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Transient receptor potential ankyrin 1 (TRPA1) is functionally expressed in primary human osteoarthritic chondrocytes

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Abstract

Background: Transient receptor potential ankyrin 1 (TRPA1) is a membrane-associated cation channel, widely expressed in neuronal cells and involved in nociception and neurogenic inflammation. We showed recently that TRPA1 mediates cartilage degradation and joint pain in the MIA-model of osteoarthritis (OA) suggesting a hitherto unknown role for TRPA1 in OA. Therefore, we aimed to investigate whether TRPA1 is expressed and functional in human OA chondrocytes.

Methods: Expression of TRPA1 in primary human OA chondrocytes was assessed by qRT-PCR and Western blot. The functionality of the TRPA1 channel was assessed by Ca^{2+} -influx measurements. Production of MMP-1, MMP-3, MMP-13, IL-6, and PGE_2 subsequent to TRPA1 activation was measured by immunoassay.

Results: We show here for the first time that TRPA1 is expressed in primary human OA chondrocytes and its expression is increased following stimulation with inflammatory factors IL-1 β , IL-17, LPS, and resistin. Further, the TRPA1 channel was found to be functional, as stimulation with the TRPA1 agonist AITC caused an increase in Ca^{2+} influx, which was attenuated by the TRPA1 antagonist HC-030031. Genetic depletion and pharmacological inhibition of TRPA1 downregulated the production of MMP-1, MMP-3, MMP-13, IL-6, and PGE_2 in osteoarthritic chondrocytes and murine cartilage, respectively.

Conclusions: The TRPA1 cation channel was found to be functionally expressed in primary human OA chondrocytes, which is an original finding. The presence and inflammatory and catabolic effects of TRPA1 in human OA chondrocytes propose a highly intriguing role for TRPA1 as a pathogenic factor and drug target in OA.

Keywords: Osteoarthritis, Chondrocyte, TRPA1, Inflammation, Matrix metalloproteinase

Background

Transient receptor potential ankyrin 1 (TRPA1) is a membrane-associated cation channel which mediates pain and hyperalgesia [1, 2] and functions as a chemosensor of noxious compounds [3–5]. TRPA1 was first discovered in 1999 [6] and has since then been found to be widely expressed in afferent sensory neurons, especially in A δ and C fibers of nociceptors [7, 8]. In addition to pain, TRPA1 also has a role in mediating neurogenic inflammation [9, 10]. More recently, TRPA1

has been found to be expressed also in some nonneuronal cells such as keratinocytes [11] and synoviocytes [12] but the functional roles of nonneuronal expression remain to be studied.

TRPA1 is activated by numerous exogenous pungent compounds such as allyl isothiocyanate (AITC) from mustard oil [5], acrolein from exhaust fumes and tobacco smoke [9], and allicin from garlic [3]. Interestingly, TRPA1 is also activated and sensitized by agents formed endogenously in inflammatory reactions, such as nitric oxide [13], hydrogen peroxide [14] and nitro-oleic acid [15]. The activation of TRPA1 causes an influx of cation ions, particularly Ca^{2+} , into the activated cells [16] and this elevation of intracellular Ca^{2+} has been

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shown to trigger an action potential in neuronal cells [16, 17]. Interestingly, among the many regulatory effects of the alterations of intracellular Ca^{2+} concentration, its increase has also been shown to affect the gene expression of inflammatory mediators [18–20].

Recent evidence suggests TRPA1 to have a role in inflammation through exogenous activation by TRPA1 agonists and also through endogenous mechanisms. TRPA1 has been shown to mediate carrageenan-induced inflammatory edema [21], tumor necrosis factor (TNF)-triggered hyperalgesia [22], airway hyperreactivity and inflammation [23, 24], and to relate to acute gouty arthritis [25, 26]. Very recently we found that TRPA1 has a role in mediating acute inflammation, cartilage destruction, and joint pain in monosodium iodoacetate (MIA)-induced inflammation and osteoarthritis in the mouse [27].

Osteoarthritis (OA) is the most common cause of musculoskeletal disability and pain worldwide and its prevalence is constantly increasing as the population ages. OA is a degenerative disease of the joints, which is characterized by inflammation and hypoxia within the joint, leading to cartilage degradation, joint deformity, disability, and pain [28, 29]. OA-related cartilage degradation is caused by a growing imbalance between the production of catabolic, anabolic, and inflammatory mediators within the joint driven by the increased expression of matrix-degrading metalloproteinases and proinflammatory mediators such as interleukin (IL)-6 and prostaglandin E_2 (PGE_2) [28].

TRPA1 has not previously been investigated in chondrocytes. However, factors involved in hypoxia and inflammation, such as hydrogen peroxide (H_2O_2), nitric oxide (NO), and IL-6 have been shown to upregulate the expression or activation of TRPA1 in some other cells [12–14]. Furthermore, the activation of TRPA1 has been reported to enhance the production of inflammatory factors [12, 21, 26, 30]. Since there is a hypoxic and inflammatory state in OA joints [28, 31], and TRPA1 has been shown to be involved in the mediation of acute inflammation and cartilage degradation in MIA-induced osteoarthritis [27], we hypothesized that TRPA1 is expressed in the chondrocytes in osteoarthritic joints, where its activation could play a vital part in the inflammation and pathogenesis of OA. In the present study, we tested that hypothesis by measuring the expression and function of TRPA1 in primary human OA chondrocytes.

Methods

Cell culture

Primary chondrocyte cultures were carried out as previously described [32]. Leftover pieces of OA cartilage from knee joint replacement surgery were used under full patient consent. The patients in this study fulfilled

the American College of Rheumatology classification criteria for OA [33] and the study was approved by the Ethics Committee of Tampere University Hospital, Tampere, Finland (reference number R09116), and carried out in accordance with the Declaration of Helsinki. The procedures to isolate and culture the primary chondrocytes are described in the supplementary data (Additional file 1). During experiments the cells were treated with IL-1 β (R&D Systems Europe Ltd, Abingdon, UK), IL-17 (R&D Systems Europe Ltd.), lipopolysaccharide (LPS) (Millipore Sigma, St. Louis, MO, USA), resistin (BioVision Inc., Milpitas, CA, USA), the TRPA1 antagonist HC-030031 (Millipore Sigma) or with combinations of these compounds as indicated.

Immortalized human T/C28a2 chondrocytes [34] were cultured as described in the supplementary data (Additional file 1). During the experiments T/C28a2 chondrocytes were treated with IL-1 β (R&D Systems Europe Ltd), IL-17 (R&D Systems Europe Ltd.), LPS (Millipore Sigma), HC-030031 (Millipore Sigma) or with combinations of these compounds as indicated.

HEK 293 human embryonic kidney cells (American Type Culture Collection, Manassas, VA, USA) were cultured as described in the supplementary data (Additional file 1). The cells were transfected using 0.42 mg/cm² of human TRPA1 plasmid DNA (pCMV6-XL4 by Origene, Rockville, MD, USA) with lipofectamine 2000 (Invitrogen, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

Animals

Wild-type (WT) and TRPA1 knockout (KO) male B6;129P-Trpa1(tm1Kyk)/J mice (Charles River Laboratories, Sulzfeld, Germany) aged 19–22 days were used in mouse cartilage culture experiments. Mice were housed under standard conditions (12–12 h light–dark cycle, 22 \pm 1 °C) with food and water provided ad libitum. Animal experiments were carried out in accordance with the legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU) and the experiments were approved by The National Animal Experiment Board (reference number UTA 845/712-86). Animals were sacrificed by carbon monoxide followed by cranial dislocation.

Mouse cartilage culture

After mice were euthanized, full-thickness articular cartilage from femoral heads were removed and cultured as described in the supplementary data (Additional file 1). The cartilage pieces were exposed to IL-1 β (R&D Systems Europe Ltd.) or its vehicle for 42 h and thereafter culture media were collected and matrix metalloproteinase (MMP)-3, IL-6, and PGE_2 concentrations were measured by immunoassay.

Western blot measurements

After the cell culture experiments, total protein was extracted, and TRPA1 was immunoprecipitated and analyzed with Western blot as described in the supplementary data (Additional file 1). TRPA1 antibody NB110-40763 (Novus Biologicals, LCC, Littleton, CO, USA) was used as the primary antibody and goat anti-rabbit HRP-conjugate (sc-2004, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) as the secondary antibody in the Western blot analysis.

Immunoassay

Concentrations of IL-6, MMP-1, MMP-3, MMP-13 and PGE₂ in medium samples were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (PGE₂: Cayman Chemical Co., Ann Arbor, MI, USA; human IL-6: eBioscience Inc. San Diego, CA, USA; MMP-1, MMP-3, MMP-13 and mouse IL-6: R&D Systems Europe Ltd).

RNA extraction and quantitative RT-PCR

At the indicated time points, total RNA was extracted and analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR) for the expression of TRPA1 mRNA as described in the supplementary data (Additional file 1).

Ca²⁺-influx measurements

TRPA1-mediated Ca²⁺ influx was measured in HEK293 cells [35] transfected with human TRPA1 plasmid, in human T/C28a2 chondrocytes, and in primary human OA chondrocytes as described previously [36]. Briefly, the cells were loaded with 4 μM fluo-3-acetoxymethyl ester (Fluo-3-AM, Millipore Sigma) and 0.08 % Pluronic F-127[®] (Millipore Sigma in Hanks' balanced salt solution (HBSS, Lonza, Verviers, Belgium) containing 1 mg/ml of bovine serum albumin, 2.5 mM probenecid and 25 mM HEPES pH 7.2 (all from Millipore Sigma) for 30 min at room temperature. The intracellular-free Ca²⁺ levels were assessed by Victor3 1420 multilabel counter (Perkin Elmer, Waltham, MA, USA) at excitation/emission wavelengths of 485/535 nm. In the experiments, the cells were first preincubated with the TRPA1 antagonist HC-030031 (100 μM, Millipore Sigma) or the vehicle for 30 min at +37 °C. Thereafter, the TRPA1 agonist allyl isothiocyanate (AITC, 50 μM, Millipore Sigma) was added and the measurements were continued for 30 s after which a robust Ca²⁺ influx was induced by application of the control ionophore compound ionomycin (1 μM, Millipore Sigma).

Statistical analysis

Data were analyzed using Graph-Pad InStat version 3.00 software (GraphPad Software, San Diego, CA, USA).

The results are presented as mean + standard error of the mean (SEM) unless otherwise indicated. Unpaired *t* test, paired *t* test, one-way analysis of variance (ANOVA) or repeated-measures ANOVA, followed by Dunnett's test were used in the statistical analysis. Differences were considered significant at *p* < 0.05, *p* < 0.01, and *p* < 0.001.

Results

TRPA1 is expressed in primary human OA chondrocytes and in immortalized human T/C28a2 chondrocyte cell line

Primary human OA chondrocytes and immortalized human T/C28a2 chondrocyte cell line expressed TRPA1. The expression was measured by quantitative RT-PCR on isolated total mRNA using a specific TaqMan assay. The proinflammatory cytokine IL-1β was found to increase TRPA1 expression in a time-dependent manner: in primary chondrocytes the expression of TRPA1 increased up to 48 hours and declined thereafter (Fig. 1a), whereas in the human T/C28a2 chondrocytes the expression maximum was at 6 hours (Fig. 1b). In addition, TRPA1 expression was also enhanced by inflammatory factors IL-17, LPS, and resistin (Fig. 2).

To verify the translation of TRPA1 mRNA into protein, we extracted total protein from primary human OA chondrocytes and human T/C28a2 chondrocytes and performed Western blot analysis. HEK293 cells transiently transfected with TRPA1 plasmid were used as positive control and the protein was detected with a specific human TRPA1 antibody. Remarkably, both cell types were found to express TRPA1 protein as seen in Fig. 3.

Human chondrocytes express a functional TRPA1 channel

To confirm that TRPA1 mRNA and the subsequent protein expressed by human chondrocytes produces a functional channel, Ca²⁺-influx measurements were carried out. Primary human chondrocytes and T/C28a2 chondrocytes were cultured with IL-1β, which was found to stimulate TRPA1 expression, or with its vehicle for 24 h, and thereafter TRPA1 was activated with the TRPA1 agonist AITC. IL-1β stimulation resulted in an increased responsiveness to AITC as seen as an enhanced Ca²⁺ influx, and the selective TRPA1 antagonist HC-030031 was shown to prevent this effect (Fig. 4).

MMP, IL-6 and PGE₂ production is downregulated by genetic depletion and pharmacological inhibition of TRPA1

After finding that functional TRPA1 was indeed expressed in chondrocytes, we aimed to further examine the possible arthritogenic role of the TRPA1 channel. We investigated the effect of genetic depletion of TRPA1 on the production of OA-related factors MMP-3, IL-6, and PGE₂ by using articular cartilage samples from TRPA1-deficient (knockout, KO) and corresponding wild-type (WT) mice. IL-1β treatment increased MMP-

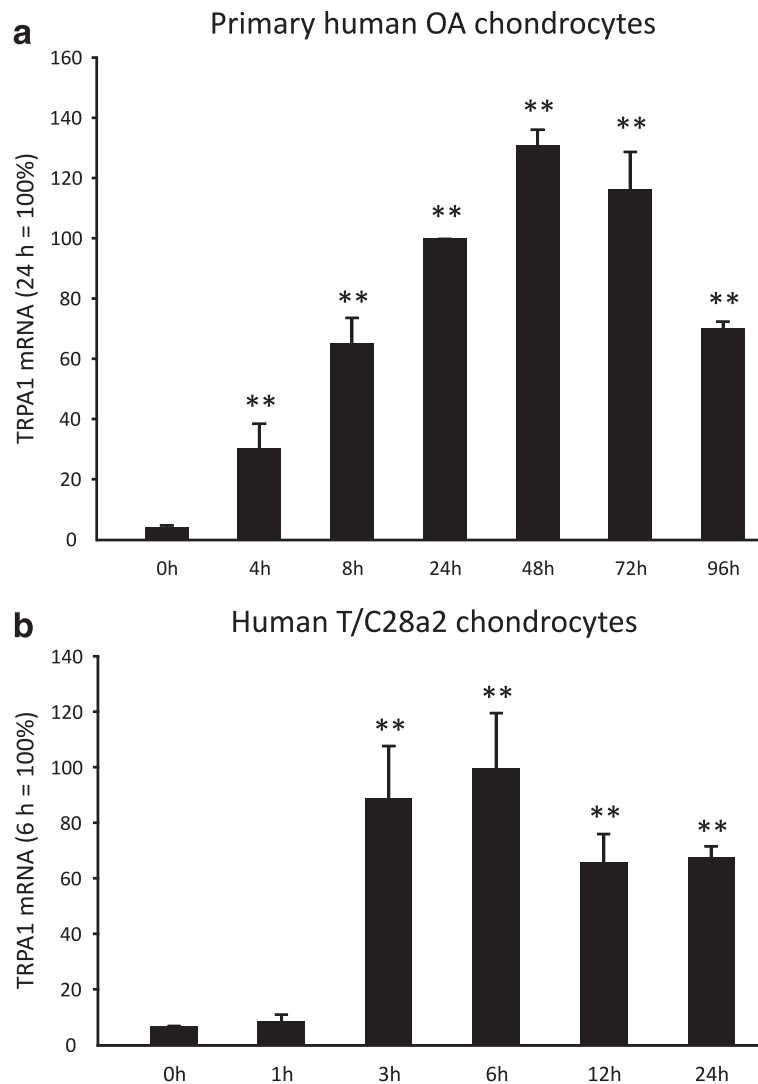


Fig. 1 Primary human OA chondrocytes (**a**) and human T/C28a2 chondrocyte cell line (**b**) express TRPA1 mRNA and its expression is enhanced by IL-1 in a time-dependent manner. Cultures of primary human OA chondrocytes (**a**) and human T/C28a2 chondrocytes (**b**) were stimulated with IL-1 β (100 pg/ml) for 0–96 h and 0–24 h, respectively, and thereafter total RNA was extracted. TRPA1 mRNA levels were measured by qRT-PCR, and the results were normalized against GAPDH mRNA. The mRNA levels are expressed as arbitrary units with the levels measured at 24 h (**a**; primary OA chondrocytes) or 6 h (**b**; T/C28a2 chondrocytes) set as 100%; and the values at the other time points are related to those values. Primary chondrocyte samples were obtained from three to five different donors and the experiments were carried out in duplicate. Human T/C28a2 chondrocyte experiments were carried out in quadruplicate. Results are expressed as mean + SEM. One-way ANOVA followed by Dunnett's post-test was used in the statistical analysis; ** indicates $p < 0.01$ compared to the control (0 h) sample. OA osteoarthritis, TRPA1 transient receptor potential ankyrin 1

3, IL-6, and PGE₂ production in cartilage as expected. Remarkably, this response was significantly attenuated in the cartilage from the TRPA1 KO mice as compared to the corresponding WT mice (Fig. 5). Further, we treated primary human chondrocytes with IL-1 β alone and together with the selective TRPA1 antagonist HC-030031 for 24 h. Interestingly, the selective TRPA1 antagonist HC-030031 downregulated IL-1 β -enhanced MMP-1, MMP-3, MMP-13, IL-6, and PGE₂ production by 25–45 % (Fig. 6), suggesting that TRPA1 plays a role in the

upregulation of these catabolic and inflammatory factors in OA cartilage.

Discussion

The findings of the present study suggest a hitherto unknown role for TRPA1 in the pathogenesis of OA. We have shown for the first time the expression of the TRPA1 channel in primary human OA chondrocytes and in the human T/C28a2 chondrocyte cell line. We showed the expression of TRPA1 mRNA and protein by

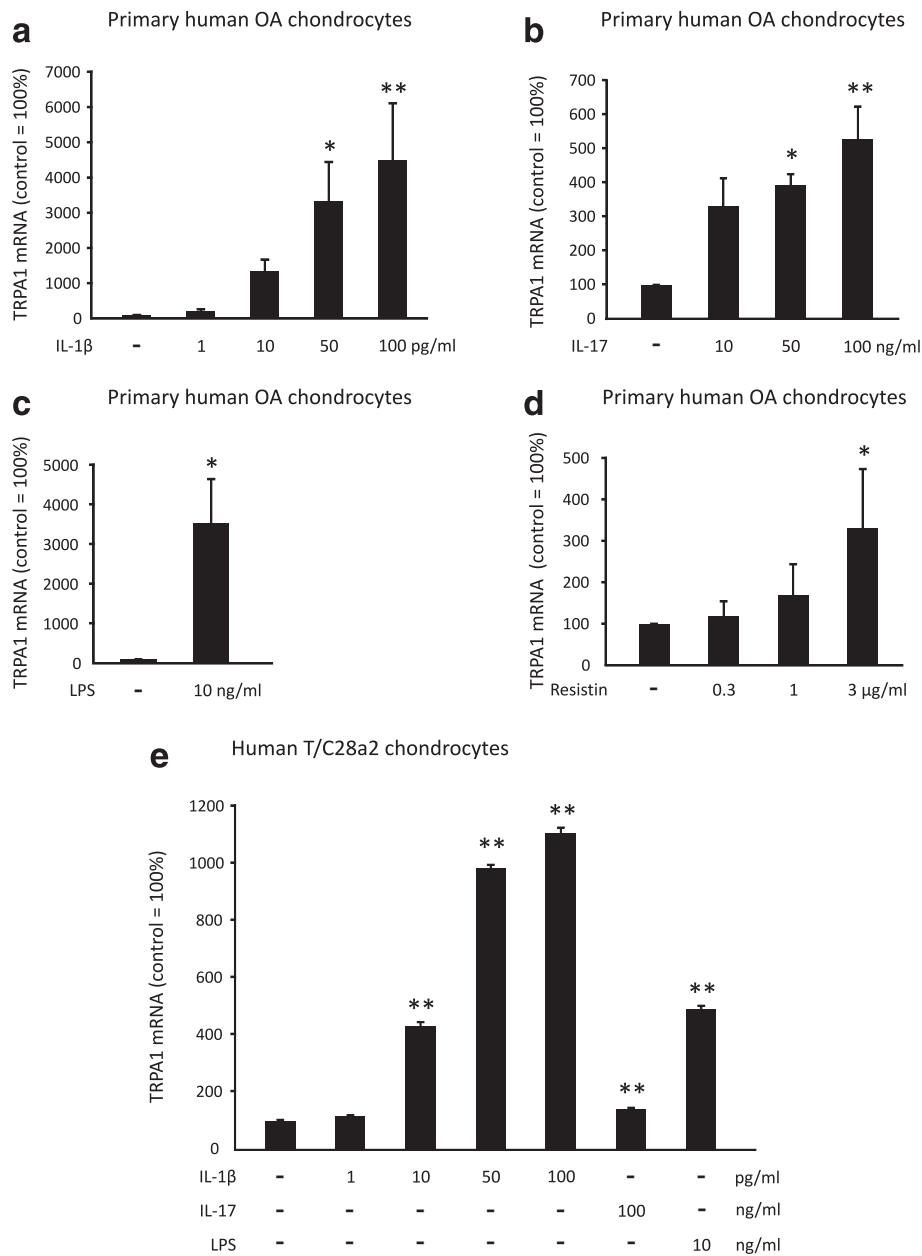
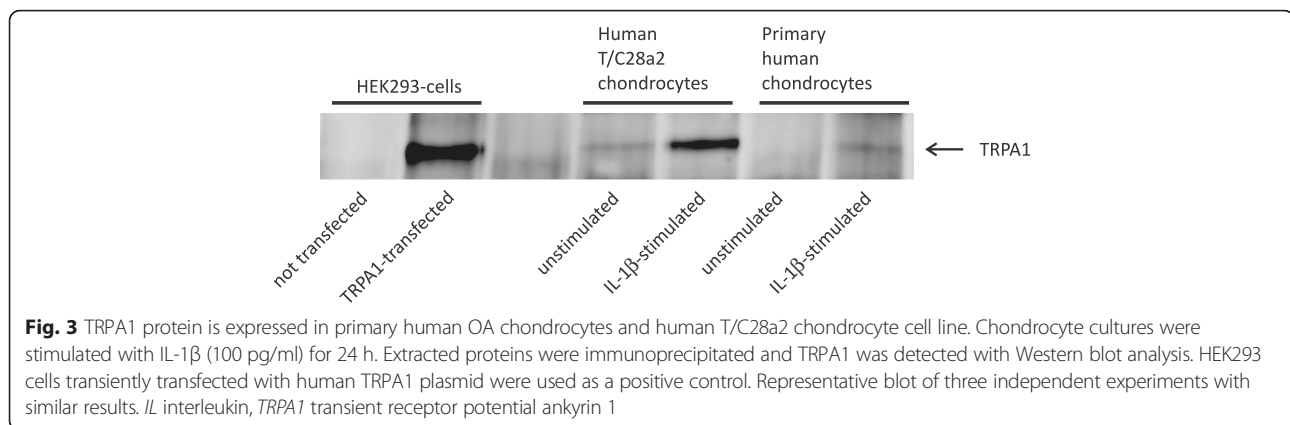


Fig. 2 TRPA1 mRNA expression is increased following stimulation with inflammatory factors IL-1 β , IL-17, LPS, and resistin in primary human OA chondrocytes (a-d) and in human T/C28a2 chondrocyte cell line (e). Isolated primary human OA chondrocytes were stimulated with IL-1 β (1–100 pg/ml) (a), IL-17 (10–100 ng/ml) (b), LPS (10 ng/ml) (c), and resistin (0.3–3 μ g/ml) (d); and human T/C28a2 chondrocytes with IL-1 β (1–100 pg/ml), IL-17 (100 ng/ml) and LPS (10 ng/ml) (e) for 24 h; and thereafter total RNA was extracted. TRPA1 mRNA levels were measured by qRT-PCR, and the results were normalized against GAPDH mRNA levels. The results are expressed as a percentage in comparison to untreated control samples, which was set as 100%. Primary chondrocyte samples were obtained from four different donors and the experiments were performed in duplicate. Human T/C28a2 chondrocyte experiments were carried out in quadruplicate. Results are expressed as mean + SEM. Repeated measures ANOVA (a, b, d) and one-way ANOVA (e) followed by Dunnett’s post-test or paired *t* test (c) was used in the statistical analysis; **p* < 0.05 and ***p* < 0.01, compared to the untreated control samples. IL interleukin, LPS lipopolysaccharide, OA osteoarthritis, TRPA1 transient receptor potential ankyrin 1

qRT-PCR and Western blot, respectively. We were also able to show that the expressed TRPA1 was functional, as evidenced by Ca²⁺-influx measurements. Further, we found TRPA1 to have a role in mediating the production of OA-related factors MMP-1, MMP-3, MMP-13, IL-6,

and PGE₂ as evidenced by pharmacological inhibition and genetic depletion of TRPA1.

TRPA1 was first discovered in 1999 in fetal lung fibroblasts [6]. Since then it has been mainly studied in different afferent sensory neurons such as A δ and C fibers of



nociceptors [7, 8]. More recently, however, TRPA1 has also been found to be expressed in some nonneuronal cells such as keratinocytes [11, 37, 38], synoviocytes [12, 39] and airway epithelial and smooth muscle cells [30]. It is noteworthy, that not all of these studies have shown functionality of the TRPA1 ion channel and some have only reported the expression of TRPA1 at the mRNA level. In the present study, we have comprehensively shown the expression and activation of TRPA1 in human chondrocytes, to support the criteria set by Fernandes et al. [40]. We were able to show for the first time the expression of both TRPA1 mRNA and protein and the functionality of the TRPA1 channel in primary human OA chondrocytes and in human T/C28a2 chondrocyte cell line. This finding is particularly interesting as in OA joints there is a hypoxic [31] and inflammatory [28, 41] state and related factors, H₂O₂, NO, and IL-6, have previously been shown to upregulate the expression and activation of TRPA1 [12–14]. According to Hatano et al. [12] the human *TRPA1* promoter has at least six putative nuclear factor kappa B (NF- κ B) binding sites and ten core hypoxia response elements (HREs), which are binding sites for hypoxia-inducible factor (HIF) transcription factors. HIFs are known to mediate adaptive responses to hypoxia as well as to be activated by inflammation [42, 43] and the binding of HIFs to consensus HREs on their target genes regulates gene transcription.

After discovering TRPA1 expression in chondrocytes, we aimed to investigate whether inflammatory factors/mechanisms related to the pathogenesis of OA [28, 29] regulate expression of TRPA1, which would indicate a role for TRPA1 as a mediator in OA. IL-1 β is considered as a major player in OA associated with cartilage destruction. IL-1 β is elevated in OA joints and it suppresses type II collagen and aggrecan expression, stimulates the release of MMP-1, MMP-3, and MMP-13, and induces the production of IL-6 and some other cytokines as well as PGE₂ [28]. In part IL-17 feeds forward

these mechanisms as it further induces IL-1 β , TNF, and IL-6 production, upregulates NO and MMPs and downregulates proteoglycan levels related to the pathogenesis of OA [28]. Based on our results, IL-1 β and IL-17 both also induce TRPA1 expression and intriguingly, some of the IL-1 β -induced inflammatory and catabolic effects are partly mediated by TRPA1. In OA the innate immune system and in particular toll-like receptors (TLRs) activated by cartilage matrix degradation products, also play a significant part in disease progression. Chondrocytes express TLRs, which trigger major inflammatory pathways and are activated by bacterial lipopolysaccharide (LPS) and damage-associated molecular patterns [29], and also the adipocytokine resistin known to be expressed in OA joints [44] has been shown to transduce its effects through toll-like receptor 4 [45]. In the present study, we found that both LPS and resistin increased expression of TRPA1 in human chondrocytes, suggesting a TLR-mediated mechanism to enhance TRPA1 expression in OA cartilage. In support of the present results, Hatano et al. showed that TRPA1 gene expression was enhanced in synoviocytes by inflammatory factors TNF- α and IL-1 [12], and the present study together with that of Hatano et al. [12] suggests a previously unrecognized mechanism that links TRPA1 as an inducible factor to joint inflammation.

Activation of TRPA1 results in a substantial influx of Ca²⁺ into the stimulated cells [46]. Here we verified the functionality and activation of the TRPA1 channel in human chondrocytes by measuring Ca²⁺ influx using the TRPA1 agonist AITC as well as the TRPA1 antagonist HC-030031. As shown previously, elevated intracellular Ca²⁺ concentration may affect the expression of inflammatory genes both in a direct or indirect manner [20]. In the present study, we found that TRPA1 regulated the production of inflammatory and catabolic factors, namely MMP enzymes, IL-6, and PGE₂ in chondrocytes. IL-1-induced MMP-3, IL-6, and PGE₂ production in the cartilage from TRPA1-deficient mice was less than half

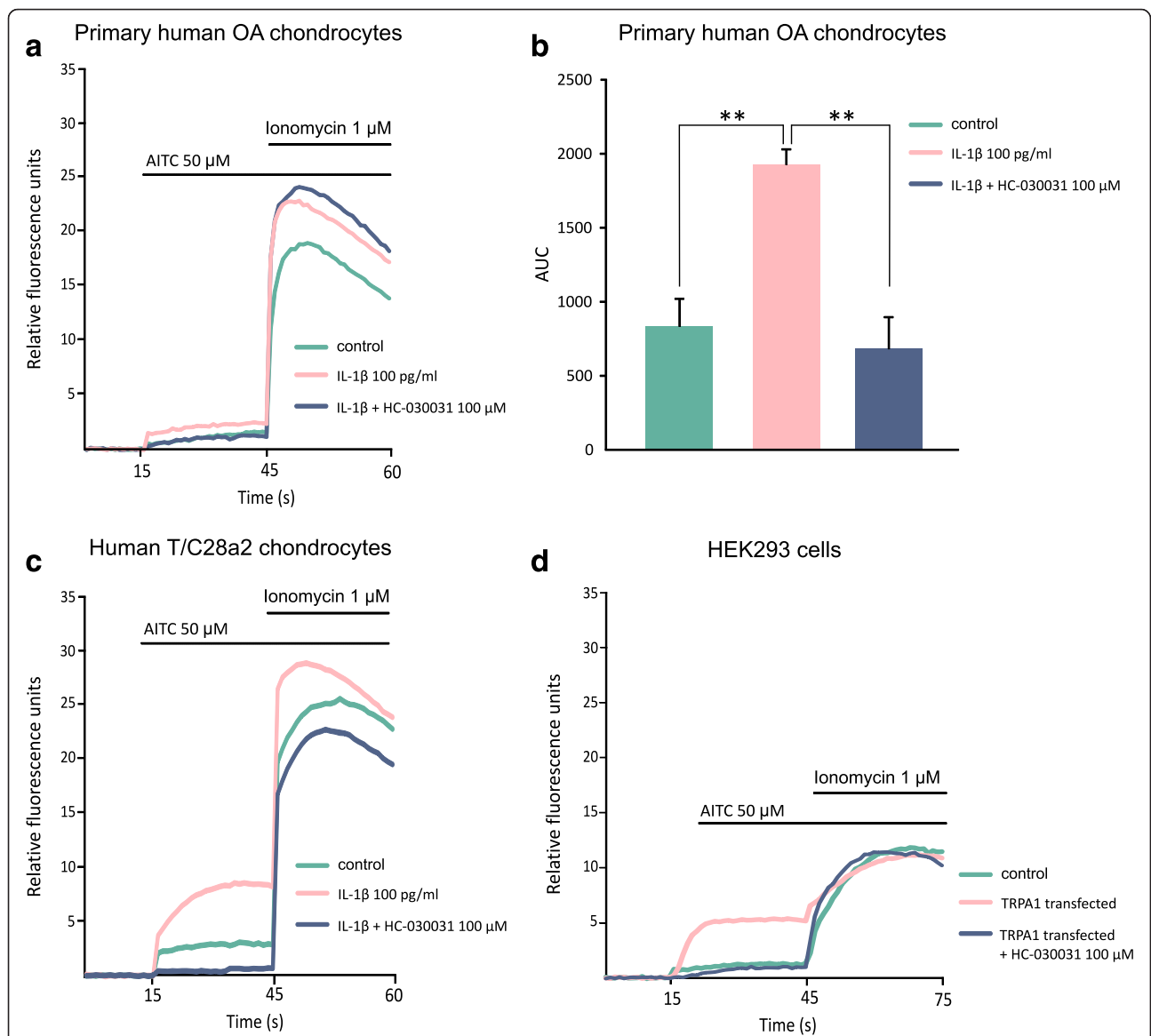
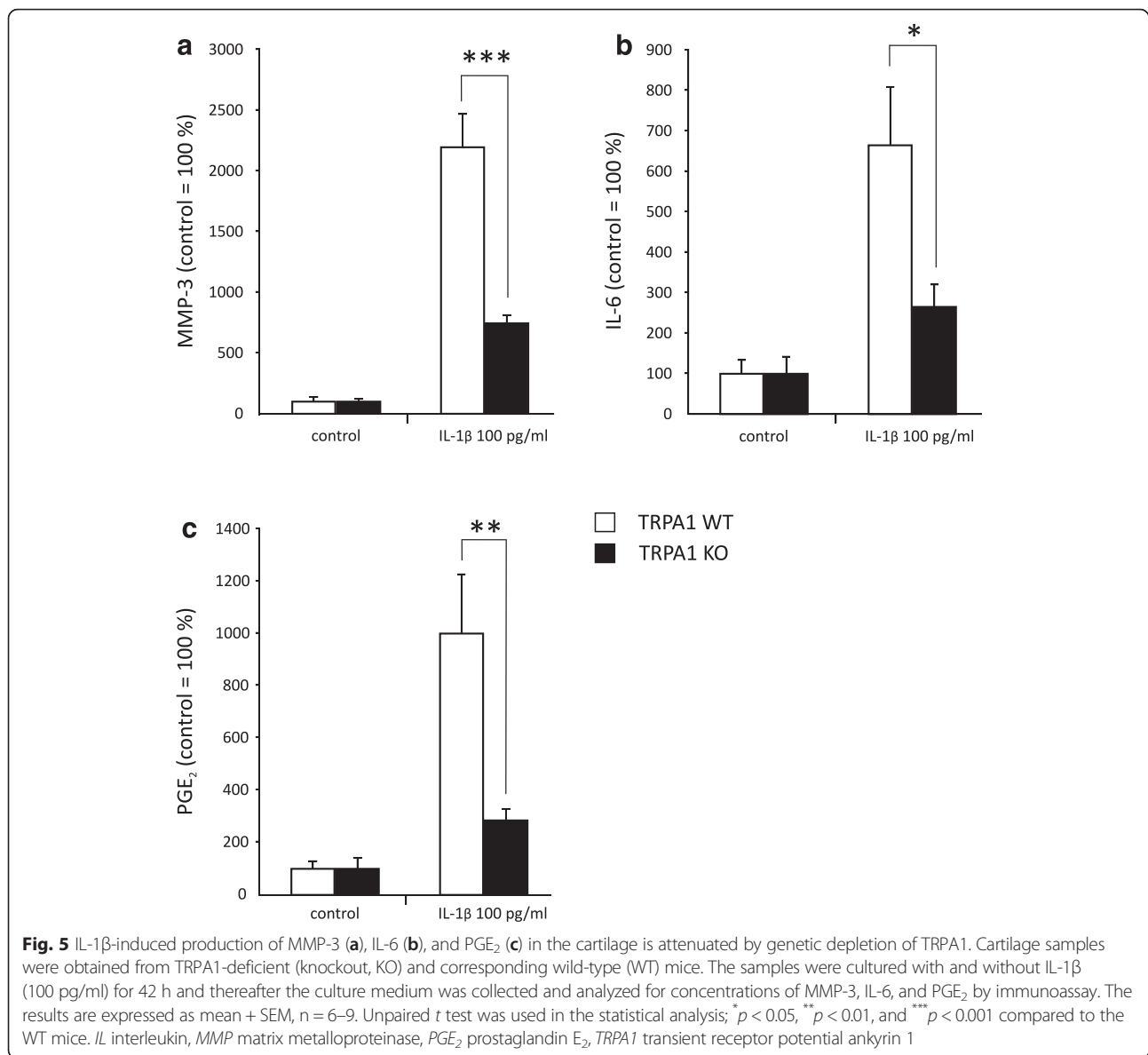


Fig. 4 The TRPA1 ion channel is functional in primary human OA chondrocytes (**a, b**) and human T/C28a2 chondrocyte cell line (**c**) as shown by TRPA1-mediated Ca²⁺ influx. Primary human chondrocytes (**a, b**) and human T/C28a2 chondrocytes (**c**) were cultured with or without (control) IL-1β (100 pg/ml) for 24 h. HEK293 cells transfected with plasmids encoding human TRPA1 were used as positive control cells (**d**). The cells were loaded with Fluo-3-AM and the TRPA1-mediated Ca²⁺ influx was measured by Victor3 multilabel counter at excitation/emission wavelengths of 485/535 nm at 1/s frequency. The cells were first preincubated with the TRPA1 antagonist HC-030031 (100 μM) or the vehicle for 30 min at +37 ° C. In the measurements, basal fluorescence was first recorded for 15 s and thereafter the selective TRPA1 agonist allyl isothiocyanate (AITC; 50 μM) was added and the measurement was continued for 30 s after which the control ionophore compound ionomycin (1 μM) was introduced to the cells. IL-1β stimulation resulted in an elevation in AITC-induced Ca²⁺ influx compared to unstimulated control cells, and it was attenuated by the selective TRPA1 antagonist HC-030031. The results were normalized against the background and expressed as mean of eight simultaneous measurements. Curves in **A, C** and **D** express results from one representative experiment. In (**b**) area under the curve (AUC) from 15 to 45 s was calculated from measurements of primary chondrocyte from four donors (each with eight repeats). Results are expressed as mean + SEM. Repeated measures ANOVA followed by Dunnett’s post-test was used in the statistical analysis; ***p* < 0.01 compared to the IL-1β-treated samples. IL interleukin, OA osteoarthritis, TRPA1 transient receptor potential ankyrin 1

of that found in the cartilage from wild-type mice. Accordingly, the selective TRPA1 antagonist HC-030031 reduced IL-1-induced MMP-1, MMP-3, MMP-13, IL-6, and PGE₂ production by 25–45 % in primary human OA chondrocytes. In the latter experiment, the cells

were incubated in the presence of IL-1 and HC-030031 for 24 h; therefore the result may be an underestimate of the effect of total inhibition of TRPA1 in OA chondrocytes because HC-030031 is a reversible TRPA1 antagonist with a relatively short half-life [47]. These findings



are supported by previous studies indicating that TRPA1 activation regulates the production of IL-1 in keratinocytes [38], IL-6 and IL-8 in synoviocytes [12], and PGE₂ along with leukotriene B₄ in fibroblasts and keratinocytes [48]. We have recently found that TRPA1 also regulates the expression of cyclooxygenase-2 (COX-2) [21, 27] and the production of monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-1 β , myeloperoxidase (MPO), MIP-1 α and MIP-2 in inflammatory conditions [26]. The detailed molecular mechanisms of this regulation remain, however, to be studied.

TRPA1 is shown to be involved in pain, hyperalgesia, and neurogenic inflammation [10, 16, 49, 50]. In OA-related pain, the role of TRPA1 has been investigated in studies by Moilanen et al. [27] McGaraughty et al. [51] and Okun et al. [52] using the MIA-model of OA. The two first-mentioned studies [27, 51] concluded TRPA1 to contribute

to joint pain in experimental OA. In addition, Moilanen et al. [27] reported that TRPA1-deficient mice developed less severe cartilage changes following MIA injections. Accordingly, we showed here that TRPA1 is functionally expressed in chondrocytes. We also examined the possible functions of the channel by treating primary chondrocyte cultures with IL-1 β and the selective antagonist HC-030031 [2, 53, 54]. Our results suggest an inflammatory and catabolic role for TRPA1 in human chondrocytes, as we found inhibition of TRPA1 to suppress the production of OA-related factors MMP-1, MMP-3, MMP-13, IL-6, and PGE₂. These results were supported by experiments with cartilage from WT and TRPA1-deficient mice: following stimulation with IL-1 β MMP-3, IL-6, and PGE₂ production was lower in the cartilage from TRPA1-deficient mice than from WT animals. These results together suggest that TRPA1-activating

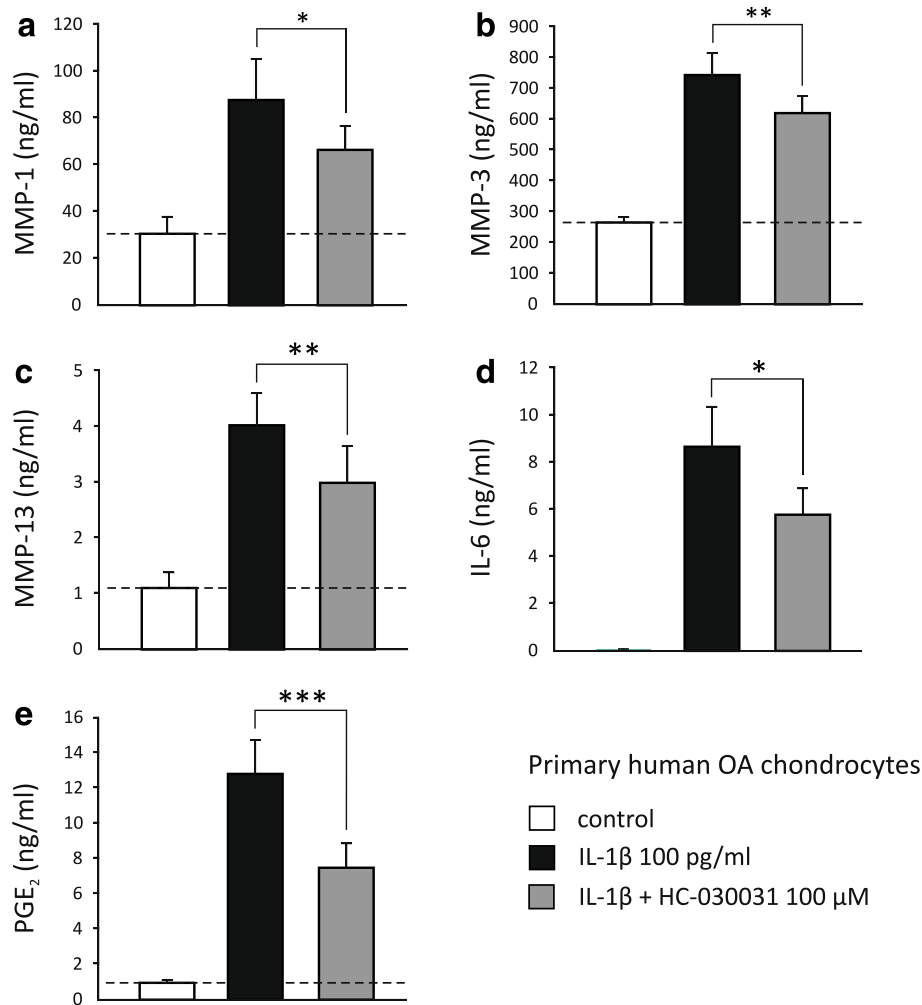


Fig. 6 IL-1 β -enhanced expression of MMP-1 (a), MMP-3 (b), MMP-13 (c), IL-6 (d), and PGE₂ (e) in primary human OA chondrocytes is attenuated by pharmacological inhibition of TRPA1. Primary human OA chondrocytes were stimulated with IL-1 β (100 pg/ml) in the presence and absence of the selective TRPA1 antagonist HC-030031 (100 μ M) for 24 h. MMP-1, MMP-3, MMP-13, IL-6, and PGE₂ concentrations in the culture media were measured by immunoassay and the results are expressed as mean + SEM. Samples were obtained from eight patients and the experiments were carried out in duplicate. Paired *t* test was used in the statistical analysis; **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 compared to the IL-1 β -treated samples. *IL* interleukin, *MMP* matrix metalloproteinase, *OA* osteoarthritis, *PGE₂* prostaglandin E₂, *TRPA1* transient receptor potential ankyrin 1

factors are present in OA joints, and that TRPA1 mediates, at least partly, OA-related pain, inflammation, and cartilage destruction in neuronal and nonneuronal cells in the joint.

Conclusions

In conclusion, we found the TRPA1 cation channel to be functionally expressed in primary human OA chondrocytes and in part to mediate inflammatory and catabolic effects, which are both original findings. The inflammatory and hypoxic environment in the OA joint is conducive to enhance the expression and activation of TRPA1. The presence and effects of TRPA1 in human OA cartilage as found in the present study, together with the previous findings on TRPA1 in experimentally induced OA [27, 51] propose an intriguing role for TRPA1 as a mediator and drug target in OA.

Additional file

Additional file 1: Supplementary data. Supplementary information to the "Methods". (DOCX 23 kb)

Abbreviations

AITC, allyl isothiocyanate; ANOVA, analysis of variance; COX-2, cyclooxygenase-2; ELISA, enzyme-linked immunosorbent assay; H₂O₂, hydrogen peroxide; HIF, hypoxia-inducible factor; HRE, hypoxia response element; IL, interleukin; KO, knockout; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIA, monosodium iodoacetate; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NF- κ B, nuclear factor-kappa B; NO, nitric oxide; OA, osteoarthritis; PGE₂, prostaglandin E₂; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SEM, standard error of the mean; TLR, toll-like receptor; TNF, tumor necrosis factor; TRPA1, transient receptor potential ankyrin 1; WT, wild-type

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Availability of supporting data

All the data is reported in the manuscript.

Authors' contributions

EN, MH, LJM, E-LP, RMN, TM, KV, and EM contributed to the design of the study and to the acquisition, analysis and interpretation of the data. EM conceived and supervised the study. EN drafted the manuscript and all authors revised the manuscript critically for important intellectual content and have approved the final version of the manuscript for submission.

Authors' information

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The patients in this study fulfilled the American College of Rheumatology classification criteria for OA [33] and the study was approved by the Ethics Committee of Tampere University Hospital, Tampere, Finland (reference number R09116), and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. Animal experiments were carried out in accordance with the legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU) and the experiments were approved by The National Animal Experiment Board (reference number UTA 845/712-86).

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