Therapeutic nuclear magnetic resonance therapy (NMRT) influences the miRNA profile and the hypoxic behavior of human chondrocytes stimulated by IL-1β

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BACKGROUND

Treatment options for osteoarthritis (OA), known as most common joint disease and being an exceptional socio-economic burden, are of exceptional interest. It is known that NMRT in the treatment of patients with OA attains pain reduction as the main clinical outcome, mechanisms at the cellular level are still unexplained. Although our previous work demonstrated an influence of NMRT on Ca^{2+} homeostasis, ATP level and NFκB activity under inflammatory conditions, greater clarity of the mechanisms of action should be given. Within the presented study we investigated the influence of NMRT on the gene expression of TC28/2a cells treated with IL1β/TNFα and grown under hypoxic conditions. In addition NMRT induced changes of the miRNA profile of human healthy and OA chondrocytes were investigated. We also analyzed the respective miRNA targets histone deacetylase 4 (HDAC4) and cyclo-oxygenase2 (COX2). This study aims to reach a better understanding how this noninvasive treatment works and would be able to underpin NMRT applications.

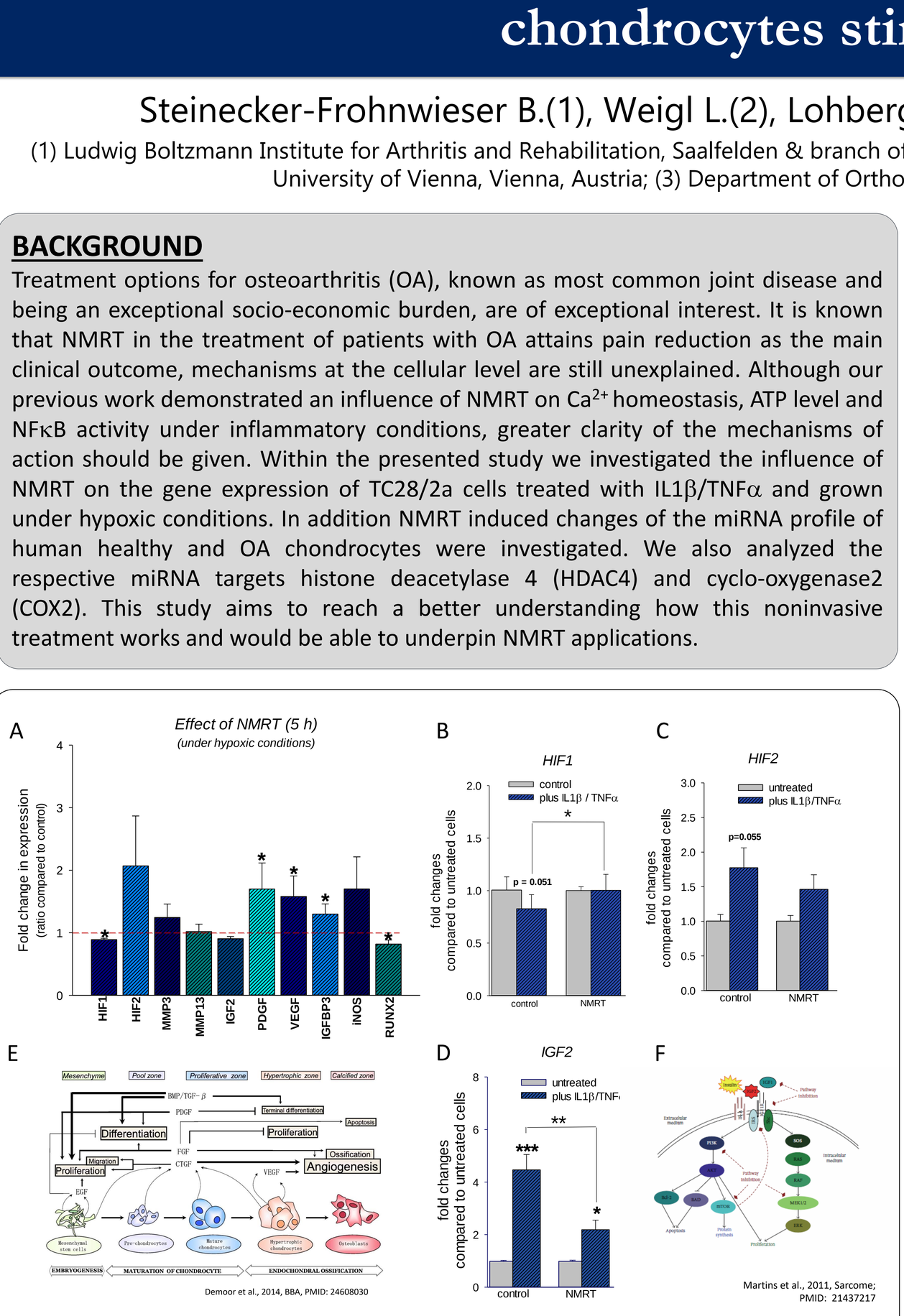

Fig. 1. Tc28/2a chondrocytes cultivated under hypoxic conditions changed their expression profile regarding several cell regulatory proteins under NMRT (A-D). Illustrations from the literature show mechanisms possbly involved in realising the NMRT modulation (E-F). Reduced expression of AMPK by NMRT is given (G).

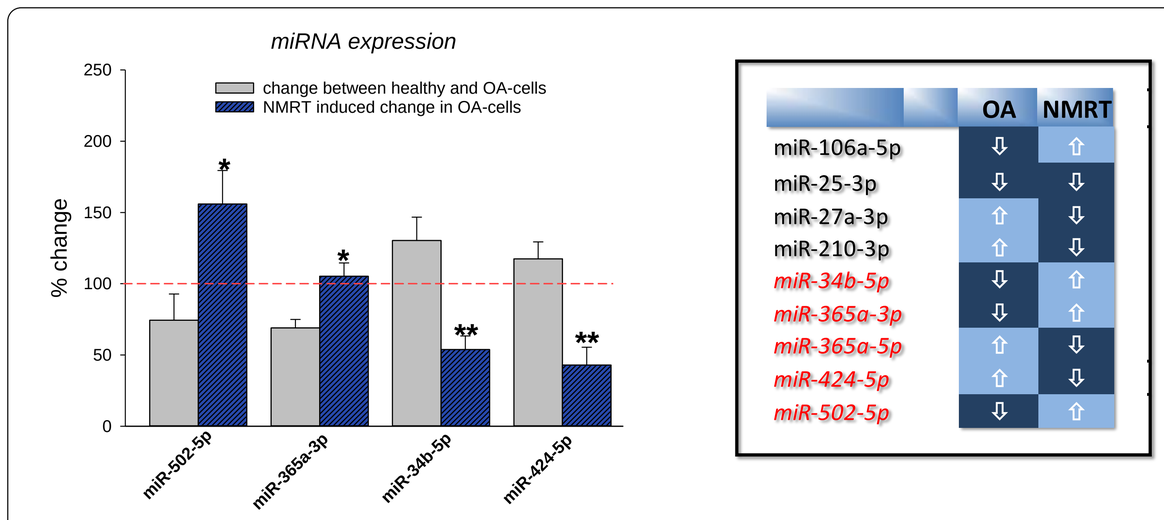

Fig.2. Changes in the expression of specific miRNAs of primary chondrocytes are shown.

METHODS

Human primary chondrocytes and the chondrocyte cell line Tc28/2a were used for the experiments while NMRT treatment was applied for 5x1 h within a time-span of two days. RNA was extracted using RNeasy Mini Kit and was used as input for the Thermo Fisher Ion Total RNA-Seq Kit v2. Sequencing was performed on Ion Proton sequencer using the Ion PI Hi-Q™ Sequencing 200 system. Signal processing and base calling was performed using Torrent Suite version 5.6. Hypoxic conditions were established and enabled cell growth in presence of 1-5% O_2. Expression of miRNAs and target proteins were studied by a standard PCR procedure as well as protein detection by western blot. Histon-Deacetylasen (HDAC) activity was measured by HDAC-Glo I/II assay.

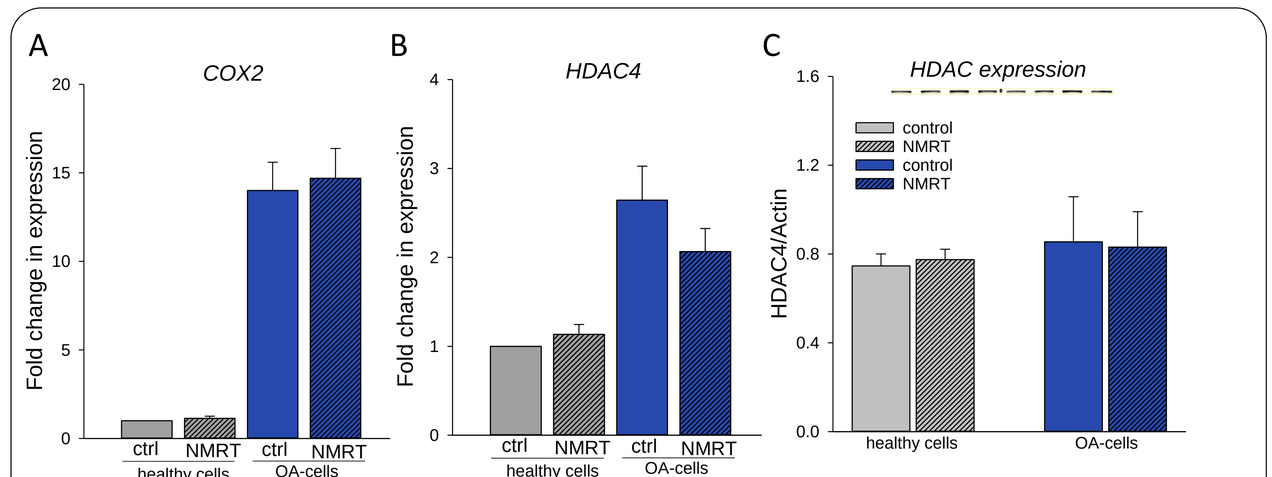

Fig. 3. NMRT tested on the expression of COX2 and HDAC4 is outlined (A-C). Changes in HDAC4 activities by NMRT with and without trichostatin are presented within the bar charts (D, E). Thoughts from the literature about the connection between HDAC, NFkB and osteoarhtritis are illustrated (F, G).

RESULTS

NMRT treatment of chondrocytes under hypoxic conditions (1-5 % O2) changed the expression profile with respect to NOS, IGF2, PDGF and IGFBP and a change in the expression of Hif1/2 under the influence of IL1β/TNFα was observed (Fig. 1A-C). NMRT reversed the IL1β/TNFα induced IGF2 expression and activation of AMP activated kinase (AMPK) (Fig. 1D,E). Characterization of the miRNA profile showed a slight upregulation of miR-365a-3p, miR-24-1-5p and miR-502-5p while miR-25-5p and miR-365a-5p was downregulated after NMR treatment (Fig. 2). For miR-365a-5p known to directly targeting HDAC and NFkB a decrease of HDAC activity by NMRT was detected Fig. 3). The miR-25-5p targeting COX2 was changed in expression by NMRT whereas no influence on CDK4 was detected known to be controlled by miR-24-1-5p. Our data once more demonstrated NMRT to counteract inflammatory mechanisms in chondrocytes we were further able to demonstrate that NMRT modulation seems to be more pronounced under hypoxic conditions.

CONCLUSION

Our investigations concerning the influence of the NMRT at the cellular level revealed a modulatory effect on miRNA and their regulatory units as well as chondrocyte function under hypoxic conditions. The results underline our former findings indicating that NMRT counteracts IL-1β induced changes probably by influencing the NFκB axis. Therefore we deduce that the often observed NMRT induced pain reduction may be a result of NMRT holding against inflammatory mechanisms under OA.