1531 Stem cell activation through non-invasive biophysical applications

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INTRODUCTION

Significant new findings on the role of **endogenous bioelectric** and **electromagnetic fields (EMF)** are about to revolutionize the understanding of collective cell behaviors required for major biochemical processes in multicellular organisms.[1,2,3] Ion fluxes through channels and gap junctions alter membrane potentials as well as extracellular and intracellular electric milieus. Already in the last century, renowned researchers correlated EMF alterations with key biological processes from embryonic development to tissue regeneration and disease.[4,5] Today, frontier of science technologies confirm a major role of bioelectric and bioelectromagnetic fields in these processes,[1] shown e.g. in seminal works for skin wound healing.[6] **Exogenous EMF** generated through natural phenomena **also impact** human cells and body**;** e.g. a major EMF with beneficial effects on the human circadian rhythm, brain activities and health arises through electric gradients between ionosphere and earth surface.[7] Taking advantage of this collective knowledge, we used a multi-omics approach to identify **an electromagnetic platform technology** with activating, stress-reducing and anti-aging properties for stem cells, which we also tested for stress-reducing abilities on the human body (Patent WO 2021191443).

Approach: Using various frequencies along the EMF spectrum generated by a Helmholtz Coil, a modulated low-frequency EMF (LF EMF; 7.83 Hz/432 Hz) has been identified with initial read-out of enhanced proliferation of juvenile, primary stem and progenitor keratinocyte cultures pooled from three donors. A multi-omics approach confirmed a significant signaling response during proliferation but not, as requested for no-risk EMF, on differentiating, contact-inhibited keratinocytes. In a pilot study, the EMF was also shown to impact the neuro-immunological axis.

RESULTS

Fig 1. The LF EMF enhances keratinocyte proliferation

Sustained beneficial effect on cell growth occurred for human primary keratinocytes continuously exposed over 6 passages (1 month; nB=3, nT=3, ±SEM) to the identified modulated LF EMF. Control cells were in a Helmholz coil without current (no LF EMF).

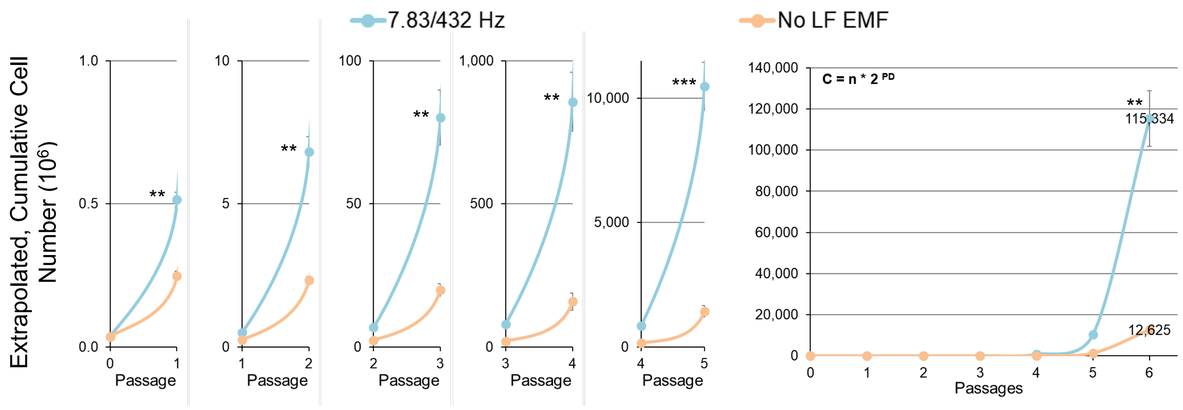
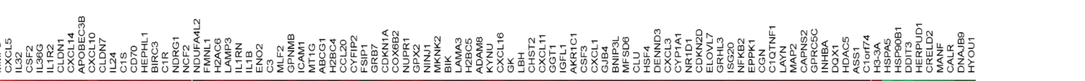


Fig 1 – Note that keratinocyte growth rate in the LF EMF over time was constant, therefore not exhausting cells despite enhanced proliferation. Furthermore, morphologically, these cells were healthier with less dead cells in the culture medium, yielding 10x more live cells after 6 passages.

Fig 2. Bulk RNA-seq : confirmation of beneficial impact of LF EMF on proliferation not affecting contact inhibition and differentiation



Primary data – proliferation / differentiation:

Cross-sectional RNA-seq analysis (nB=3, nT=1 replicates) via DESeq2 shows large differences during proliferation (144 DEGs: 21 up, 123 down; adj p≤0.05, log2 FC ≥2) and only 1 DEG after contact inhibition (S100A: down). This demonstrates a major impact of the LF EMF on stem and progenitor cell activation and proliferation, and no tumorigenic overgrowth during differentiation (contact inhibition).

2a

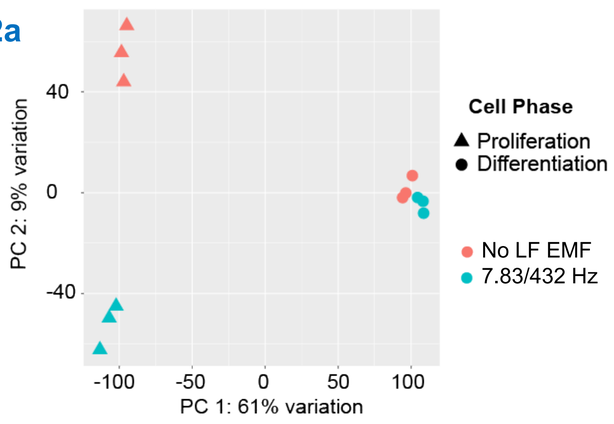


Fig 2a – PCA Plot revealed separate clusters of exposed and non-exposed cells in proliferation phase but not in differentiation phase.

2b

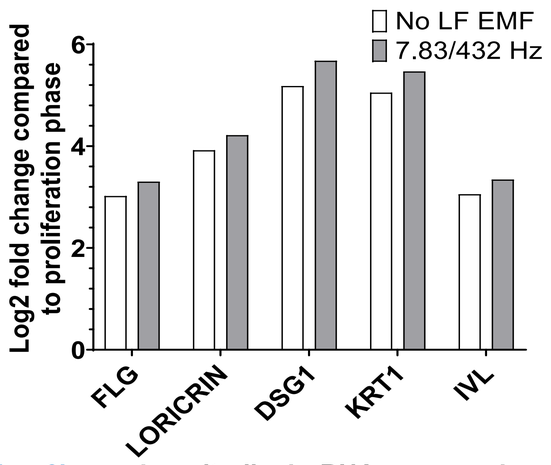


Fig 2b – Longitudinal RNA-seq analyses confirmed significant relative increase in expression of known differentiation-dependent markers over the course of 8-9 days.

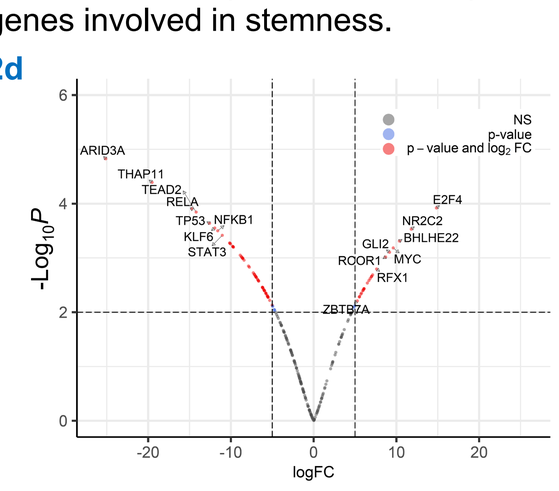
2c

Fig 2c – Heat Map of the 100 most deregulated genes during proliferation reveals a "black and white" response to LF EMF in terms of gene regulation and inferred biological pathway modulation (GSEA). Besides reduced inflammation and cellular stress, cancer pathway-encoding genes (N=94 Genes) were also downregulated. Conversely, increased proliferation was accompanied by an increase in replication, transcriptional and translational activities, as well as DNA repair (N=285 Genes) and DNA double strand break repair (N=130 Genes) pathways.

Inferred data – proliferation/volcano plots:

Volcano plots of transcription factors showed e.g. highly upregulated E2F4 and Myc and inhibition of p53 and NF-kB, paralleled by a significant upregulation of PLURINET genes involved in stemness.

2d



2e

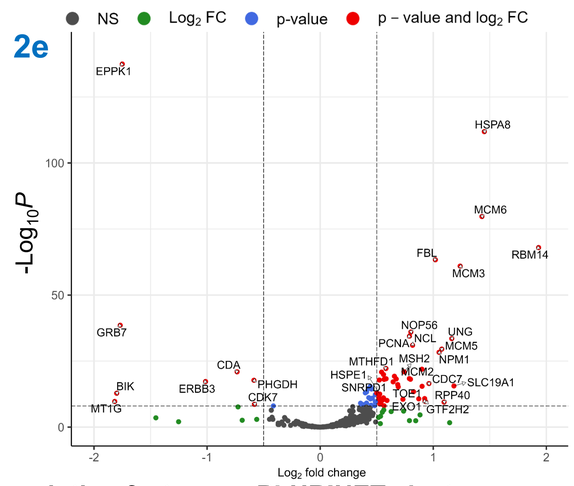
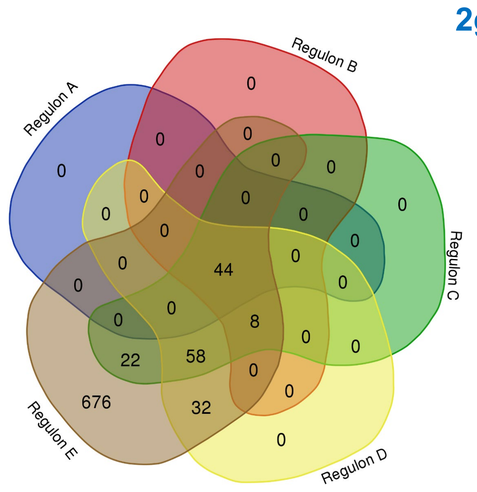


Fig 2d/2e – Volcano plots; d, most deregulated transcription factors; e, PLURINET cluster genes.

Inferred data – proliferation/Master regulators (msVIPER)

Master regulators in response to LF EMF suggest direct DNA activation *via* a major transcription factor family: Zn2+ Finger TF and the tumor suppressor *BARX2*

2f



2g

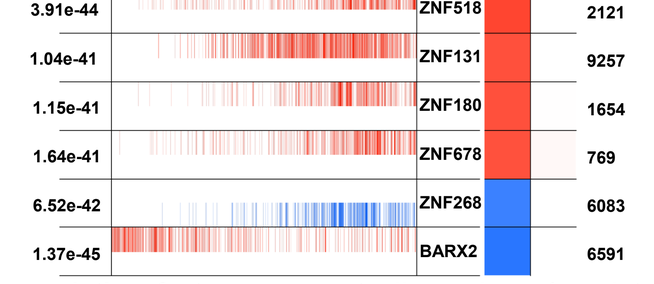


Fig 2f/2g – f, Venn diagram of common transcription factors amongst all regulons (regulatory network). g, msVIPER plot showing the enrichment of transcription factors in regulon E.

Fig 3. Anti-aging activity of LF EMF paired with reduced cellular stress

qPCR measures of telomere lengths in keratinocytes (nB=3, nT=3 replicates; ±SEM) showed no significant shortening after exposure to LF EMF for 6 passages. In contrast, telomeres were 50% shorter in control keratinocytes. Accordingly, the stress response in terms of Hsp47 (and Hsp90 (data not shown)) to starvation attenuated in LF EMF keratinocytes.

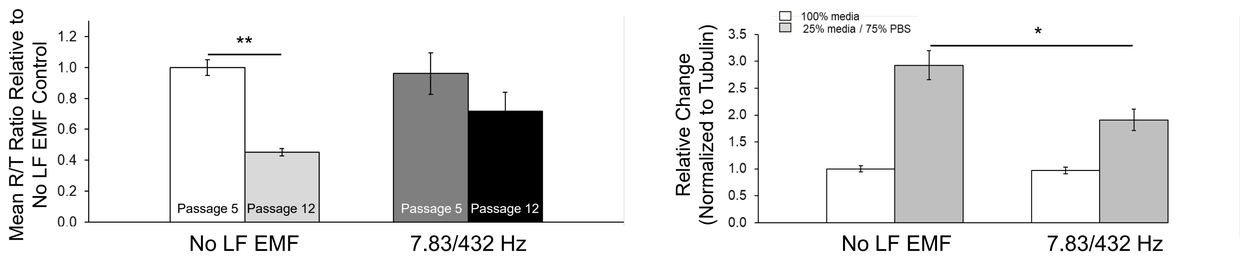


Fig 3a/3b – a, RT-qPCR to measure telomere length; b, Quantitative Western blot analysis of Hsp47

Fig 4. Electroencephalogram (EEG): Single-blinded cross-over pilot study: LF EMF entrain brain activities indicative for relaxation

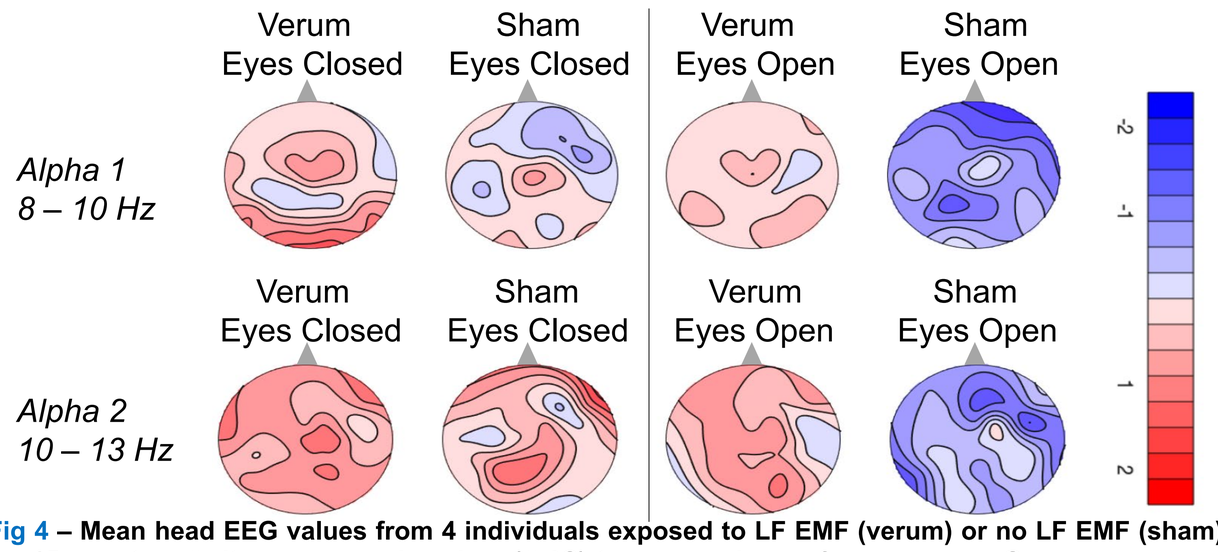


Fig 4 – Mean head EEG values from 4 individuals exposed to LF EMF (verum) or no LF EMF (sham) for 15 minutes. Multi-dimensional scaling (MDS) t-maps analysis of combined EEG recordings: red is increased, blue is decreased brain wave activity for "post" minus "pre" measures. On a scale of 2 to -2, anything above 1 or below -1 is significantly different. n=18 sessions, 4 test subjects.

Conclusion

The LF EMF technology has a significant two-pronged effect of activation and stress reduction: directly on stem cells and indirectly on bodily functions through the neuro-immunological axis.

References: (1) Levin et al., Cell, 2021. (2) Levin, Bioelectromagnetics, 2003. (3) Funk & Scholkmann, Prog Biophys Mol Biol, 2023. (4) Burr, The Yale journal of biology and medicine, 1944. (5) Marino et al., Annals of the New York Academy of Sciences, 1974. (6) Zhao et al., Nature, 2006. (7) Price et al., Int J Biometeorol, 2021.