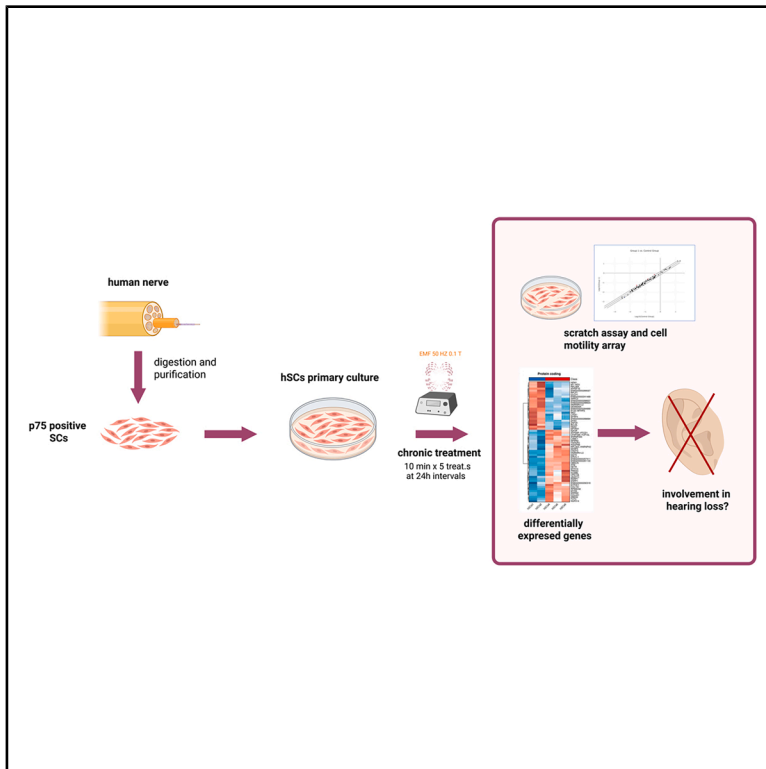


Electromagnetic exposure changes human Schwann cell motility and transcriptomic profile of hearing-loss-related genes

Graphical abstract



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In brief

Molecular biology; Neuroscience; Cell biology

Highlights

- hSCs were successfully isolated, purified, and characterized *in vitro*
- Chronic EMF exposure altered hSCs' motility and the expression of adhesion proteins
- Simultaneously, genes associated with pathways related to hearing loss were activated
- Exposure to EMF predisposes the hSCs to oncotransformation



Article

Electromagnetic exposure changes human Schwann cell motility and transcriptomic profile of hearing-loss-related genes

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SUMMARY

Schwann cells (SCs) are glial cells of the peripheral nervous system with functional plasticity in physio-pathological conditions, such as injury and oncotransformation. Interestingly, human SCs seem to develop, mature, and respond differently to other species to environmental challenges. Recently, concerns have been raised about the expansion of environmental electromagnetic fields (EMFs) and the increasing incidence of vestibular schwannoma (VS). We previously demonstrated that SCs respond to EMF by acquiring a state prone to oncotransformation. However, the biodependence of such transformation on the species (rodents versus humans) deserves clarification. Here, we obtained and characterized human SCs, showing that EMF affects proliferation, migration, and transcriptomic profile. The expression of motility proteins increased, while that of cell-to-cell adhesion proteins decreased. The functional clustering of genes differentially expressed matched those involved in hearing loss. In summary, we provide insights into the effects of chronic EMF on human SCs, highlighting its potential role in VS onset.

INTRODUCTION

Environmental exposure of human cells to electromagnetic fields (EMFs), emitted by electronic devices and appliances, has recently been indicated as a potential risk factor for human health.^{1,2} Although this issue is controversial and requires further investigation, data collected from case-control and case-case studies have suggested a correlation between exposure to EMF and the development of central tumors, such as the vestibular schwannoma (VS).^{2–7} VS, also named acoustic neuroma, is a benign tumor of the VIII cranial nerve, arising from the oncotransformation of Schwann cells (SCs) and most commonly associated with neurofibromatosis type 2 (NF2), but may also arise sporadically. It is a slow-growing brain tumor that negatively impacts the quality of life, mainly causing hearing loss, facial palsy, and loss of balance.^{8,9}

SCs are the major glial component of the peripheral nervous system (PNS), mostly forming myelin in the peripheral nerves. In fact, SCs are involved in peripheral physiological functions, such as nerve development, myelination, and axonal conduction, and in some pathophysiological conditions of the PNS,

ranging from demyelinating diseases to nerve regeneration and schwannoma growth. In the last decades, most of the studies on SCs have been conducted on rodent cell cultures, while human SCs (hSCs), isolated in the early '80s,¹⁰ have been little considered. hSCs showed some biological and behavioral differences from their non-human counterparts;^{11–13} therefore, their study deserves *a priori* a complete and in-depth characterization.

Recently, we speculated on the oncotransformation of rat SCs. Namely, we demonstrated that rat SCs, when exposed to environmental EMF challenge, changed their biological features (e.g., morphology, proliferation, migration, and myelinating capacity), shifting the phenotype toward a proliferative/migrating condition.^{14,15} In those cells, the oncosuppressor merlin was downregulated, leading to the activation of the intracellular signaling pathways MAPK/ERK-PI3/Akt and Hippo.¹⁴ We suggested that changes in rat SCs were pathologically relevant to the mechanisms underlying VS development. Furthermore, we found that chronic EMF exposure also affected a human VS cell line (i.e., the HEI-193 cells).¹⁶ Indeed, EMF-exposed HEI-193 cells altered cell proliferation, in parallel with changes in



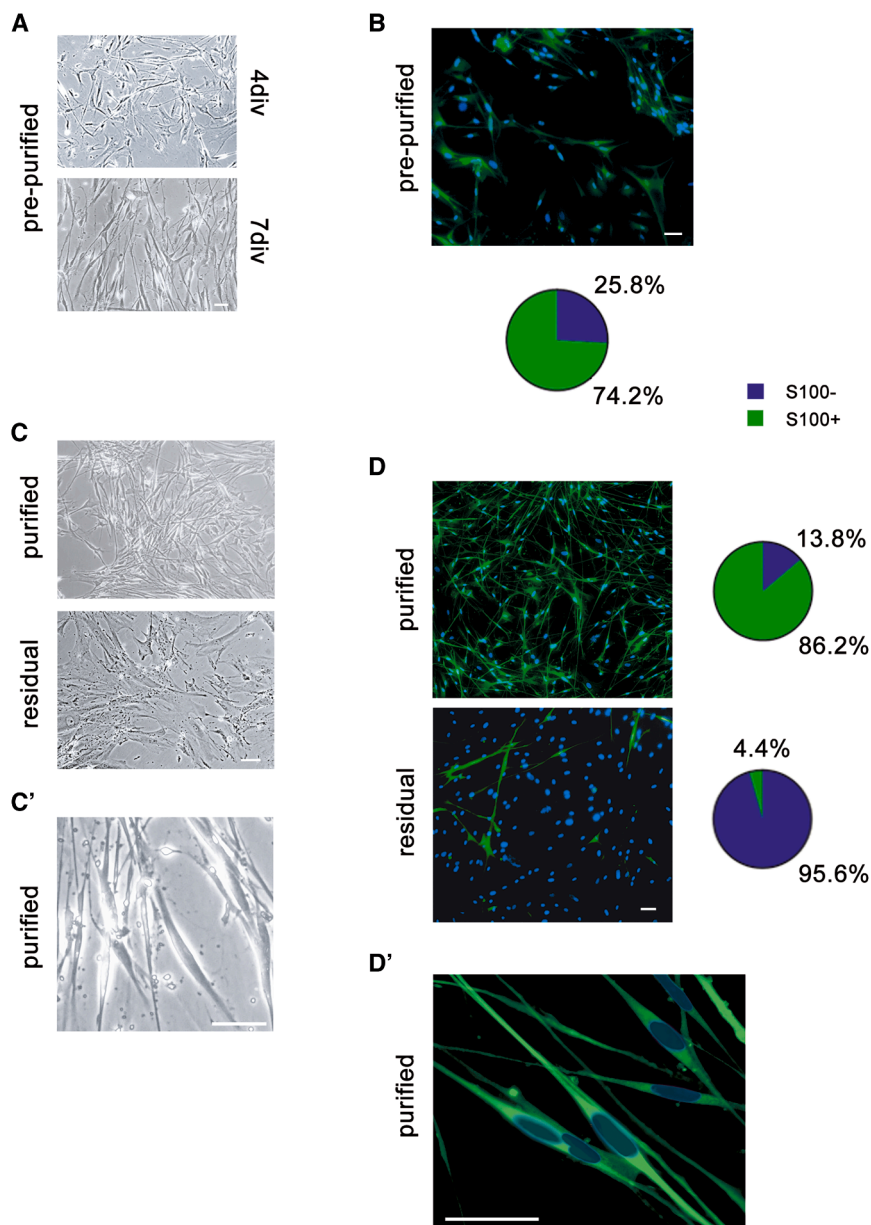


Figure 1. Characterization of hSC morphology and purification

(A) Light microscopy images of pre-purified hSCs after 4 and 7 days *in vitro* (d.i.v.). Scale bars, 10 μ m.

(B) Representative immunofluorescence image and pie chart of the immunolabeling quantification for the SCs marker S100, showing the percentage (about 74%) of immunopositive cells in the pre-purified condition. Scale bar, 10 μ m.

(C) Light microscopy images of purified and residual hSCs obtained by QuadromaCS positive selection for the specific surface marker p75 neurotrophin receptor (CD271). In C', a higher magnification image of purified hSCs showing the characteristic spindle shape. Scale bars, 10 μ m.

(D) Representative immunofluorescence image and pie chart of the immunolabeling quantification of purified and residual hSCs for the SC marker S100. About 86% and 4% of immunopositive cells were present in purified and residual hSCs, respectively. In D', a higher magnification immunofluorescent image of purified hSCs, labeled for S100, shows the typical spindle-shaped morphology. Scale bars, 10 μ m.

the expression of genes related to cell motility was altered. The RNA sequencing analysis also showed changes in the transcriptomic profile of hSCs following chronic EMF exposure. Overall, our findings suggest that chronic exposure of hSCs to EMF may change the expression of genes correlated to nervous system development, RNA metabolic pathways, and mitochondrial changes, leading to hearing loss and oncotransformation.

RESULTS

Isolation and assessment of human Schwann cells

Primary hSCs were first characterized for the expression of S100 protein, a marker of cells of glial origin and typical of SCs.

the intracellular signaling and metabolic pathways, mainly related to translation and mitochondrial activities. Accordingly, the expression of some hearing loss-related genes (e.g., *NEFL*, *TPRN*, *OTOGL*, *GJB2*, and *REST*) appeared to be deregulated following the chronic EMF exposure.¹⁶

Based on the potential discrepancies between SCs from different species, in principle we hypothesized that the effects of the EMF exposure on naive human SCs, i.e., the potential impact on their oncotransformation into VS, deserve further investigation.

In the present study, we obtained purified hSCs, which were primarily characterized. Then, the effect of EMF-exposure on these cells was analyzed. We found that chronic EMF exposure affected the proliferation and migration of hSCs; in accordance,

As shown in the Figure 1A, most of the cells presented a classical spindle-like morphology (at 4 and 7 d.i.v., respectively), even at the pre-purified stage, and about 74% of the cells were immunopositive for the SC marker S100 (Figure 1B). However, to obtain an enriched culture, primary hSCs were purified by a positive selection, based on their surface expression of p75 neurotrophin receptor (CD271), using QuadromaCS. After 1 week in culture, the fraction of purified cells was enriched in SCs, better organized with the typical spindle-shaped morphology (Figures 1C and 1C'), while the residual cells showed a completely different morphology (Figure 1C). Both purified and residual SCs were further assessed by checking for a primary antibody against S100. As shown in Figure 1D, S100+ cells appeared fusiform, with the typical morphology of differentiated SCs, giving further

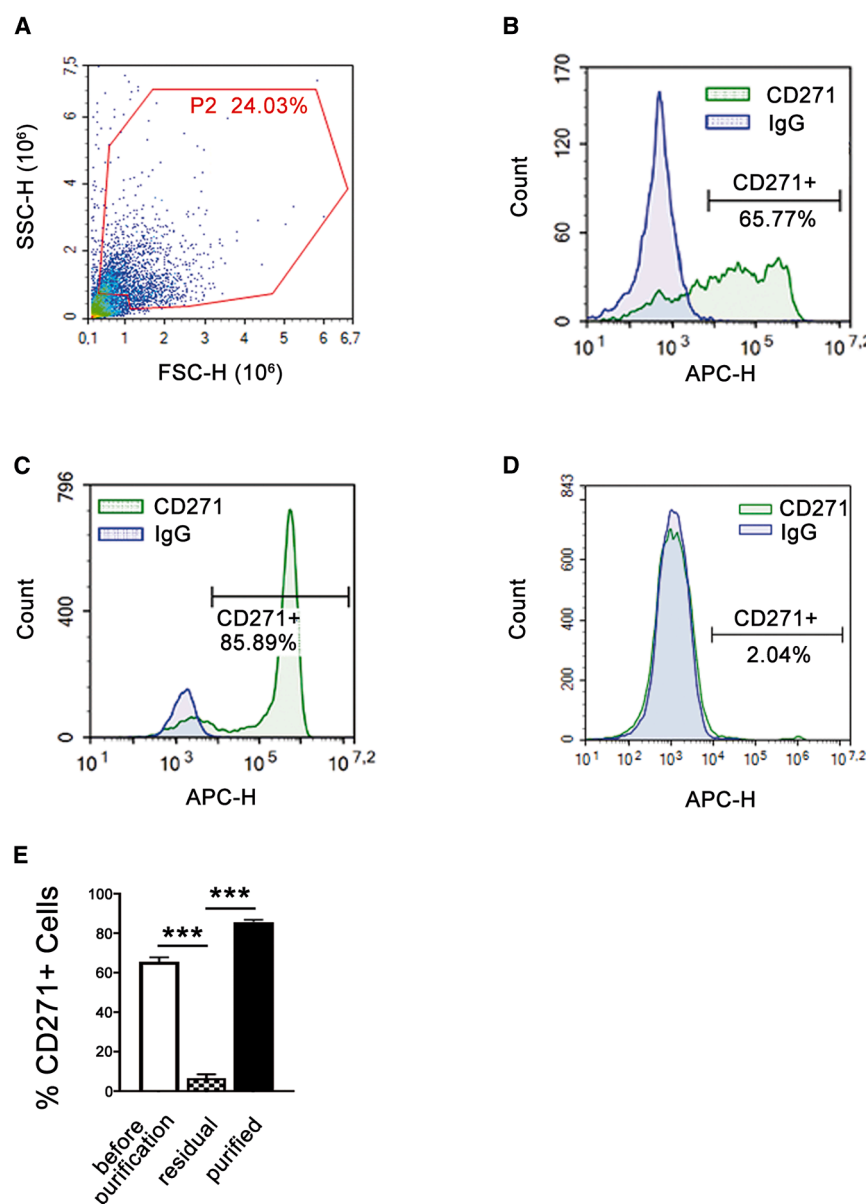


Figure 2. Flow cytometry characterization of hSCs

(A) Color dot plot in which P2 area (24.03%) represents the cell population analyzed. SSC-H: side scatter histogram; FSC-H: forward scatter histogram.

(B) CD271+ hSCs percentage (65.77%) after 1 week in culture, before purification. Surface staining for IgG was used as a reference. APC-H: allophycocyanin H (APC-H) fluoroprotein.

(C and D) The percentage of CD271+ hSCs in purified (85.89%) and residual cell culture, respectively.

(E) Histogram representation of the different percentages of CD271+ hSCs before and after purification with QuadroMACS; residual hSCs are shown. Values of columns are means $\pm$ SEM ($n = 3$). One-way ANOVA using Sidak's post-hoc test. *** $p < 0.001$.

non-purified hSCs showed about 66% of CD271+ cells (Figure 2B). Then, following purification the percentage of CD271+ hSCs increased around 86% (Figure 2C), while the residual cells showed only 2% of CD271+ cells (Figure 2D). In summary, the percentage of CD271+ cells is represented by the diagram in Figure 2E, confirming that more than 85% of purified cells are CD271+.

Chronic electromagnetic field exposure induces changes in human Schwann cells proliferation and motility

To evaluate the effects of EMF on hSCs, we applied a chronic EMF protocol, consisting of a treatment of 50 Hz, 0.1 T, 10 min, repeated for five consecutive days¹⁶ (Figure 3A). When cell proliferation was assessed, we found that five days of chronic EMF exposure induced a significant decrease ($p < 0.01$) in hSCs proliferation

compared to untreated control cells (Figure 3B; time point 0); however, at later time points, i.e., 24, 48, and 72 h after the last treatment, this effect normalized to the control levels (Figure 3B). This data was replicated by BrdU incorporation, which confirmed the decrease in hSCs proliferation following five days of chronic EMF treatment (time point 0), but not 24 h after withdrawal of EMF exposure (Figure 3C). The effect was not dependent on an increase in cell death, tested by MTT assay, since chronic 5-day EMF did not induce any change in cells viability (Figure 3D).

Flow cytometry characterization of human Schwann cells

Pre-purified, purified, and residual SCs were assessed by flow cytometry analysis for the expression of the surface marker CD271. The P2 area, highlighted in red in Figure 2A, represents the cell population analyzed (24.03%), from which cell debris has been excluded. One week after growing in culture, the

confirmation of the SCs enrichment following purification. Immunolabeling quantification showed around 86% S100+ cells in the purified culture, while in the residual cells, the percentage was very low (about 4%; Figure 1D), further confirming a high percentage of hSCs in our primary culture. The typical spindle-shaped morphology of purified SCs *in vitro* is shown in Figure 1D', at high magnification.

Interestingly, the hSC migratory capacity was modified by chronic EMF exposure. We used the wound-healing assay, applied on a cell monolayer, to measure hSC migration ability (Figure 4A), finding that the EMF exposure promoted hSC motility, repopulating the wounded region within the time points

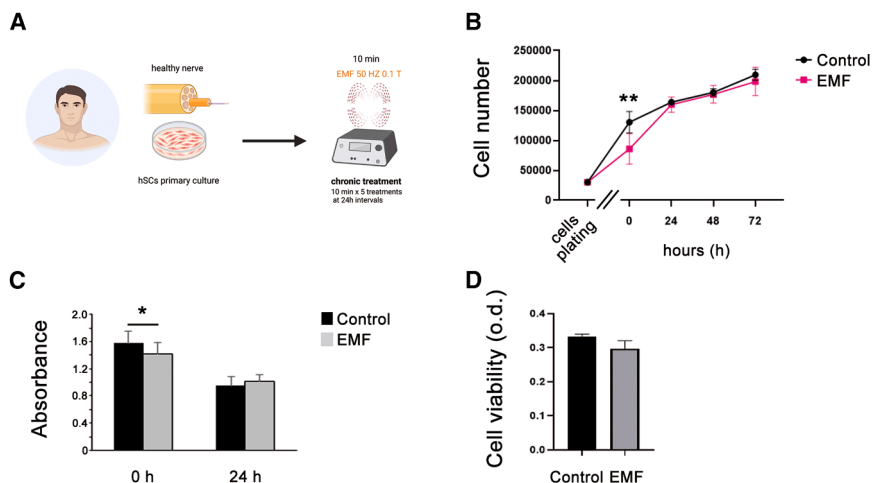


Figure 3. Proliferation and viability in hSCs exposed to chronic EMF

(A) Scheme of the experimental protocol applied. hSCs were exposed to EMF of 50 Hz, 0.1 T, for one 10-min treatment/per day for five days (every 24 h at the same time; chronic protocol).

(B) Cell proliferation assessed following a 5-day chronic EMF exposure. Time point 0 is immediately after the last EMF treatment; cell count was then done at 24, 48, and 72 h following the last EMF exposure. Experimental points were quadruplicated and replicated at least three times. Data are expressed as mean of cell number $\pm$ SEM; two-way ANOVA using Sidak's post-hoc test; $**p < 0.01$.

(C) BrdU incorporation at five days following chronic EMF treatment (time point 0; $*p < 0.05$; Student's t-test). Experimental points were quadruplicated and replicated at least three times. Data expressed as absorbance $\pm$ SEM of the mean.

(D) MTT assay for the vitality of exposed hSCs (EMF) vs. control, at five days following chronic EMF treatment (time point 0). Values are means $\pm$ SEM ($n = 3$).

analyzed (Figure 4A). A complete closure of the two sides was achieved at the longest time-point, i.e., 24 h (Figure 4A). Quantification of the covered area indicated a significant increase at 2 h

($p < 0.0001$) and 6 h ($p < 0.001$) following the last five-days exposure (Figure 4B). Moreover, the single-cell tracking analysis confirmed the migratory capability of hSCs (black arrow in

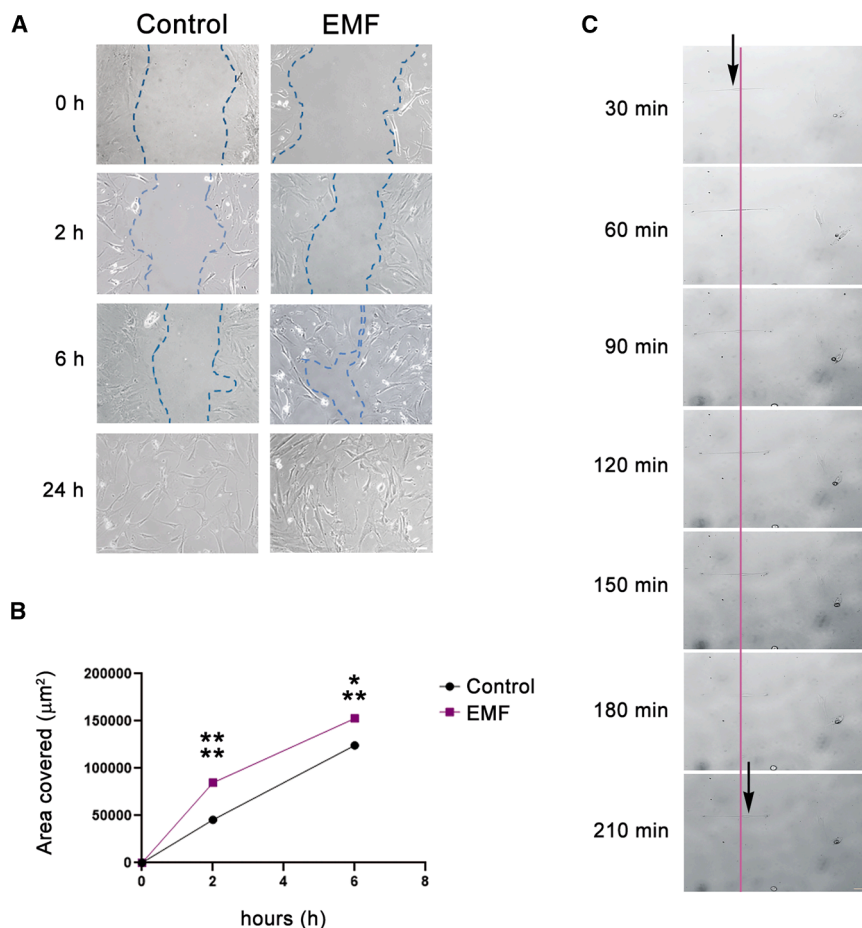


Figure 4. Migration effects of chronic EMF on hSCs

(A) Wound-healing assay, showing the hSCs migration ability: light microscope pictures were acquired at 0, 2, 6, and 24 h after the wound, in Control vs. EMF. Bar: 10 μ m.

(B) Quantification of the area covered by migrating hSCs (in squared μ m) at 2 and 6 h after the wound. Data were calculated as the difference between measurements of empty space at time 0 and the following time points (2 and 6 h). Data were expressed in μ m² $\pm$ SEM of the mean; ($n = 3$). $***p < 0.001$ and $****p < 0.0001$; two-way ANOVA using Sidak's post-hoc test.

(C) Single-cell tracking of hSCs following chronic EMF exposure. Representative frames of an hSC (black arrow), every 30 min (from 30 to 210 min following the last exposure), confirming the cell migration capability. Scale bar, 10 μ m. The whole registration (.avi file) is provided as supplementary material.

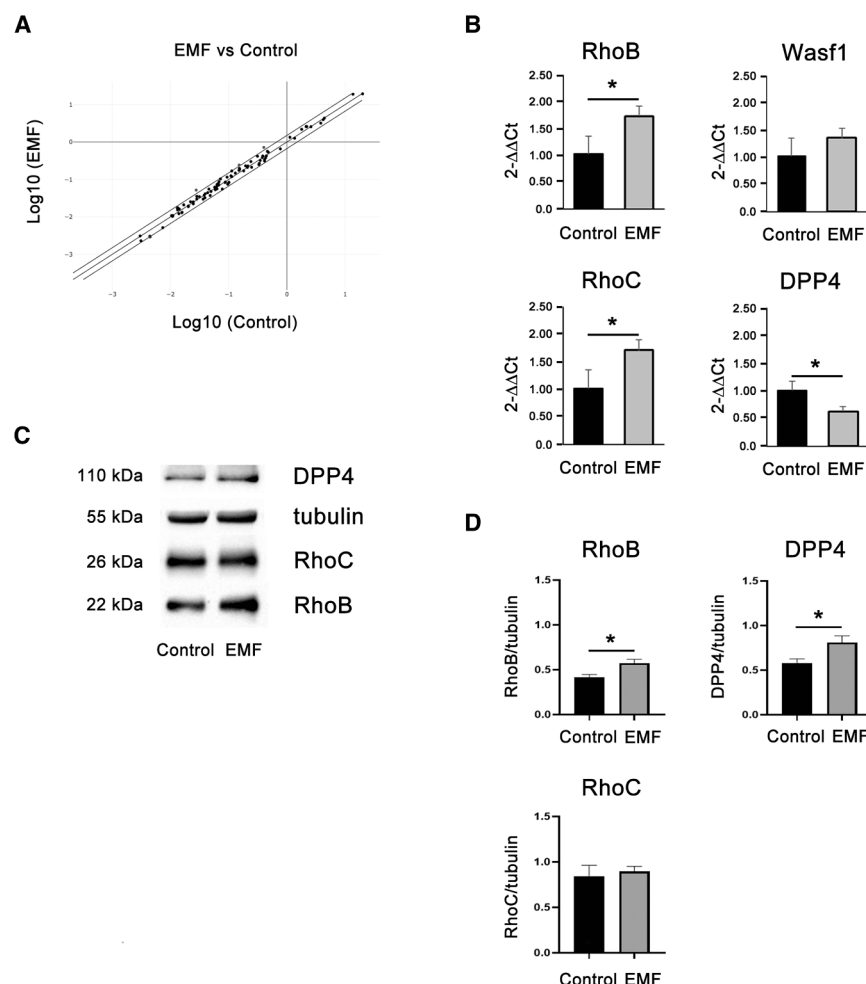


Figure 5. Changes in migration-related genes

(A) Scatterplot of the differentially expressed genes in hSCs, Control vs. EMF chronically exposed, as shown by the RT² profiler PCR array for Human Cell Motility ($n = 3$ for Control and EMF, respectively).

(B) Expression of the modified genes (*RhoB*, *RhoC*, *Wasf1* and *DPP4*) by real-time PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ using Control samples as reference ($n = 3$ for Control and EMF, respectively). * $p < 0.05$. One-way ANOVA using Sidak's post-test.

(C) Representative immunoblots of RhoB, RhoC, and DPP4 proteins after EMF chronic exposure.

(D) Quantitative analysis of protein levels, normalized per tubulin, showed a significant increase (* $p < 0.05$; Student's t test; the experiment was repeated 3 times; data are mean $\pm$ SEM) of RhoB and DPP4, while RhoC was unchanged.

sure, while RhoC seemed unchanged (Figure 5C). Quantitative blot analysis corroborated these data, showing a significant increase ($p < 0.05$) of RhoB and DPP4 (Figure 5D), respectively.

In summary, these results indicate that chronic EMF exposure is associated with a decrease in hSC proliferation and a concurrent increase in cell migration.

Differentially expressed genes in human Schwann cells following chronic electromagnetic field exposure

Primary hSCs were next analyzed for transcriptomic changes after chronic EMF exposure by RNA sequencing (RNA-Seq). Principal component analysis (PCA), a computational approach for the maximization of differences among samples, confirmed the quality of data and the separation of the two experimental conditions based on their transcriptomic profiles (Figure 6A). The higher the distances between experimental conditions (EMF vs. control), the higher the difference in terms of transcriptional profile; in parallel, more replicates are similar (reproducibility), and even more the dots plot lies in the same PCA region. We found that PC1 included 99.27% of system variability (Figure 6A), and this tends to separate the sample replicates of the EMF condition (lower side) from replicates of the control condition (upper side).

Differentially expressed genes (DEGs) were then determined from sample replicates of each condition (EMF vs. control). The analysis was performed on 15,724 genes, distinguishing between protein coding or non-coding RNAs; the DEGs were above the defined, considering a detection limit (Transcript per Million, TPM>1), and a p -value of 0.01 from DESeq2 was set as the statistical threshold (Table S1). The volcano plot represents the result of this comparison (Figure 6B), indicating the up-regulation of some genes (red dots, lower side) following chronic

Figure 4C) following chronic EMF exposure (see the whole registration as.avi file, supplementary material).

The RT² profiler PCR array for human cell motility was then performed to corroborate the possible involvement of specific proteins in migration. Among a total of 84 genes, the bioinformatic analysis revealed mostly an upregulation of three of these genes, differently involved in cell motility pathways (Figure 5A). In detail, some proteins of the Rho family, especially regulating the cellular projections, such as RhoB (Ras Homolog Family Member B) and RhoC (Ras Homolog Family Member C) showed a significant increase in gene expression ($p < 0.05$; Figure 5B), confirming the RT² profiler outcomes. Wasf1 (WASP Family Member 1), a protein involved in Rac signaling and lamellipodia formation, rose at limit of statistical significance (Figure 5B). Other proteins, such as DPP4 (dipeptidyl peptidase-4), a surface protein involved in cell-cell adhesion, showed a significant decrease in gene expression ($p < 0.05$; Figure 5B). The results from the RT² profiler PCR array, therefore, have been confirmed also by real-time PCR performed with specific set of primers. Moreover, to support these results, the protein levels of RhoB, RhoC, and DPP4 were evaluated. The immunoblots showed that RhoB and DPP4 rose after EMF expo-

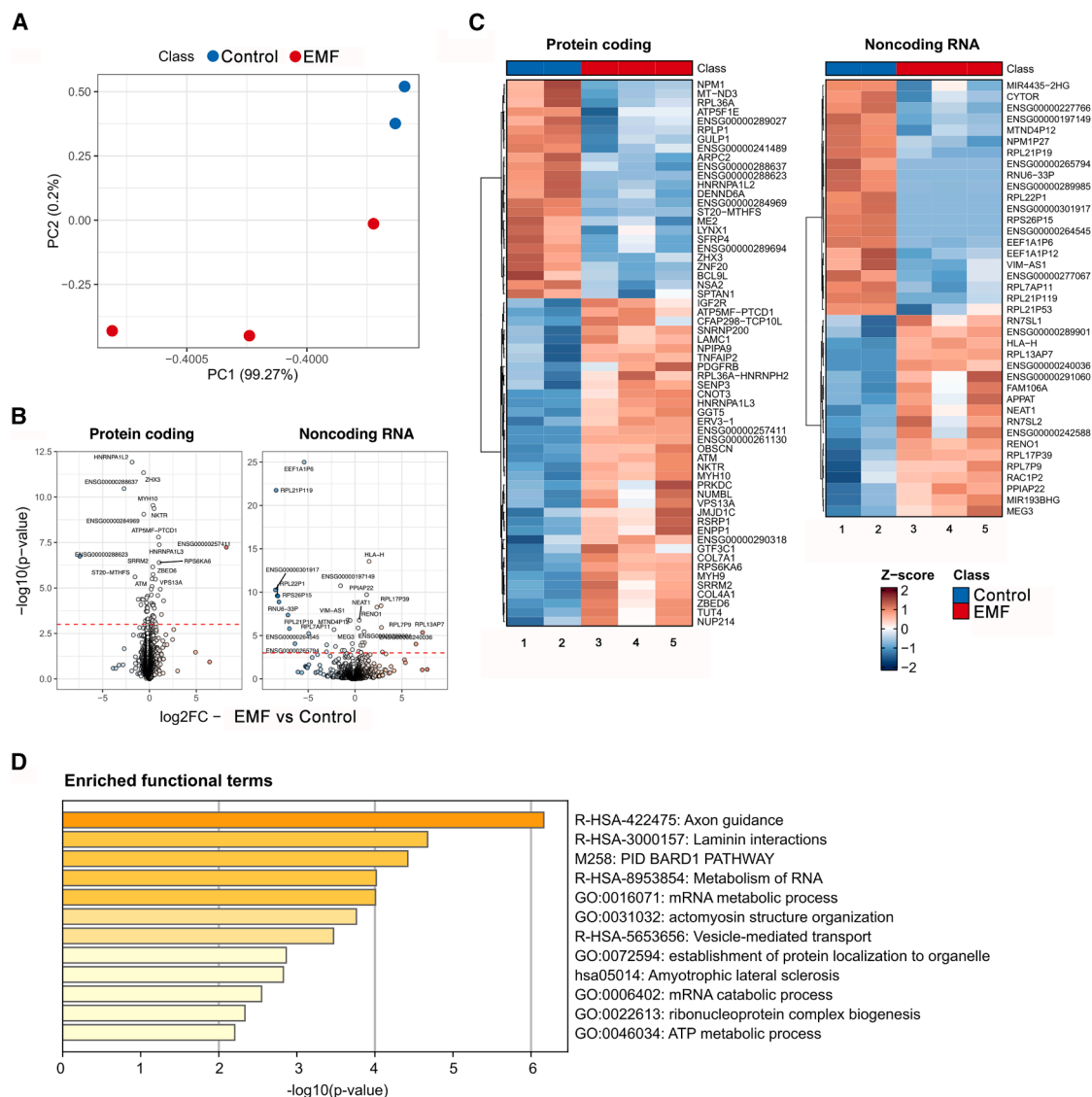


Figure 6. Bioinformatic analysis of hSCs following chronic EMF

(A) PCA of the gene expression levels measured by RNA-Seq in control (blue) and EMF-treated (red) cells.

(B) Volcano plot of the differential expression analysis results reporting the analysis log₂FC and significance. Data are reported separately for protein-coding and non-coding RNA genes. The red dashed lines represent the significance threshold of 0.01 selected in the analysis.

(C) Heatmap of the Z-score-normalized levels of protein-coding (left) and non-coding RNA (right) genes identified as differentially expressed between the two study conditions. Genes are organized based on the hierarchical clustering result.

(D) Bar plot of the statistical significance of the DEG enrichment analysis performed with Metascape.¹⁷

EMF exposure, either protein-coding or non-coding RNA. On a hierarchical clustering plot, we identified 60 DEG for protein coding (36 and 24 genes up- and downregulated, respectively) and 39 DEGs for non-coding RNA (18 and 21 genes up- and downregulated, respectively), distinguishing the control from the EMF samples (Figure 6C). Interestingly, some genes related to mitochondrial dynamics, such as *ATP5MF-PTCD1*, and promoting cell migration, tumorigenesis and tumoral invasion, such as TNF-alpha-induced protein 2 (*TNFAIP2*) and SUMO specific peptidase 3 (*SEN3*), were upregulated in the EMF cells. Furthermore, other genes in passing related to hearing loss

were upregulated in the EMF cells: myosin heavy chain 9 (*MYH9*),^{18,19}; cilia and flagella associated protein 298 (*CFAP298*),^{20,21}; platelet-derived growth factor receptor beta (*PDGFRB*),²²; natural killer cell triggering receptor (*NKTR*).²³ At once, genes involved in mitochondrial disorders, leading to hearing loss, such as NADH-ubiquinone oxidoreductase chain 3 (*MT-ND3*) and ATP synthase F1 subunit epsilon (*ATP5F1E*) were downregulated in EMF cells (Figure 6C); similarly other genes regulating growth arrest, as GULP PTB domain containing engulfment adaptor (*GULP1*), cell cycle, as ribosome biogenesis factor (*NSA2*), and cell-cell adhesion, as DENN Domain

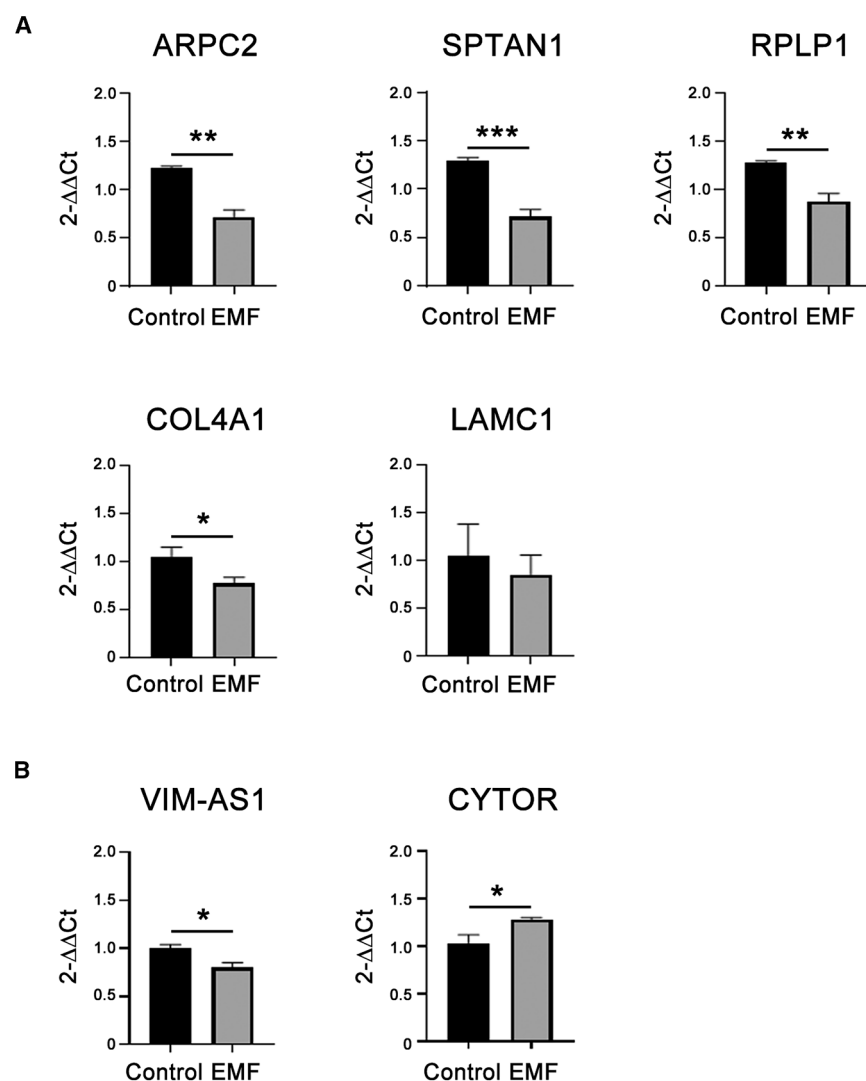


Figure 7. Validation of coding and non-coding DEGs

(A) Expression of some modified coding DEGs (*RPLP1*, *SPTAN1*, *ARPC2*, *COL4A1*, and *LAMC1*) by real-time qRT-PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ using control samples as reference ($n = 3$ for control and EMF, respectively). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. One-way ANOVA using Sidak's post-test.

(B) Expression of 2 modified non-coding RNAs (*VIM-AS1* and *CYTOR*) by real-time qRT-PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ using control samples as reference ($n = 3$ for control and EMF, respectively). * $p < 0.05$. One-way ANOVA using Sidak's post.

two other non-coding RNA were also checked to validate the strength of complex transcriptomic findings (Figure 7B): vimentin antisense RNA 1 (*VIM-AS1*) and cytoskeleton regulator RNA (*CYTOR*). As shown in Figure 7B, *VIM-AS1* resulted in significant downregulation ($p < 0.05$), further validating the clustering plot of the DEGs, whereas *CYTOR* showed a different trend, being upregulated ($p < 0.05$).

To further profile the DEGs in EMF-exposed hSCs, we performed an enrichment analysis, clustering the functional terms against the canonical pathways and biological process gene sets using the Metascape web tool.¹⁷ A list of the enriched terms is shown in Figure 6D, indicating that following EMF chronic exposure, about 12 intracellular signaling and metabolic pathways were changed in hSCs. The full list of terms is reported in Table S2. The nervous system develop-

ment and axon guidance pathway (R-HAS-422475) as well as the BARD1 pathway (M258) were the most significantly changed in EMF-exposed hSCs. In addition, some RNA metabolic pathways (R-HSA-8953854; GO:0016071), accounting for RNA processing and localization (GO:0051236), were upregulated in EMF cells (Figure 6D). Interestingly, all these pathways are complex systems participating in cell adhesion and oncotransformation. Further detailed investigations are ongoing to corroborate these changes. Then, the DEGs were explored with respect to a gene expression profiling of our hSCs chronically exposed to EMF and malignant peripheral nerve sheath tumor (MPNST) cells (ST88-14) that showed similar changes in migration.²⁴ Among them, 49 (42 protein-coding genes and 7 long non-coding RNAs) were detected in the microarray data used in the study and among them, 33 were characterized by a significant differential expression between the EMF-exposed hSCs and the MPNST cell lines ($p < 0.05$) (Table S3). Notably, 18 genes (16 protein-coding and 2 long non-coding RNAs) were associated with a coherent dysregulation between the two studies (Table 1).

Table 1. Log2FC and p-value of the DEGs

Symbol	Specific biotype	log2FC		p-value	
		This study	Sun et al. 2023 (GSE39764)	This study	Sun et al. 2023 (GSE39764)
HLA-H	lncRNA	1,50	0,89	2,83E-14	4,32E-04
GGT5	protein_coding	1,16	0,79	1,18E-05	2,81E-02
VPS13A	protein_coding	0,64	0,24	3,28E-06	8,26E-03
NKTR	protein_coding	0,49	0,16	4,29E-10	4,75E-03
JMJD1C	protein_coding	0,41	0,19	7,79E-05	5,82E-05
RSRP1	protein_coding	0,32	0,32	7,61E-04	1,54E-03
CNOT3	protein_coding	0,31	0,11	3,77E-04	7,13E-03
ATM	protein_coding	0,28	0,19	3,16E-06	5,63E-03
IGF2R	protein_coding	0,24	0,48	7,00E-05	5,65E-04
COL4A1	protein_coding	0,21	0,20	2,41E-05	6,67E-03
MYH9	protein_coding	0,19	0,31	1,17E-05	1,55E-02
LAMC1	protein_coding	0,17	0,69	4,82E-04	7,34E-04
COL7A1	protein_coding	0,14	1,43	9,50E-04	7,70E-05
NPM1	protein_coding	-0,17	-0,37	7,24E-04	1,30E-05
CYTOR	lncRNA	-0,29	-0,19	8,48E-05	1,15E-02
LYNX1	protein_coding	-0,31	-0,55	2,02E-04	8,14E-05
ZHX3	protein_coding	-0,63	-0,11	4,58E-12	3,99E-02
HNRNPA1L2	protein_coding	-1,85	-0,25	1,20E-12	1,67E-03

Log2FC and p-value of the DEGs associated with a coherent significant dysregulation in this study and in the comparison between EMF-exposed SCs and ST88-14 cell line (peripheral nerve sheath tumor cell line), which showed comparable migratory changes *in vitro*.²⁴

¹HLA-H, Human Leukocyte Antigen H; GGT5, Gamma-glutamyltransferase 5; VPS13A, Vacuolar Protein Sorting 13 Homolog A; NKTR, Natural Killer Cell Triggering Receptor; JMJD1C, Jumoni Domain Containing 1C; RSRP1, Arginine and Serine Rich Protein 1; CNOT3, CCR4-NOT Transcription Complex Subunit 3; ATM, Ataxia Telangiectasia Mutated; IGF2R, Insulin-like Growth Factor 2 Receptor; COL4A1, Collagen Type IV Alpha 1 Chain; MYH9, Myosin Heavy Chain 9; LAMC1, Laminin Subunit Gamma 1; COL7A1, Collagen Type VII Alpha 1 Chain; NPM1, Nucleophosmin 1; CYTOR, Cytoskeleton Regulator RNA; LYNX1, Ly6/Neurotoxin 1; ZHX3, Zinc Fingers and Homeobox 3; HNRNPA1L2, Heterogeneous Nuclear Ribonucleoprotein A1 Like 2.

DISCUSSION

Despite general evidence that exposure to environmental EMFs is harmless, the question of whether EMFs affect human nervous cells is still largely unexplored. Recently, great importance has been attributed to microenvironmental challenges, such as the inflammatory milieu,²⁵ but also the exposure to EMFs,²⁶ as potential risk factors and pathogenic causes for tumor development in the nervous system. It is against that background that the increased cell phone use paradoxically led to earlier diagnosis of central tumors, such as the VS.^{7,27} The issue becomes even more controversial when considering that EMF are clinically recommended for treating certain pathologic conditions. For instance, the therapeutic use of low-frequency (20 Hz) EMFs has long been proposed as a reliable therapeutic tool to promote re-myelination and peripheral nerve regeneration.^{28,29} To our knowledge, Gamboa O.L. et al.³⁰ investigated the potential biological effects of EMF exposure on SCs, reporting a modest increase in proliferation without significant morphological alterations. Similarly, another study by Siwak M. et al.³¹ showed that pulsed EMF can enhance markers of SCs proliferation, and reduce inflammation, but only fulfilling some specific conditions: (1) the pulsed EMF was particularly incisive only in combination with low-intensity ultrasound; (2) the EMF was pulsed, that

is 20 s-pulse delivered every 20 s, repeated 20 times; (3) the EMF frequency ranged from 100 to 1000 Hz; and (4) the hSCs used were from a cell line, not primary naive cell culture. Given these methodological differences, it is not surprising that our findings using human naive SCs differ. Recent work by Tai G. et al.³² elegantly reviewed the multiple cellular signaling pathways activated by the EMF (e.g., PI3K-AKT) that play key roles in guiding cell migration. Supporting this, murine neural stem cells exposed to EMF (100 Hz) were induced to differentiate into neurons, astrocytes, and oligodendrocytes,³³ while murine mesenchymal stem cells, which play a key role in tissue regeneration and wound healing, exhibited very rapid migration, within 1 min following the EMF exposure.³⁴ Overall, these studies corroborate our observation that EMF exposure can influence the directional migration of human naive SCs.

Given the complexity, however, the international scientific community has been proposing the more prudent hypothesis of “electromagnetic sensitivity” state, to indicate occasional symptoms in humans, including headaches, concentration difficulties, sleep problems, depression, and fatigue due to EMF exposure.³⁵ In any case, the topic is highly debated, and no consensus or conclusion has been drawn.

Notably, in the last decades, only a limited number of studies have investigated the hSCs, compared to rodent models (rat or

mouse origin), due to difficulties in isolation and purification. However, hSCs are of great relevance for studies addressing the biological and etiopathological causes of several peripheral diseases, including neurofibromatosis, peripheral neuropathies, and tumor growth, such as VS or peripheral schwannoma.^{36,37} In this regard, we highlight that further clarifications are necessary to unveil the biodependence of those hSCs transformation on the species (i.e. rodents versus humans).

In this study, we addressed our main efforts to purify and to characterize the naive hSCs, then demonstrating that these cells respond to the environmental chronic EMF exposure. Such a cellular and biological behavior was only partially resembling that of tumoral SCs of human origin.¹⁶ Previous studies performed on hSCs originating from the commonest VS tumor, indeed, showed that chronic EMF exposure modified the cell proliferation and migration, in parallel with intracellular signaling and metabolic pathways changes, mostly related to translation and mitochondrial activities. In particular, the expression of some genes related to hearing loss (e.g., *NEFL*, *TPRN*, *OTOGL*, *GJB2*, and *REST*) was deregulated following chronic EMF exposure. However, here we found that the proliferative effect observed in naive hSCs was transient and disappeared over time, indicating that continuous EMF exposure is necessary to sustain long-term effects. Previous research has suggested that prolonged exposure to certain types of EMFs may pose health risks, potentially contributing to conditions such as Alzheimer's disease, amyotrophic lateral sclerosis, and male infertility.^{26,38} Nonetheless, these outcomes might depend on EMF parameters, duration of exposure time, and biologic background of targets. In this context, we suggest that the different behavior of hSCs (tumoral vs. naive) to potentially harmful EMF exposure may be related to the tumor state and/or predisposition.

Hereby, the analysis of the human cell motility profile (performed by wound healing assay and supported by single-cell tracking analysis) corroborated the hypothesis that chronic EMF exposure might also stimulate the capability of hSCs to migrate. Indeed, the expression of some proteins involved in lamellipodia and stress fibers formation, such as *Wasf1* and *RhoB*/*RhoC*, was upregulated, whereas only the *RhoB* protein levels were increased. Meanwhile, the expression levels of proteins for cell-to-cell adhesion, such as *DPP4*, were downregulated, whereas this was coupled to an increase in protein. Although *DPP4* has a well-known role in cell migration, its action is context-dependent; for example, *DPP4* inhibition increases breast cancer cell migration, and *DPP4* rising promotes the migration of endometrial cancer cells.³⁹ Importantly, our findings demonstrate that chronic EMF exposure significantly alters hSC behavior, enhancing the migratory capacity, which is a critical process in tumorigenesis. This change was accompanied by transcriptomic alterations, with the upregulation of genes associated with tumorigenesis (e.g., *TNFAIP2* and *SEN3*) and hearing loss (e.g., *MYH9* and *CFAP298*), as well as the downregulation of mitochondrial genes linked to cellular metabolism (e.g., *MT-ND3* and *ATP5F1E*). In general, the transcriptomic analysis of DEGs was validated, replicating the expression changes of selected coding (*ARPC2*, *SPTAN1*, *RPLP1*, *COL4A*, and *LAMC1*) and non-coding (*VIM-AS1* and *CYTOR*) RNAs. For example, in accordance with RNAseq data, the expression of

ARPC2 and *SPTAN1* was downregulated. *ARPC2* encodes part of a protein complex crucial to the actin cytoskeleton, and implicated in hearing loss,⁴⁰ so its decrease may be related to migratory cellular rearrangements. Similarly, *SPTAN1* plays a role in SCs, where it integrates actin-dependent cytoskeletal rearrangements required for myelination⁴¹; its decrease, therefore, is consistent with the migratory state of SCs. Gene ontology analysis further revealed the involvement of pathways related to nervous system development, axon guidance, and RNA processing, suggesting that EMF exposure may induce widespread intracellular signaling changes.

The role of SCs in tumor development is not new, and the literature on this topic is constantly growing. New evidence supports the vision that the SCs play important roles in initiating tumors, then contributing to the microenvironment that can promote and maintain tumor development.^{42,43} To date, it is not clear whether this is exactly the case with VS. However, in pancreatic and colon cancer SCs can migrate toward the cancer cells, colonizing neoplastic sites before the onset of cancer invasion and exerting paracrine effects on tumor development.^{44,45} Although the EMF parameters used may not fully reflect real-world exposure scenarios, given our current and previous^{14–16} findings, we suggest that chronic EMF exposure represents a type of hit affecting SC development in vulnerable human subjects, specifically those bearing *NF2* mutations or changes in merlin expression.²⁶

In summary, we acknowledge that no preclinical *in vivo* data from other mammal species nor clinical evidence currently support the hypothesis that EMF exposure contributes to the origin of VS. Therefore, further studies are needed to determine whether and how *in vitro* findings may be relevant to VS pathogenesis. However, our preclinical results align with the assumption that, at least in line of principle, chronic EMF exposure may affect hSCs, contributing to VS and promoting oncogenic transformation.

Limitations of the study

The study's reliance on an *in vitro* model limits the extension of conclusions to *in vivo* tissues and target organs (e.g., central and/or peripheral nerves). It remains unclear whether the EMF-induced changes of hSC proliferation are transient or long-lasting, so that the possibility that the cells may regain their ability to proliferate at a later stage cannot be ruled out. Although further analysis may be needed, we speculate that prolonged EMF exposure exert lasting effects on hSC proliferation.

Overall, future research (e.g., mitochondrial functional test or *in vivo* validation) would corroborate our findings, also in other human tissues, and explore the mechanisms underlying EMF-induced oncotransformation of primary hSCs. It is arguable that the EMF parameters used are highly specific and may not reflect real-world exposure to appliances and electronics. Nevertheless, the variability of EMF conditions (e.g., pulsed or continuous, frequencies, and intensities) or simultaneous exposure to multiple EMF sources (as in the human environment) is rather complicated to study but warrants further investigation. All these conditions would help to better understand the biological effects of EMF exposure, particularly in the context of public health.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the Lead Contact, Prof. Valerio Magnaghi (valerio.magnaghi@unimi.it).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data: RNA-seq files (Fastq data and quantitation matrix) have been deposited at NCBI GEO: GSE322769 and are publicly available as of the date of publication. Accession numbers are also listed in the [key resources table](#). Original western blot images have been deposited at Mendeley (<https://data.mendeley.com/drafts/2nybr66g9c>) and are publicly available as of the date of publication. The DOI is also listed in the [key resources table](#). Microscopy data reported in this article will be shared by the [lead contact](#) upon request.
- Code: All original code has been deposited at Zenodo (<https://doi.org/10.5281/zenodo.18200711>) and is publicly available as of the date of publication. DOIs are also listed in the [key resources table](#).
- Additional information: Any additional information required to re-analyze the data reported in this article is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.C. and V.M.; investigation, A.C., T.M., A.J.R., and A.F.; bioinformatic analysis, G.F. and V.M.; data curation, A.C., T.M., A.F., G.F., and V.M.; writing and original draft preparation, A.C. and V.M.; writing, and review and editing, A.J.R., A.F., A.C., and V.M.; supervision and project administration, V.M.; funding acquisition, A.C. and V.M. All authors have read and agreed to the published version of the article.

DECLARATION OF INTERESTS

The authors declare no competing financial interests and no conflicts of interest.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti S100	Dako Agilent, Santa Clara, CA, USA	Cat# GA504
Anti CD271 - APC	Miltenyi Biotec, Bologna, Italy	Cat# 130-112-602; RRID:AB_2725865
FITC Alexa 488	Thermo Fisher Scientific, Monza, Italy	Cat# A-11008; RRID:AB_143165
Anti-RhoB	Proteintech, Manchester, UK	Cat# 14326-1-AP; RRID:AB_2179092
Anti-RhoC	Proteintech	Proteintech Cat# 67542-1-Ig; RRID:AB_2882761
Anti-CD26	Proteintech	Proteintech Cat# 68383-1-Ig; RRID:AB_3085101
Anti-tubulin	Merk Life Science	Sigma-Aldrich Cat# T6199; RRID:AB_477583
DAPI	Thermo Fisher Scientific, Monza, Italy	Thermo Fisher Scientific Cat# D3571; RRID:AB_2307445
Chemicals, peptides, and recombinant proteins		
MTT	Merck Life Science, Milan, Italy	Cat# 475989
TriFast II	Euroclone, Milan, Italy	Cat# EMR517100
GGF-2	Biotechnie, Milan, Italy	Cat# 377HB050
FSK	Merck Life Science, Milan, Italy	Cat# F6886-10 MG
WESTAR ETA C ULTRA 2.0	Cyanagen Bologna, Italy	Cat# XLS075
Critical commercial assays		
RT ² ProfilerPCR Array	Qiagen, Milan, Italy	Cat# PAHS-128Z
iScript TM Reverse Transcription Supermix	Bio-Rad Laboratories, Segrate, Milan, Italy	Cat# 1708841
Sso Advanced Universal SYBR [®] Green Supermix	Bio-Rad Laboratories, Segrate, Milan, Italy	Cat# 1725270
BRDU Cell Proliferation Assay Kit	Cell Signaling Technology, Milan, Italy	Cat# 6813
Luna [®] One-Step RT-qPCR Kit	New England Biolabs, Ipswich, MA, USA	Cat#E3005S
Deposited data		
RNA-seq files	NCBI GEO	GSE322769 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE322769)
Code of bioinformatic analysis	Zenodo	https://doi.org/10.5281/zenodo.18200711
Original WB	Mendeley	https://data.mendeley.com/drafts/2nybr66g9c
Experimental models: cell lines		
ST88-14 (malignant peripheral nerve sheath tumor)	Cellosaurus.org	RRID CVCL_8916 (https://www.cellosaurus.org/CVCL_8916)
Oligonucleotides		
VIM-AS1 FW: ATGTGAGGCCCGAGGCATTGA RV: ACTAGTACACCCCGACGTGTT	Merck Life Science, Milan, Italy	–
CYTOR FW: ATAACGGGAACGAGCGGACA RV: AGCTGCATTCCGGCTGTGAT	Merck Life Science, Milan, Italy	–
COL4A1 Fw:GACCCCGGGAGAAATAGGTTT Rv:ACCTTTGAGCCGCAAGTCGAA	Merck Life Science, Milan, Italy	–
LAMC1 FW:TAGCCCTGTGCTGCAGGAAT Rv:AACTCGCTTGCCTGTCCAT	Merck Life Science, Milan, Italy	–

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
RPLP-1 FW:TGCACGACGATGAGGTGACAGT RV:AGGCTCAACATTTACACCGGCT	Merck Life Science, Milan, Italy	–
SPTAN1 FW:CCCACCAACATCCAGCTTTCCA RV:GGCATGCAGCTCTGCTTCAA	Merck Life Science, Milan, Italy	–
ARPC2 FW:TGGAGGTGAACAACCGCATCA RV:AGAGGACCCCATCGAAATCTGC	Merck Life Science, Milan, Italy	
Software and algorithms		
GeneGlobe software	Qiagen, Milan, Italy	http://www.qiagen.com/geneglobe
NovoExpress	Agilent Technologies, Santa Clara, CA, USA	https://www.agilent.com/en/product
Zen software	Zeiss, Oberkochen, Germany	https://www.zeiss.com/microscopy/en/products
Image lab software	Bio-Rad Laboratories, Segrate, Milan, Italy	https://www.bio-rad.com/it-it/product/image-lab-software?ID=KRE6P5E8Z
MetaVue software	Molecular Devices	www.moleculardevices.com

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Tissue collection and ethical issues

Human sensory nerves were isolated from two Caucasian patients (female; 40–50 years) undergoing reconstructive surgery at Manchester University NHS Foundation Trust, UK, after informed consent was granted. Tissue samples were collected by the ComplexWounds Biobank@Manchester under ethical permissions from the National Research Ethics Committee, UK (NRES 23/NW/0320) and conformed to the World Medical Association Declaration of Helsinki. In compliance with ethical standards and data protection regulations, sensitive patient information is not disclosed in this study.

Primary cell culture preparation

Primary naive hSCs were obtained as per Colciago et al.,¹⁶ accordingly to Piovesana et al.⁴⁶ No RRID is available for these cell cultures, as they are primary cells established in our laboratory and no commercially available. Briefly, sensory nerves were dissected, and single extracted fibers were cut into small pieces, then cultured in 60 cm² dishes with SC media supplemented with 10 μM forskolin (fsk; Merck Life Science, Milan, Italy) and 60 ng/mL glial growth factor 2 (GGF-2, Biotechne, Milan, Italy) for 2 weeks. Nerves were digested with dispase (Life Technologies, Carlsbad, CA, USA) and collagenase IV (Life Technologies) for 24 h, then the cell suspension was gently triturated and passed through a sterile 70 μm mesh. After centrifugation, the cell pellet was gently suspended in SCs media supplemented with 10 μM fsk (Merck Life Science, Milan, Italy) + 60 ng/mL GGF-2 and seeded onto poly-D-lysine-coated (Merck Life Science, Milan, Italy) plates for experiments. At confluence, cells were splitted or frozen (DMEM +20% FBS +10% DMSO) for later use.

METHOD DETAILS

Cell purification

Cells were purified using the MACS MicroBeads immunomagnetic selection system (Miltenyi Biotec, Bologna, Italy), based on the specific surface expression of the p75 neurotrophin receptor CD271 in SCs. Mixed cell suspensions were incubated with anti-CD271-conjugated MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions, and subsequently passed twice through LS separation columns mounted on a magnetic separator to increase purity. CD271⁺ hSCs were collected by elution, immediately counted, and seeded onto poly-D-lysine-coated plates for further *in vitro* expansion. Cells were maintained in DMEM supplemented with 10% FBS, 10 μM fsk, and 60 ng/mL GGF-2, under standard culture conditions (37 °C, humidified atmosphere, 5% CO₂). Medium was refreshed every 2–3 days.

Flow cytometry

Cells (about 1 × 10⁵ per tube) were incubated for 15 min in the dark at 4 °C with anti-CD271 antibody (Miltenyi Biotec, Bologna, Italy) at the concentration recommended by the manufacturer. After incubation, cells were washed once with cold phosphate-buffered saline (PBS) to remove unbound antibody and suspended in 200 μL of PBS. The suspension was transferred into conical-bottomed

polystyrene tubes for flow cytometric analysis. Fluorescence of the allophycocyanin H (APC-H) fluorochrome was measured using a Novocyte 3000 flow cytometer (Agilent Technologies, Santa Clara, CA, USA), acquiring at least 10,000 events per sample, with a maximum volume of 70 μ L. An average flow rate was set to accommodate the medium-to-large size of the cells. Data were analyzed with NovoExpress software (Agilent Technologies, Santa Clara, CA, USA). Each condition was performed in triplicate, and an additional control sample (blank), incubated with PBS only and no antibody, was included to determine background fluorescence. Gating strategies were applied to exclude debris and doublets prior to analysis of CD271 expression.

Immunofluorescence (IFL) and cell characterization

Microscopy and IFL were applied for SC morphologic characterization. Cells were plated on glass coverslips at a density of approximately 4×10^4 cells per coverslip. After 24 h, cells were fixed for 20 min in 4% paraformaldehyde (Merck Life Science, Milan, Italy) and washed three times with PBS without Ca^{2+} and Mg^{2+} . Cells were permeabilized with 0.2% Triton X-100 (Merck Life Science, Milan, Italy) and blocked for 1 h in 0.25% bovine serum albumin (BSA; Merck Life Science, Milan, Italy) at room temperature. Primary antibody against the SC marker S100 (1:150; Dako Agilent, Santa Clara, CA, USA), characteristic of early-stage SCs, was applied overnight at 4 °C in a humidified chamber. The following day, coverslips were washed in PBS without Ca^{2+} and Mg^{2+} and incubated with Alexa Fluor 488-conjugated secondary antibody (Thermo Fisher Scientific, Monza, Italy) for 1 h at room temperature, protected from light. After washing, cells were mounted with Vectashield containing DAPI (Vector Laboratories, Oxford, UK) for nuclear staining. Negative controls omitting the primary antibody were included to assess background fluorescence. Imaging was performed using a Zeiss confocal laser scanning microscope, and images were analyzed with Zen software (Zeiss, Oberkochen, Germany). Quantification of S100⁺ cells was performed on five independent samples per cell type. A grid was superimposed on each image, and at least eight areas were randomly selected per sample. S100⁺ cells were expressed as a percentage of total cells, determined by DAPI-stained nuclei.

EMF exposure

Cells were exposed to 50 Hz 0.1 T EMF (at room temperature) for 10-min treatment per day for 5 consecutive days (every 24 h, at the same daytime) mimicking the chronic exposure (Figure 3A). The EMF was generated using a magnetic field generator, specifically designed and customized by Ugo Basile (Gemonio, Italy) to meet the experimental requirements. The temperature set at 37 °C and monitored during the exposure; no significant changes were observed. Following EMF treatment, the cells were then analyzed at different time points, according to the specific assays. Cells used as controls (i.e., sham-exposure) were plated in the same culture conditions, maintained in the same temperature conditions, but were not exposed to the EMF.

In vitro cell proliferation

Cells were plated in petri dishes and analyzed for viability, proliferation and migration. To assess proliferation, approximately 6×10^4 cells were plated into 35 mm petri dishes and analyzed 24, 48, and 72 h following the last treatment (time 0) of the 5-day chronic EMF. Cells were collected with trypsin 0.05%-EDTA 0.02% in PBS (Euroclone, Pero, Italy), then suspended in DMEM (Euroclone, Pero, Italy) and counted with a hemocytometer. Each experimental point was in quadruplicate and experiments replicated at least three times. Cell proliferation was also assessed by BrdU incorporation with a commercial assay kit (Cell Signaling Technology, Danvers, MA, USA). hSCs were plated in 96-well plate (1.5×10^3 of cells/well), and left to adhere for 24 h, then exposed to chronic EMF. 10x BrdU solution was added to each well, incubated overnight, and the assay performed at time point 0 (immediately after the last EMF), and 24h following the last EMF exposure, according to the manufacturer instructions. The absorbance was read at 450 nm (EnSpire, Perkin Elmer, Waltham, MA, USA).

In vitro cell viability

Cell viability was assessed using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 0.5 mg/mL; Merck Life Science, Milan, Italy]. Cells were seeded in 96-well plates at a density of 5×10^3 – 1×10^4 cells per well and allowed to adhere overnight. MTT solution was added to each well and incubated for 30 min at 37 °C under standard culture conditions (humidified atmosphere, 5% CO_2). Following incubation, the formazan crystals were solubilized by adding 100 μ L of isopropanol per well and gently shaking for 10 min at room temperature. Absorbance was measured at 570 nm using a microplate reader. Each experimental condition was performed in triplicate wells, and data were expressed as mean absorbance $\pm$ SEM. Background absorbance from wells containing medium and MTT but no cells was subtracted from all measurements.

Migration assay

Migration was tested by the wound healing assay, making a wound scratch on the cell monolayer. Cells were cultured with very low serum concentration (1% FBS) to avoid the effect of cell proliferation. Cells were photographed with a light microscope (Axiovert 200; Zeiss, Oberkochen, Germany) at different time points (2, 6, and 24 h) after the scratch at the final magnification of 600 $\times$. Images were acquired using MetaVue software and the 2D area covered by the cell migration was measured. Each experimental point was in triplicate. Each data point was calculated as the difference of the 2D scratched area (at time 0) minus the 2D remaining area at each time point. Data were expressed in $\mu\text{m}^2 \pm$ SEM of the mean.

Single-cell tracking analysis

hSCs were cultured in DMEM supplemented with 10% serum, 10 μ M forsk; and 60 ng/mL GGF at 37 °C and 5% CO₂. For time-lapse imaging, cells were seeded in imaging dishes (35 mm glass-bottom, P35G-1.5-20-C, MATTEK) at a density of 20,000 cells/cm², 24 h prior to treatment.

Live-cell imaging was performed on confocal microscope Zeiss Confocal System (Zeiss, Oberkochen, Germany) equipped with a stage-top incubator maintaining 37 °C, 5% CO₂, and humidity. Objectives: 20 $\times$ /0.8 N.A. One frame was taken every 5 min for 8 h after 5 days of EMF treatments.

Western blotting

Cells were lysed with RIPA buffer (0.05 mol/L Tris-HCl pH 7.7, 0.15 mol/L NaCl, 0.8% SDS, 10 mmol/L EDTA, 100 μ M/L NaVO₄, 50 mmol/L NaF, 0.3 mmol/L PMSF, and 5 mmol/L iodoacetic acid), containing protease inhibitors (all by Merck Life Science): leupeptin (50 μ g/mL), aprotinin (5 μ L/mL), and pepstatin (50 μ g/mL). Cell lysates were then centrifuged at 12,500 rpm for 15 min at 4 °C, and the supernatant protein content was determined using a BCA protein assay kit (Thermo Fisher). An equal amount of proteins (20–30 μ g) was separated through SDS-PAGE electrophoresis and transferred to a nitrocellulose membrane (GE Healthcare, Milan, Italy). After blocking, the membranes were exposed to primary antibodies, respectively: rabbit anti-RhoB (1:5000; # 14326-1-AP, Proteintech, Manchester, UK); mouse anti-RhoC (1:5000; # 67542-1-Ig, Proteintech); mouse anti-DPP4/CD26 (1:3000; #68383-1-Ig, Proteintech); mouse anti- α -tubulin (1:2000; T6199, Merck Life Science). Then, the membranes were incubated with HRP-conjugated anti-rabbit or anti-mouse secondary antibody for 1 h at room temperature and revealed using an enhanced chemiluminescence kit Cyanogen Ultra (Cyanogen, Bologna, Italy). In each experiment, tubulin expression was evaluated as a loading control. Blots were visualized using the Chemidoc MP Imaging System (Bio-Rad Laboratories) and analyzed by the Image Lab software (Bio-Rad Laboratories).

RNA preparation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from SCs cultures by TriFast II (Euroclone, Pero, Italy), according to the manufacturer's protocol, then quantified with Nano-Drop2000 (Thermo Fisher Scientific, Monza, Italy). One-step reverse transcription and qRT-PCR was performed using the Luna One-Step RT-qPCR Kit (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's protocol. Briefly, 10 ng of total RNA was added to the 8 μ L reaction mix containing Luna RT-qPCR Reaction Mix, Luna WarmStart RT Enzyme Mix, and gene-specific primers (final concentration 200 nM each). Reactions were run in CFX 96 Real Time System-C1000 (Bio-Rad Laboratories, Milan, Italy) under the following cycling conditions: 55 °C for 10 min for reverse transcription, 95 °C for 1 min for initial denaturation, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Fluorescence was recorded at the end of each annealing/extension step. All samples were run in triplicate, and relative gene expression was calculated using the 2^{− $\Delta\Delta$ Ct} method with 18s as internal control.

Expression profile of cell motility markers

RT² Profiler PCR array human cell motility (Qiagen, Milan, Italy) was used to profile the expression of 80 genes related to proteins involved in human cell motility pathways. Total RNA was extracted from hSCs, 2 h after chronic EMF treatment. Single-stranded cDNA was synthesized from 500 ng of total RNA by using iScript reverse transcription Supermix (Bio-Rad Laboratories, Milan, Italy), and then amplified with SYBR Green/ROX PCR master mix (Bio-Rad Laboratories, Milan, Italy) according to the manufacturer's protocol. Thermal cycling and fluorescence detection was done with a CFX 96 Real Time System-C1000 (Bio-Rad Laboratories, Milan, Italy). Data analysis was done with the 2^{− $\Delta\Delta$ Ct} method for relative quantification, and calculation also performed by GeneGlobe software (<http://www.qiagen.com/geneglobe>).

NGS technology RNA-Seq

RNA-Seq was performed on hSCs exposed to EMF or control cells, which were not exposed to EMF. For each condition, experiments were done in triplicate to perform statistics for differential expression studies. For each sample, quantity control was performed using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) to validate RNA integrity. Each sample of 500 ng total RNA was processed to obtain a library of indexed fragments using an Illumina Stranded mRNA kit (San Diego, CA, USA). Equimolar quantities of indexed libraries for each sample were loaded for sequencing on two flow cells Illumina MidOutput 150 cycles and sequenced using Illumina NextSeq550dx (RUO mode). About 40–60 $\times$ 10⁶ fragments each sample were sequenced in paired-ends (PE) modality, which characterized 75 nucleotides of both ends of each fragment of the library. The raw data were generated as fastq.gz. For quality control of sequencing, two rounds of reading generated optimal metrics, with >85% of reads passing the filtering and 93% of reads with Q score>30; technical error rate was 0.32%. Thus, no problems in sequencing occurred. Another quality control of reads was done using the tool FastQC (v 0.11.8), which analyzed some other parameters, including multiplex balance of samples, distribution of specific characteristics of each base, representativeness of nucleotides, mean size of reads and presence of adapters.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

Statistical analyses were done by using GraphPad Prism 8.0 (San Diego, CA, USA). Data are presented as mean $\pm$ standard error of the mean (SEM) from at least three independent experiments, unless otherwise indicated. Comparison between two groups was

performed using an unpaired two-tailed Student's *t* test. For multiple groups comparison, one-way or two-way ANOVA were applied, followed by Sidak's multiple comparisons post-hoc test. A *p*-value <0.05 was considered statistically significant, and exact *p*-values are reported in figure legends when appropriate. All graphs show mean values with error bars representing SEM.

Bioinformatic analysis of transcriptome

Read pre-processing was performed with FastP (v.0.21) in default setting. The filtered reads were aligned on the reference human transcriptome annotations from Gencode v48 (Build GRCh38) to be converted into quantifications of different transcripts present in the samples. The alignment was performed with Salmon tool (v.1.4) with default settings and with seqBias and gcBias options to correct for hexamer priming and GC biases, respectively. A mean alignment rate of 95% was observed. The read counts were then normalized in transcripts per million (TPM) with the txImport R package (v.1.22). Differential expression analysis was performed with DESeq2 package (v1.46) using the Wald test. To define the DEGs, a selection of genes with TPM>1 was performed, considering 15,296 expressed genes. Among them, those associated with a *p*-value <0.01 were considered as significantly DEGs. The enrichment analysis was performed with Metascape (v3.5.20250101),¹⁷ in default settings.

Differential expression analysis between EMF-exposed hSCs and ST88-14 cell line from GSE39764²⁴ was performed using GEO2R tool. Specifically, the results from the analysis were compared with the DEGs obtained in this study, considering for each gene, the probe associated to most significant differential expression.