Piezo1-induced DNA Damage Reveals Novel Stress Response Mechanism in Chondrocytes

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INTRODUCTION: Osteoarthritis (OA), a highly prevalent disease characterized by progressive cartilage loss and joint dysfunction, affects millions of people in the United States, but the limited understanding of its underlying causes and mechanisms has constrained the development of effective treatment options[1]. Chondrocytes, the only cell type in cartilage, respond to mechanical forces via mechanosensitive ion channels such as transient receptor potential vanilloid 4 (TRPV4) and Piezos[2]. Piezo1 in particular can be activated by supraphysiologic loading (>50% cellular deformation) or by its selective small-molecule agonist Yoda1[3], leading to rapid increases in cytosolic calcium (Ca^{2+}). Previous studies have underscored the pathological significance of Piezo1 activation in the progression of joint diseases such as OA[4,5]. Piezo1 has also been found to induce cellular senescence, which can contribute to OA pathogenesis through the proinflammatory senescence-associated secretory phenotype[6,7]. One of the major drivers of cellular senescence is DNA damage[1]; however, the role of Piezo1 activation in this process has not been investigated in chondrocytes and may provide a novel therapeutic target. Here, we examined the hypothesis that Piezo1 activation leads to DNA damage in primary chondrocytes.

METHODS: Fresh chondrocytes were harvested from articular cartilage of femoral condyles of 5-to-6-month-old pigs and experiments were performed within 48h. 5 μM Yoda1 was applied every 30min for a total of 4h to activate Piezo1 chemically. Chondrocytes were embedded in 1% agarose gel and compressed to 80% gel volume (hold for 1min) in DMEM to activate Piezo1 mechanically. The comet assay (Fig. 1) was performed after Piezo1 activation to assess the level of DNA damage, with 30min 400μM H_2O_2 used as the positive control. Damage was assessed by comet tail length or percentage of total DNA in the tail. To determine if the DNA damage was due to non-specific Ca^{2+} signaling or other mechanosensitive pathways, 1-10nM GSK101 (to activate TRPV4) was added every 30min for 4h, followed by comet assay to assess DNA damage. To further confirm the DNA damage was Piezo1-driven, Piezo1-specific siRNA and non-targeting control (NC) siRNA were delivered to chondrocytes via nucleofection (Fig. 2A) followed by gel-embedding and 80% compression. To investigate the role of Piezo1-mediated Ca^{2+} influx in DNA damage, Ca^{2+} free media (Ca[-]) supplemented by 10mM EGTA was applied during Yoda1 treatment and during 80% compression. The effect of Yoda1 on human chondrocytes was also explored using de-identified surgical waste tissue from knee replacement for OA chondrocytes (68 year-old male) and a cadaveric donor for chondrocytes from macroscopically healthy cartilage (69 year-old female). IRB confirmed appropriate use of human tissue. One-way ANOVA or Kruskal-Wallis test and unpaired t-tests with Mann-Whitney test were used to determine statistical significance (GraphPad Prism version 10).

RESULTS: Treatment with the Piezo1 selective agonist Yoda1 at 5μM for 4h significantly increased comet tail length in chondrocytes (Fig. 2B), GSK101 treatment for the same duration and frequency did not cause significant tail length change compared to the negative control (Fig. 2C), supporting the role of Piezo1 in DNA damage. Piezo1-knock-down by siRNA demonstrated decreased tail length compared to control group that received non-targeting siRNA, which further supported the specificity of the DNA damage to Piezo1 activation (Fig. 2D). Moreover, treating chondrocytes with 5μM Yoda1 in Ca^{2+} free media reduced tail length significantly, compared to chondrocytes received Yoda1 in regular media with Ca^{2+}, and reduced the tail length to the same level as the negative control (Fig. 2E). While 80% compression induced DNA damage as shown by higher tail length, loading in Ca^{2+} free media reduced the tail length (Fig. 2F). In addition, 24h of 5μM Yoda1 treatment in human primary chondrocytes also demonstrated increasing DNA damage level compared to the vehicle control (Fig. 2G).

DISCUSSION: Our findings indicate that activation of Piezo1 leads to DNA damage in primary chondrocytes. This effect was shown following Piezo1 activation with the selective agonist Yoda1 or by high-strain mechanical compression. Furthermore, this effect is specifically mediated by the Piezo1-dependent Ca^{2+} influx, as confirmed by Piezo1 siRNA and Ca^{2+} free experiments. Furthermore, the DNA damage response was not activated by other Ca^{2+} signaling pathways such as TRPV4. These findings suggest a novel role for Piezo channels in regulating DNA damage and downstream effects in cartilage, which may contribute to senescence or other pathophysiologic processes in OA.

SIGNIFICANCE: By revealing the direct effect of Piezo1 activation on DNA damage, this work provides novel insight into downstream cellular responses to supraphysiological mechanical stress. Targeting Piezo1-mediated Ca^{2+} influx offers a potential therapeutic strategy to mitigate DNA damage in OA.

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Fig. 1

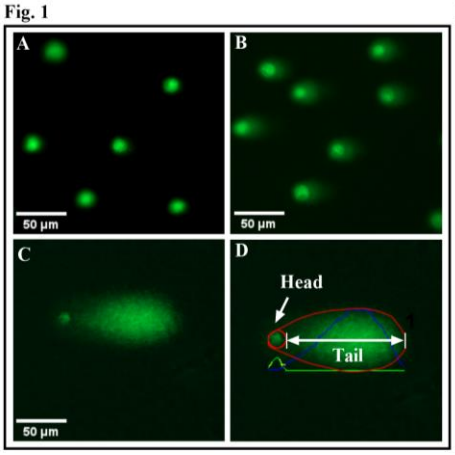

Fig. 2

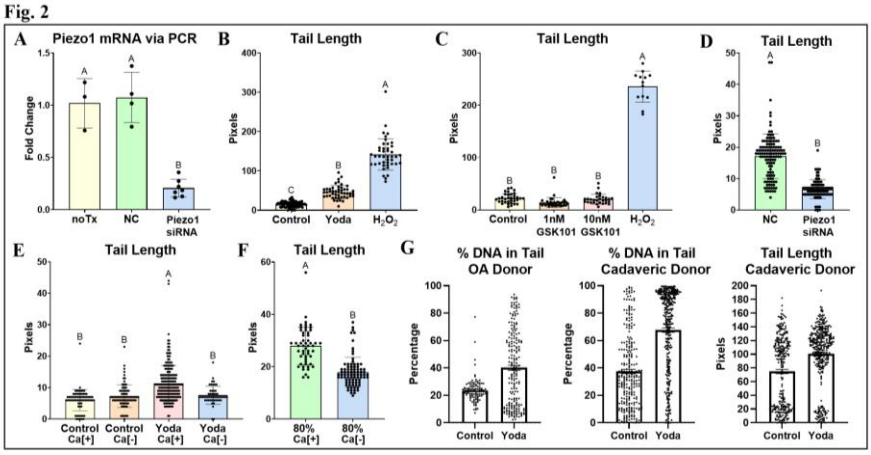

Fig. 1. Comet assay sample data. A) Negative control cells. B) DNA damage cells from Piezo1-activation. C) Positive control cells. D) An illustration for comet head and tail. Comet assay data were analyzed by OpenComet in FIJI. Longer tail length suggests higher DNA damage level.
Fig. 2. A) Piezo1 was knocked down by siRNA at around 80%. B) Yoda1 led to significantly increased tail length. C) TRPV4 activation via GSK101 did not cause increasing tail length. D) Piezo1 knockdown reduced tail length from 80% compression. E) Ca^{2+} free media during Yoda1 treatment reduced tail length. F) Ca^{2+} free media during 80% compression reduced tail length. G) Human chondrocytes showed higher damage after 24h with 5 μM Yoda1.