NFAT-modulation in bone and cartilage cells by nuclear magnetic resonance therapy

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Issue:

Nuclear magnetic resonance (NMR), based on magnetic resonance tomography technology, has recently started to be used in orthopaedics and rheumatology in gonarthrosis, arthrosis of the finger joints and low back pain. At cellular level, NMR effects could be observed with regard to proliferation rate, morphology, protein concentration, DNA content and collagens I, III and IV [1]. There are as yet few studies on the effects of therapeutic NMR on cellular factors. Ca2+ regulated NFAT (nuclear factor of activated T cells) pathway acts as an important signal transmitter, responsible for the differentiation and growth of cells and the associated balance between bone formation and loss.

Types of NFAT	Other names	Regulation	Expression in the immune system
NFAT1	NFATc2 and NFATp	Calcium calci-neurin	Yes
NFAT2	NFATc1 and NFATc	Calcium calci-neurin	Yes
NFAT3	NFATc4	Calcium calci-neurin	No
NFAT4	NFATc3 and NFATx	Calcium calci-neurin	Yes
NFAT5	TonEBP and OREBP	Osmotic stress	Yes

Table 1: NFAT, nuclear factor of activated T-cells; TonEBP, tonicity-responsive enhancer-binding protein.

Transcription factors of the NFAT family (Tab. 1) mediate the Ca2+- and calcineurin-dependent transcription of many cytokines as part of T-cell activation. Basic therapeutic agents (e.g. Cyclosporine A) are in use for inflammatory-rheumatic diseases which are associated with the NFAT pathway. The calcineurin/NFAT signal pathway is influenced both by immunosuppressant therapy and also by inflammatory mechanisms – leading to osteopenia and fractures. Gene expression studies during NMR can supply new information about the bases and the rationale of NMR therapy at cellular level.

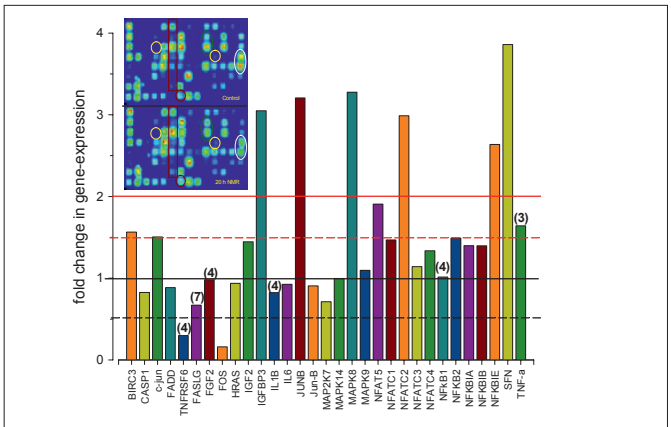


Fig. 1: Quantification of gene expression in a microarray
The insert shows a gene array of hybridisation experiments; unstimulated above and after 20 hours of NMR below. Blue indicates low expression levels and yellowish-red high expression levels.
The bar chart shows the average change in expression of Cal-72 cells after standardisation to control genes, results of array duplicates and several experiments.

Conclusions:

- Dependent on the NMR exposure time, there are different impacts on the NFAT pathway of osteocytes and chondrocytes.
- Genes of the NFAT family are conspicuously regulated in the microarray after 20 hours of NMR – double the therapeutic application.
- Neither could significant NFAT increases as a result of NMRT be verified after 20 hours in the quantitative PCR; no indication of activation of osteoclast differentiation via NFATc1 (bone loss).
- Changed expression levels of Stratifin and growth factors (IGF, IGFBPs) indicate that growth, differentiation, cellular cycles and the survival of bone and cartilage cells can be influenced as a result of the NMR.

Literature:

[1] Kullich, W.Therapeutischer Einsatz der Kernspinresonanz bei Arthrosen, German Conference for Orthopaedics and Accident Surgery, 22nd – 25th October 2008, Berlin, German Medical Science (GMS) Publishing House (2008). Published online, Doc. W152-99.

[2] Temiz-Artmann A., Linder P., Kayser P., Digl I., Artmann G.M., Lücker P. NMR In Vitro Effects on Proliferation, Apoptosis and Viability of Human Chondrocytes and Osteoblasts. Methods Find Exp Clin Pharmacol 2005; 27:391-394

Methods:

Cell culture: Cal-72 human osteosarcoma (ACC439) cells; Cal-78 human chondrosarcoma (ACC449), cells from DMSZ Germany

Nuclear magnetic resonance treatment: nuclear magnetic resonance equipment specially adapted for cell cultures (MedTec, Wetzlar, Germany); field strength: 2.3 m Teslar; 1, 5, 10 or 20 hours NMR exposure

Microarray technique: Different pathway-specific gene arrays (SA Biosciences Corp., USA) were hybridised with probes marked with biotin, synthesised via RNAs isolated from Cal 72/78 cells.

Quantitative PCR: Synthesised cDNA (Invitrogen, SuperScript First Strand) was analysed with gene-specific primers at the level of quantitative PCR (FIVE-PRIME, RealMasterMix Cyber Rox)

Reporter gene assay: Cal-72s were transfected with DNAs of the Cignal Finder 10-Pathway Reporter Assay Kits (SA Biosciences) and stimulated or non-stimulated cells tested for their Luciferase activity.

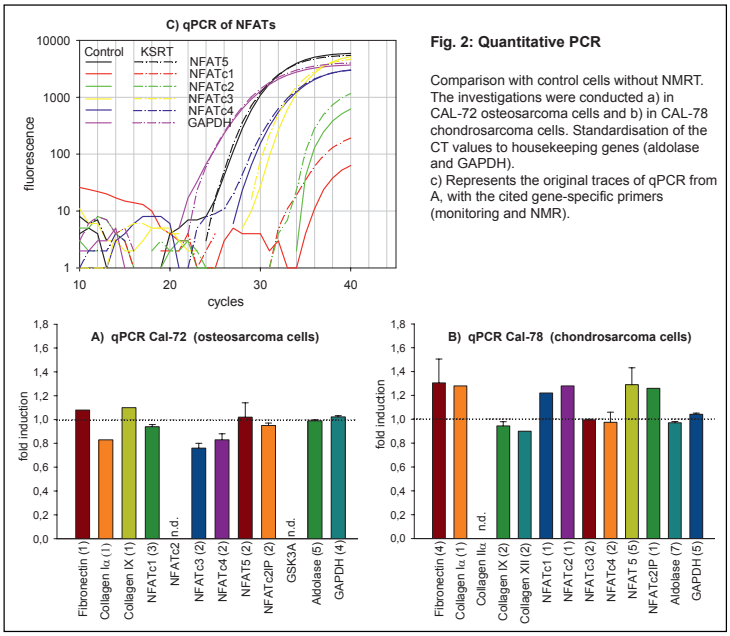
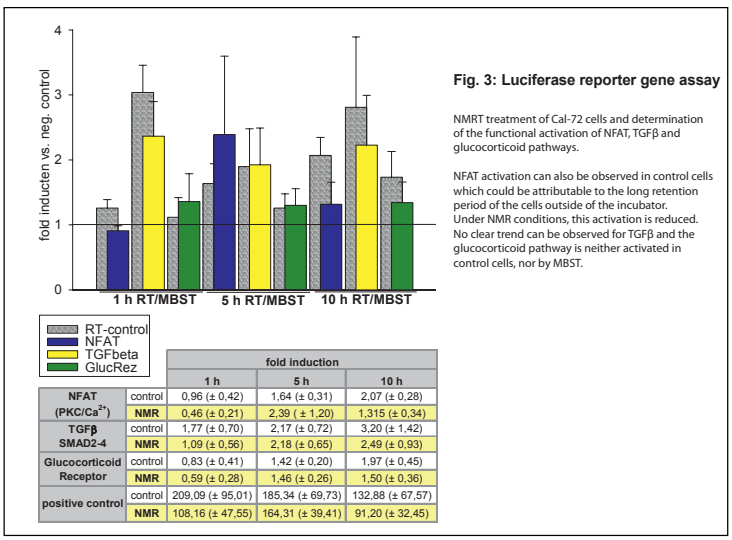


Fig. 2: Quantitative PCR

Comparison with control cells without NMRT. The investigations were conducted a) in CAL-72 osteosarcoma cells and b) in CAL-78 chondrosarcoma cells. Standardisation of the CT values to housekeeping genes (aldolase and GAPDH).
c) Represents the original traces of qPCR from A, with the cited gene-specific primers (monitoring and NMR).

Results and discussion:

The results are depicted and described in Fig. 1-3. NFAT components are changed in CAL-72 and CAL-78 cells after 20 hours of NMRT in the gene array (Fig. 1). An indirect osteoclastic effect via NFATc1 and the chemokine CCL8 is known. However, no increase of NFATc1 by NMRT could be proven in the quantitative PCR (Fig. 2). NMRT increased the expression of the protein stratifin important in cell regulation (Fig. 1). Due to the expression modulation during NMRT, there was no clear trend of an impact on bone growth via the SMAD family (small mothers against decapentaplegic proteins) which represent links in the signalling of TGFβ (growth factor of osteoblasts) to morphogenetic proteins (Fig. 3). Reporter gene investigations also show time-dependent changes in relation to NFAT.



		fold induction		
		1 h	5 h	10 h
NFAT (PKC/Ca^{2+})	control	0,96 (± 0,42)	1,64 (± 0,31)	2,07 (± 0,28)
	NMR	0,46 (± 0,21)	2,39 (± 1,20)	1,315 (± 0,34)
TGFβ SMAD2-4	control	1,77 (± 0,70)	2,17 (± 0,72)	3,20 (± 1,42)
	NMR	1,09 (± 0,56)	2,18 (± 0,65)	2,49 (± 0,93)
Glucocorticoid Receptor	control	0,83 (± 0,41)	1,42 (± 0,20)	1,97 (± 0,45)
	NMR	0,59 (± 0,28)	1,46 (± 0,26)	1,50 (± 0,36)
positive control	control	209,09 (± 95,01)	185,34 (± 69,73)	132,88 (± 67,57)
	NMR	108,16 (± 47,55)	164,31 (± 39,41)	91,20 (± 32,45)

Fig. 3: Luciferase reporter gene assay

NMRT treatment of Cal-72 cells and determination of the functional activation of NFAT, TGFβ and glucocorticoid pathways.

NFAT activation can also be observed in control cells which could be attributable to the long retention period of the cells outside of the incubator. Under NMR conditions, this activation is reduced. No clear trend can be observed for TGFβ and the glucocorticoid pathway is neither activated in control cells, nor by MBST.

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