethical Committee on Animal Experimentation (protocol number: 23/2012) from the School of Dentistry at Araraquara. Animal experiments were carried out following the recommendations of the ARRIVE guidelines.

Informed consent All donors of the PDL cells or their parents gave written informed consent.

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