A novel mitochondrial-based rAAV-mediated IGF-I gene therapy to improve the human osteoarthritic phenotype

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INTRODUCTION: While gene therapy procedures based on the use of rAAV vectors hold great promises to treat osteoarthritis [1,2],a number of challenges need to be addressed for an effective and safe clinical translation, including the possible neutralization of the vector particles by pre-existing anti-AAV capsid antibodies and the possible integration of viral sequences into the host genome [2]. Owing to the intrinsic protein translation capability of the mitochondria [3], our goal was to determine the potential of implanting rAAV-mediated genetically modified mitochondria to overexpress the insulin-like growth factor I (IGF-I) in human osteoarthritic articular chondrocytes as an innovative therapeutic tool to alleviate the altered phenotype of these cells.

METHODS: rAAV vectors were packaged, purified, and titrated as previously described [4-6]. rAAV-*lacZ* carries the *E. coli* β-galactosidase (*lacZ*) gene and rAAV-hIGF-I a human insulin-like growth factor I cDNA, both controlled by the CMV-IE promoter/enhancer [4-6]. Human osteoarthritic cartilage biopsies (n = 5; 6-mm diameter; Mankin score = 7-9) were randomly collected from the femoral condyle of patients undergoing total knee arthroplasty and the chondrocytes were isolated using established protocols [4,5]. Mitochondria were immediately extracted from freshly isolated the chondrocytes (10^7 cells) using the Mitochondria Isolation Kit (Thermo Scientific™) [7] to obtain one unit that was subsequently resuspended for 1 h at 37°C in 110 µl of serum-free DMEM medium and rAAV (40 µl). The samples were then completed with 150 µl of DMEM, 10% FBS for another incubation of 24 h at 37°C. rAAV-treated mitochondria (1 unit) were transplanted in the chondrocytes (5 x 10^4 cells in 6-well plates) for 24 h at 37°C. Transgene (IGF-I) expression was monitored by ELISA (R&D systems) and by immunocytochemistry [4]. The ATP contents were measured using the ATP Determination Kit (Invitrogen™) [8]. Cell viability was measured using the Cell Proliferation Reagent WST-1 (Sigma-Aldrich, Merck) [6]. Cell apoptosis was estimated with the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method (Invitrogen™), with histomorphometric analyses and apoptotic indices calculated as previously described [4,5]. The deposition and contents of proteoglycans were estimated by safranin O staining and by binding to the dimethylmethylene blue (DMMB) dye relative to the total protein contents measured using the BCA protein assay (Thermo Scientific™), respectively [4]. Each condition was performed in quintuplicate in 4 independent experiments. The t-test was employed, with $P < 0.05$ considered statistically significant.

RESULTS: *Efficacy and functional benefits of rAAV-mediated IGF-I gene transfer and overexpression in mitochondria*: Successful rAAV-mediated IGF-I gene transfer and overexpression was noted in mitochondria extracted as seen by the significantly achieved IGF-I levels when providing rAAV-hIGF-I over time to the mitochondria relative to the control treatments (no vector condition, rAAV-*lacZ* treatment) (up to 3.6-fold difference with rAAV-hIGF-I between 1 and 8 days *versus* controls, always $P \leq 0.05$) (**Fig. 1a**). Effective IGF-I overexpression via rAAV led to significant increases in the ATP contents of rAAV-hIGF-I-treated mitochondria over time relative to the control treatments (no vector condition, rAAV-*lacZ* treatment) (up to 1.6-fold difference with rAAV-hIGF-I between 1 and 6 days *versus* controls, always $P \leq 0.05$) (**Fig. 1b**). *Efficacy of implantation of rAAV-hIGF-I-treated mitochondria in human osteoarthritic chondrocytes*: Successful implantation of rAAV-hIGF-I-modified mitochondria was noted in human osteoarthritic chondrocytes as seen by significantly achieved IGF-I deposition (**Fig. 2a, 2b**) and IGF-I levels (**Fig. 2c**) when providing rAAV-hIGF-I-treated mitochondria to the cells relative to the control treatments (mitochondria without vector or rAAV-*lacZ*-treated mitochondria) (IGF-I immunodetection: up to 23-fold difference with rAAV-hIGF-I after 24 h *versus* controls, always $P \leq 0.05$; IGF-I ELISA: up to 9-fold difference with rAAV-hIGF-I after 24 h *versus* controls, always $P \leq 0.05$). *Functional benefits of implantation of rAAV-hIGF-I-treated mitochondria in human osteoarthritic chondrocytes*: Effective and functional implantation of rAAV-hIGF-I-modified mitochondria significantly enhanced the viability of human osteoarthritic chondrocytes as seen by the results of a WST-1 assay relative to the control treatments (no mitochondria, mitochondria without vector, or rAAV-*lacZ*-treated mitochondria) (up to 2-fold difference with rAAV-hIGF-I after 24 h *versus* controls, always $P \leq 0.05$) (**Fig. 3a**). These findings were corroborated by the results of a TUNEL assay revealing that implantation of rAAV-hIGF-I-treated mitochondria significantly decreased the apoptotic indices relative to the control treatments (no mitochondria, mitochondria without vector, or rAAV-*lacZ*-treated mitochondria) (up to 4-fold difference with rAAV-hIGF-I after 24 h *versus* controls, always $P \leq 0.05$) (**Fig. 3b, 3c**). In addition, implantation of rAAV-hIGF-I-modified mitochondria significantly increased the deposition and contents of proteoglycans in human osteoarthritic chondrocytes as seen by safranin O staining with an histomorphometric analysis (**Fig. 3d, 3e**) and by the results of a DMMB assay (**Fig. 3f**) relative to the control treatments (no mitochondria, mitochondria without vector, or rAAV-*lacZ*-treated mitochondria) (safranin O staining: up to 2-fold difference with rAAV-hIGF-I after 24 h *versus* controls, always $P \leq 0.05$; DMMB assay: up to 24-fold difference with rAAV-hIGF-I after 24 h *versus* controls, always $P \leq 0.05$).

DISCUSSION: The current evaluation shows the beneficial effects of providing rAAV-hIGF-I-treated mitochondria to human osteoarthritic chondrocytes as an effective strategy to improve the unhealthy phenotype of these cells.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates the potential of therapeutic mitochondria-based protein replacement therapy via rAAV gene transfer to counteract osteoarthritic changes in human patients.

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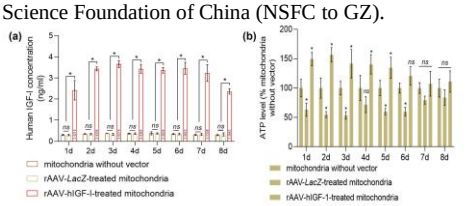

Fig. 1. Efficacy and functional benefits of rAAV-mediated IGF-I gene transfer and overexpression in mitochondria from primary human osteoarthritic articular chondrocytes. Detection of transgene (IGF-I) expression (**a**) and of the ATP contents (**b**) in mitochondria treated with rAAV-hIGF-I.

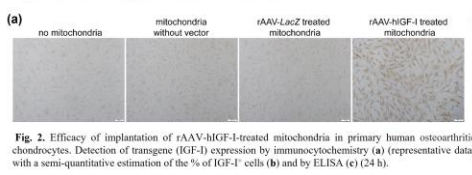

Fig. 2. Efficacy of implantation of rAAV-hIGF-I-treated mitochondria in primary human osteoarthritic chondrocytes. Detection of transgene (IGF-I) expression by immunocytochemistry (**a**) (representative data) with a semi-quantitative estimation of the % of IGF-I+ cells (**b**) and by ELISA (**c**) (24 h).

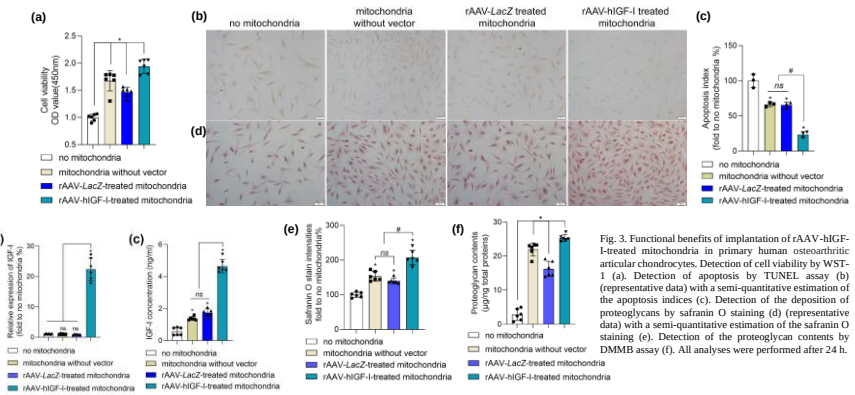

Fig. 3. Functional benefits of implantation of rAAV-hIGF-I-treated mitochondria in primary human osteoarthritic articular chondrocytes. Detection of cell viability by WST-1 (a). Detection of apoptosis by TUNEL assay (b) (representative data) with a semi-quantitative estimation of the apoptosis indices (c). Detection of the deposition of proteoglycans by safranin O staining (d) (representative data) with a semi-quantitative estimation of the safranin O staining (e). Detection of the proteoglycan contents by DMMB assay (f). All analyses were performed after 24 h.