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ORIGINAL PAPER

EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELD (ELF-EMF) INDUCED ALTERATIONS IN GENE EXPRESSION AND CYTOKINE SECRETION IN CLEAR CELL RENAL CARCINOMA CELLS

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Abstract

Background: The study aimed to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMF) on clear cell renal cell carcinoma (ccRCC) by assessing alterations in gene expression and the secretion of cytokines and chemokines. **Material and Methods:** Three ccRCC cell lines (786-O, 769-P, and CAKI-1) and a healthy HEK293 cell line were subjected to ELF-EMF exposure (frequency 50 Hz, magnetic field strength 4.5 mT) for 30 min daily for 5 days. The study examined the expression of *ADAM28*, *NCAM1*, and *VEGFC* genes, along with the secretion of 30 cytokines and chemokines. **Results:** Notably, primary tumor-derived cell lines, but not those from metastatic sites, exhibited *ADAM28* gene expression, which increased following ELF-EMF exposure. A statistically significant reduction in *VEGFC* gene expression was observed in 769-P cells after ELF-EMF exposure. Additionally, *NCAM1* gene expression was upregulated in HEK293, 769-P, and 786-O cells, representing normal embryonic kidney cells and primary tumor cells, but not in CAKI-1 cells, which model metastatic sites. After EMF exposure, there was a statistically significant decrease in transforming growth factor β1 (TGF-β1) concentration in the cell culture supernatants of HEK293 and CAKI-1 cell lines, with no other significant changes in the secretion of tested cytokines. **Conclusions:** Given the study's findings and available research, caution is warranted when drawing conclusions about the potential inhibitory effect of ELF-EMF on ccRCC progression. Standardization of experimental models is imperative when assessing the effects of EMF in a human context. Med Pr Work Health Saf. 2024;75(2):133–141

Key words: electromagnetic field, renal carcinoma, *ADAM28*, *VEGFC*, *NCAM1*, TGF-β1

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INTRODUCTION

The impact of electromagnetic fields (EMF) on the human body and its association with cancer has been extensively studied for >3 decades. It is well-established that exposure to very low-frequency electromagnetic fields (<300 Hz) can influence various cellular processes, including gene expression. Different magnetic flux densities, frequencies, and exposure patterns of EMF have been shown to induce diverse cellular responses at the mRNA level. The EMF is now recognized as a promising medical tool due to its potential anti-proliferative effects on cancer cells and its ability to facilitate drug delivery [1]. Extremely low-frequency EMF (ELF-EMF) is utilized in various industries and technologies, including power generation and distribution, where it is inherent to the operation of electrical grids and appliances. Other prevalent sources of EMF encompass radio and television broadcasts, radar

and satellite communications, magnetic resonance imaging (MRI) devices, industrial machinery, cell phones, and wireless local area networks (WLANs) [2].

The vascular endothelial growth factor (VEGF) pathway plays a pivotal role in renal cell carcinoma (RCC) by driving angiogenesis, contributing to disease progression and tumor growth. Kats-Ugurlu et al. have elucidated the close association between malignant tumor development and neoangiogenesis, with vascular endothelial growth factor C (VEGF-C) emerging as the first cytokine identified to regulate lymph angiogenesis [3]. Vascular endothelial growth factor C has been observed to bind to vascular endothelial growth factor receptor 2 (VEGFR-2), stimulating vascular endothelial cell proliferation, migration, and enhancing vascular permeability. In RCC, frequent mutations in the von Hippel-Lindau (VHL) tumor suppressor gene disrupt the degradation of hypoxia--inducible factor (HIF), resulting in the transcription of various genes including VEGF, thus promoting angiogenesis [4]. Given the highly vascular nature of RCC, one of therapeutic strategies focuses on VEGF pathway inhibition through targeted agents, such as tyrosine kinase inhibitors (TKIs), which block VEGF receptors and other angiogenesis-related receptors, alongside anti-VEGF monoclonal antibodies. Interestingly, angiogenesis is also under the modulation of ADAM28, a versatile transmembrane proteinase known to be involved in cancer cell migration and survival [5]. Among the substrates of ADAM28, connective tissue growth factor (CTGF) has been identified [6] – a potent inducer of angiogenesis, associated with *in vivo* tumorigenesis and angiogenesis [7]. Intriguingly, it can also exhibit antiangiogenic properties by interacting with vascular endothelial growth factor-A (VEGF-A) to hinder its binding to the VEGF receptor (VEGFR), thus mitigating the VEGF signaling pathway [8]. Renal cell carcinoma is significantly correlated with the occurrence of epithelial-mesenchymal transition (EMT), a pivotal mechanism in cancer advancement characterized by enhanced tumor cell migration and invasion, evasion of senescence and apoptosis, and heightened resistance to conventional radiotherapy and chemotherapy approaches [9]. In the context of kidney development, NCAM 1, known for its widespread expression, assumes a multifaceted role in these EMT processes, influencing cell migration and proliferation.

Furthermore, ELF-EMF has been observed to affect immune system cells, leading to molecular and cellular alterations [10]. The effects of EMF on the immune system appears to be dependent on factors such as frequency, amplitude, exposure time, and the specific biological characteristics of the cells being tested [11]. However, it is suspected that ELF-EMF may not significantly impact the human immune system, primarily due to its low energy level, it can modulate ongoing inflammatory responses [12]. Only a limited number of studies have investigated the effect of ELF-EMF on the secretion of cytokines produced by cancer cells without stimulating the immune system. Most research in this area has focused on cytokine serum levels [13]. Previous studies have indicated that ELF-EMF exerts its antitumor activity by reducing the levels of interleukin 9 (IL-9), which is associated with inflammatory diseases and autoimmunity, and tumor necrosis factor α (TNF-α), a cytokine involved in coordinating cellular responses and local inflammation [10,13]. Considering the crucial role of cytokines in controlling cancer development, it is essential to examine the impact of ELF-EMF on the immune function of cancer cells without stimulating the immune system. In light of this, this study aimed to investigate the effects of electromagnetic field on changes in the expression of *VEGFC*, *ADAM28*, and *NCAM1* genes, as well as alterations in cytokine secretion by ccRCC cells.

MATERIAL AND METHODS

Cell culture and ELF-EMF exposure

The research was carried out on *in vitro* cultures of 4 cell lines: HEK293, 786-O, 769-P, and CAKI-1, purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). HEK293 were selected as control cell line since these cells are widley used as a model of physiological kidney [14]. The ccRCC cell lines were chosen as an experimental model for the study on the effects of ELF-EMF due to ccRCC's prevalence as the most common type of kidney cancer in adults, and its sensitivity to ELF-EMF [15]. This choice allows for a focused investigation into how ELF-EMF exposure might influence gene expression and cytokine secretion in a cancer type of significant clinical interest. Additionally, using ccRCC cell lines alongside healthy kidney cells (HEK293) facilitates comparative analysis to discern ELF-EMF's effects on cancerous versus non-cancerous cells, enhancing the study's relevance to understanding potential therapeutic or diagnostic implications for ccRCC. All cell lines were cultured as in previous study [16] as monolayers in RPMI 1640 medium (Thermo Fisher Scientific, Warsaw, Poland) supplemented with 10% (v/v) fetal bovine serum (Thermo Fisher Scientific, Warsaw, Poland), 1% penicillin-streptomycin, and 1% L-glutamine (Thermo Fisher Scientific, Warsaw, Poland) in a humidified atmosphere,

5% CO_2 at 37°C. Moreover, the tested cell lines were studied under the same conditions as in previous study, using a Helmholtz coil that generated a field at 50 Hz, with the magnetic induction of 4.5 mT [16]. Cells were exposed for 30 min a day for 5 days at room temperature.

RNA isolation method and gene expression analysis

After the exposure, cells from the control and test group were harvested by the enzymatic method for RNA isolation with the Total RNA mini kit (AA Biotechnology, Gdańsk, Poland), according to the manufacturer's instructions. The isolated RNA was transcribed into cDNA using the TransScriba kit from AA Biotechnology. All reactions were performed in a Bio-Rad T100 thermal cycler. The obtained cDNA was used in Real-Time-PCR in which the Sensitive RT HS-PCR Mix SYBR kit was used. The expression of genes: *VEGFC, ADAM28, NCAM1,* and 2 reference genes: *GAPDH, RPL30* was studied using primers presented in Table 1. Samples in 96-well plates were prepared by combining 1 μM of appropriate primers, Master Mix containing the non-specific SYBR Green dye, and 1 μg of cDNA. In the blank sample, nuclease-free water was added instead of the cDNA.

The obtained solutions were placed in a CFX Connect Real-Time System thermal cycler (Bio-Rad, Hercules, CA, USA), and the RT-PCR reaction was performed under the following conditions: 95°C 20 s, 54°C 20 s, 72°C for *GAPDH* and *VEGFC* for 35 cycles. For the *RPL30*, *ADAM28*, and *NCAM1* genes, RT-PCR was performed under the following conditions: 95°C 20 s, 58.5°C 20 s, 72°C for 35 cycles. The analysis of the obtained results was carried out in Microsoft Excel 2016 using the $2^{-\Delta\Delta Cq}$ method according to the following formulas.

Calculating the difference for individual trials between the Cq real-time PCR values running for the test gene (Cq of the test gene) and the selected reference gene (Cq ref):

$$\Delta Cq = Cq \text{ tested gene} - Cq \text{ ref} \quad (1)$$

The mean ΔCq was drawn from all samples for a given gene:

$$\text{Mean expression} = \frac{\Delta Cq1 + \Delta q2 + \cdots + \Delta Cqn}{n} \quad (2)$$

Calculation of the normalized value of the relative expression level of the test gene in the test unknown sample against the calibrator of reference gene:

$$R = 2^{-\text{mean}\Delta\Delta Cq} \quad (3)$$

Bio-Plex Multiplex analysis

Thanks to soluble factors, including cytokines, the immune system is able to maintain homeostasis through activating and inhibiting signals while adjusting the response to environmental signals [11]. Modern platforms such as Bio-Plex Magpix by Bio-Rad allow the simultaneous determination of up to 100 proteins, peptides, and nucleic acids in a single sample [17].

In this study, each day immediately after the exposure to EMF, supernatants from the test and control groups were collected and frozen at –80°C until the analysis was performed. In total, 30 analytes were measured using 2 kits: Bio-Plex Pro TGF-β Panel 3-Plex (Bio-Rad) and Bio-Plex Pro Human Cytokine Grp I Panel 27-Plex (Bio-Rad).

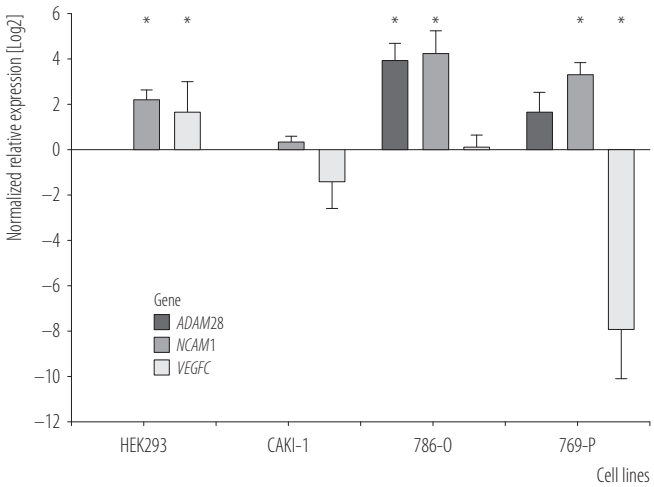
Data analysis was performed on a Bio-Plex 200 system in conjunction with Bio-Plex Manager ver. 6.1.1 using a 5-parameter (5-PL) nonlinear logistic regression curve fit model (Bio-Rad). According to the Bio-Rad Bio-Plex Multiplex Immunoassay Instruction the sensitivity of the assay was defined as analyte concentration corresponding to the median background fluorescence intensity (MFI) plus 2 standard deviations (SD) of the mean background MFI.

Statistical analysis

All results were presented as the M±SD. The data distribution was evaluated using the Shapiro-Wilk test. Obtained RT-PCR results were log2 transformed and statistically analyzed using the one-way ANOVA with

Table 1. Sequences of selected primers used for reverse transcription polymerase chain reaction (RT-PCR) testing

Gene	Forward primer	Reverse primer
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
RPL30	ACAGCATGCGGAAAATACTAC	AAAGGAAAATTTTGCAGGTTT
VEGFC	AATCACACTTCCTGCCGATG	TCTTGTTCGCTGCCTGACAC
ADAM28	GGCTGTTCAACCCCAAGAGATGAG	TTTGGATTTGAGTCCTTAGGTGTAGAC
NCAM1	GGAATTAGAGGAGCAGGTCACTCTTAC	GATGCTCTTCAGGGTCAGCGAC



* $p < 0.05$, one-way ANOVA with Holm-Šídák's multiple comparisons test, data presented as mean normalized fold change.

Figure 1. Effect of low-frequency electromagnetic field on the expression of *NCAM1*, *VEGFC*, and *ADAM28* genes

Holm-Šídák's multiple comparisons test. Effects of EMF exposition on cytokine and chemokine concentration were evaluated using the Mann-Whitney test. Differences in cytokine and chemokine secretion of unexposed cells were calculated using one-way ANOVA with Holm-Šídák's multiple comparisons test. The results at the level of $p < 0.05$ were considered statistically significant. The GraphPad Prism ver. 9.3.1 (La Jolla, CA, USA) and Microsoft Excel 2016 were used for statistical calculations. In the descriptions of results, the expression „relative to the control" means the sham group, which is a population of cells not exposed to ELF-EMF.

RESULTS

RT-PCR

Considering the variable expression of *GAPDH* between different cell lines and individual repeats, the authors opted to use *RPL30* as the reference gene. Previous research by de Jonge et al. has shown that *RPL*30 serves as a more stable reference gene [18]. Interestingly, only cell lines derived from primary tumor lesions exhibited expression of the *ADAM28* gene. Upon exposure to the electromagnetic field, a significant increase in *ADAM28* expression (4.065-fold change) was observed exclusively in the 786-O cells. Extremely low-frequency electromagnetic field exposure led to elevated expression of *NCAM1* in HEK293 (2.308-fold change), 786-O (4.375-fold change), and 769-P (3.364-fold change) cells, but it did not impact CAKI-1. Lastly, EMF exposure resulted in decreased expression of *VEGFC* solely in the 769-P cell line (–8.015-fold change). The data, shown as mean normalized fold change compared to sham-exposed controls, is presented in Figure 1.

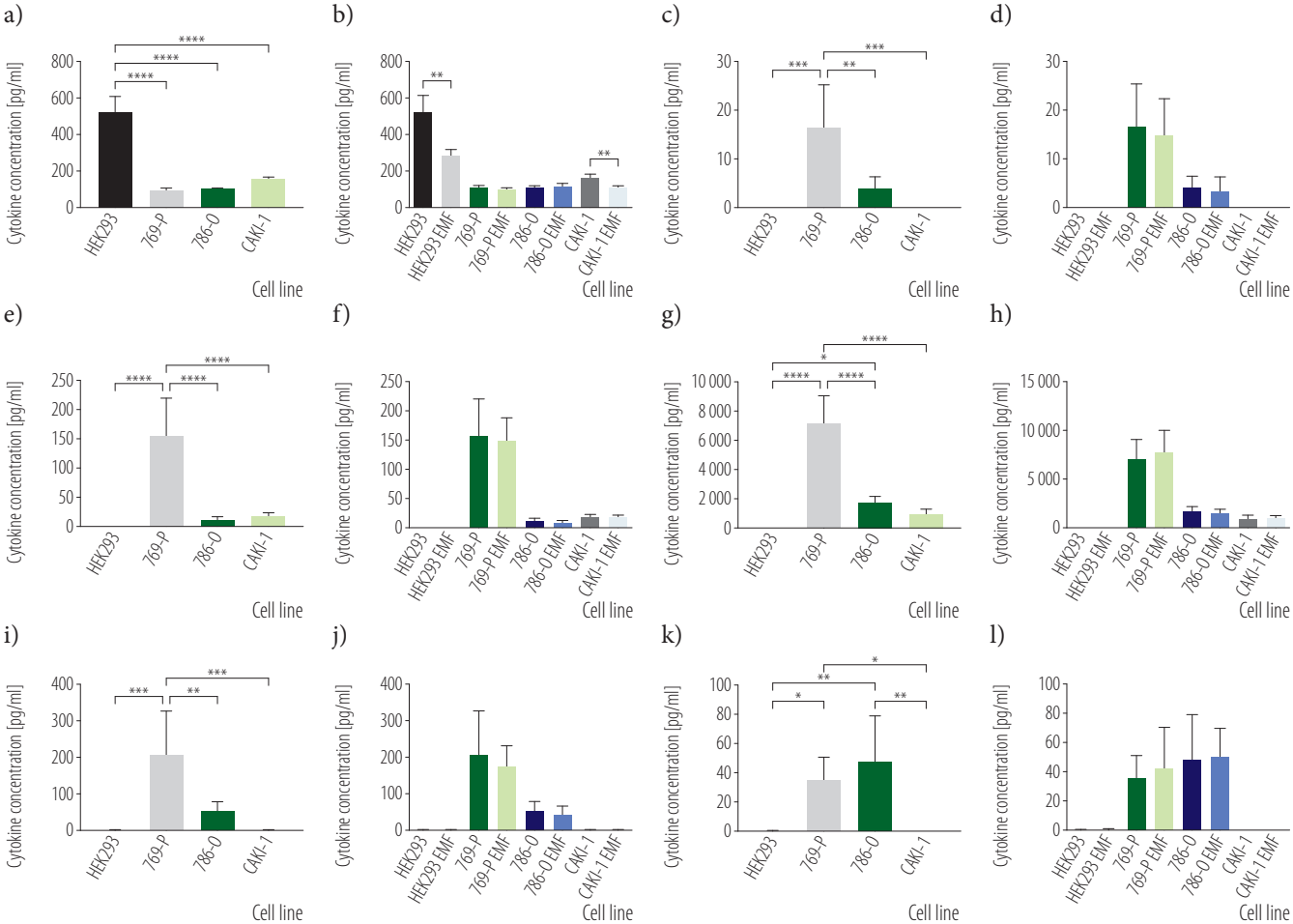
Bio-Plex analyte concentration analysis

Cytokine concentration analysis of cell culture supernatant after EMF exposure revealed a statistically significant decrease in TGF-β1 concentration in HEK293 (M±SD 282.5±33.66, M±SD 519±95.54) and CAKI-1 (M±SD 115.3±3.067, M±SD 158.1±19.74) cells. No other significant changes in the secretion of the tested cytokines were observed after EMF exposure (Figure 2).

Furthermore, a comparison of mean cytokine concentrations between different cell lines was conducted. Most significant differences between normal HEK293 and carcinogenic 786-O, 769-P and CAKI-1 cells were observed in terms of secretion of interleukin 2 (IL-2), IL-6, IL-8, TNF-α, and interferon γ (IFN-γ). Interestingly, secretion of IL-2, TNF-α, and IFN-γ was only observed in cells representing primary tumor lesions (786-O, 769-P), but not in CAKI-1 cells, which model metastatic sites.

DISCUSSION

In the previous study, a decrease in proliferation in primary cancer cell lines after ELF-EMF exposure was observed [16]. Surprisingly, this decrease did not correlate with changes in *ADAM28* expression. In fact, *ADAM28* expression was increased in cell lines derived from primary tumors but not in in HEK293 and CAKI-1. Previous research has shown that *ADAM28* is expressed in human lymphocytes, lymphoid tissues, and epithelial cells and is overexpressed in several types of cancer, including breast cancer, chondrosarcoma, head and neck cancer, B-cell acute lymphoblastic leukemia, bladder cancer, small cell lung cancer, prostate cancer, and pancreatic cancer [19]. Mochizuki et al. demonstrated that silencing the *ADAM28* gene reduced primary tumor growth and spontaneous metastasis in line 769-P cells but did not detect the production of the ADAM28 protein [20]. In previous research, it was found that exposure to ELF-EMF weakened the migration, invasion, and metastatic properties of primary tumor lines but increased *ADAM28* expression. Based on the Gerard et al. findings, *ADAM28* has been proposed as a potential therapeutic target for preventing the spread and progression of cancer [21]. However, taking into account the results from both studies, further investigation of *ADAM28* as a potential therapeutic target for the treatment of ccRCC is needed.



Results are presented as the mean ± standard deviation [pg/ml].
Data acquired using Bio-Plex MAGPIX (Bio-Rad, Hercules, CA, USA).
Results were considered significant for * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.00005$.

Figure 2. Concentration in clear cell renal cell carcinoma (ccRCC) cell lines of : a), b) transforming growth factor β1 (TGF-β1); c), d) interleukin 2 (IL-2); e), f) interleukin 6 (IL-6); g), h) interleukin 8 (IL-8); i), j) tumor necrosis factor α (TNF-α); k), l) interferon γ (IFN-γ)

The expression of the *VEGFC* gene is also indirectly linked to cell proliferation. However, the effects of this gene on cell behavior when exposed to ELF-EMF are not well understood. Some research suggests that changes in *VEGFC* expression in neoplastic cells may be linked to an increase in apoptosis induced by ELF-EMF, possibly due to an increase in reactive oxygen species, rapid influx of calcium ions, or activation of specific signaling pathways. Ndiaye found that *VEGFC* expression is higher in neoplastic cells compared to healthy cells, but the levels of expression varied [22]. They observed that *VEGFC* expression in cells from metastatic tumors reached levels similar to those in healthy cells, leading the authors to conclude that *VEGFC* plays a role in the early stages of cancer development. However, high levels of *VEGFC* may be harmful in the case of metastasis. The authors caution against targeting *VEGFC* as a therapeutic option, as they found that overexpression of *VEGFC* was associated with increased disease-free time and overall survival in patients with metastatic tumors, but in some cases, it also correlated with decreased overall survival in patients with metastases.

Furudoi et al. found that overexpression of *VEGFC* was positively correlated with the pathological stage of the tumor, lymphatic invasion and metastasis, venous invasion, liver metastasis, Duke's stage, and angiogenesis [23]. In the present research, it was observed that the level of *VEGFC* expression varied not only between the transformed and non-transformed cells but also within the test group after the cell lines were exposed to EMF. Specifically, in the 769-P cell line, *VEGFC* expression significantly decreased after ELF-EMF exposure compared to the sham-exposed cells. This is in line with the findings of Ndiaye et al. since 769-P cells had reduced

metastatic capacity after exposure to ELF-EMF [16,22]. A similar tendency was observed in the CAKI-1 cell line, although the differences were not statistically significant. In a separate study, Mochizuki et al. suggested that *ADAM28* may indirectly promote *VEGF*-induced angiogenesis [6]. In this study a significant increase in *ADAM28* expression and a decrease in *VEGFC* expression following ELF-EMF exposure were observed. These changes were associated with reduced migration and invasion properties of RCC cells, suggesting a potential involvement of *ADAM28-VEGFC* dependency. These findings underscore the significance of *VEGFC* in cancer development and emphasize the necessity for further exploration of its behavior and impact on signaling pathways in response to tumor cell exposure to electromagnetic fields.

The *NCAM1* expression is functionally associated with neoplastic disease progression. Sasca et al. found that the NCAM1 protein regulates survival, stress resistance, and self-renewal of blasts in acute myeloid leukemia *in vitro* and *in vivo*, but the expression of this gene is heterogeneous in different types of acute myeloid leukemia, suggesting a non-specific regulatory mechanism [24]. *NCAM1* re-expression has also been observed in the regeneration process in certain tubules, as well as in renal cell carcinoma and in RCC metastasis in the central nervous system and adrenal glands [25]. Cirović et al. concluded that the NCAM1 protein is present in RCC tissue regardless of histological type, but its expression is related to the nuclear tumor grade in ccRCC, making it not a useful marker for differentiating RCC but correlated with aggressive behavior and tumor metastatic potential [26]. In the present study, the *NCAM1* expression increased relative to the control group when cells were exposed to low-frequency electromagnetic radiation. Guan et al. observed that high *NCAM1* expression in ameloblastoma inhibits the migration of these tumor cells [27]. Present research suggests that ELF-EMF exposure which leads to increased *NCAM1* expression and faster aggregation of cells in the hanging drop assay in primary lesion cell model, inhibits the metastatic capacity of RCC cells [16].

In the last decade, the role of the tumor microenvironment (TME) in tumor progression and metastasis has become more evident. Moreover, recent studies have highlighted the importance of immune factors such as cytokines and chemokines in these processes [28]. Analysis of cytokine secretion showed that EMF significantly influences the secretion of TGF-β1 – a dimeric isoform of the 25 kDa TGF-β1 polypeptide that plays a role in many important cell functions, including differentiation, adhesion, migration, angiogenesis, apoptosis, proliferation, and immune surveillance. Paradoxically, despite its ability to inhibit cell proliferation, TGF-β1 is highly expressed in the neoplastic tissue of many patients with various types of cancer. As a cytokine, TGF-β1 modulates the activation of lymphocytes and can locally influence the immune response in ccRCC, inhibiting the immune action on the tumor and promoting the formation of metastasis. Many studies have confirmed the correlation between increased TGF-β1 expression and advanced cancer stage and decreased patient survival. The increased expression of TGF-β1 in these patients is associated with several specific aspects of tumor progression, including epithelial-mesenchymal transformation, angiogenesis, and metastasis [29–31]. The ELF-EMF model used in this study showed a significant effect on the CAKI-1 metastatic cell line, in which the concentration of TGF-β1 decreased, which may suggest the potential role of ELF-EMF as an adjunct therapy during immunotherapy. Therefore, low-frequency EMF may reduce the "malignancy" of the metastatic line. However, it is important to note that the increased concentration of TGF-β1 in neoplastic cells may result not only from increased secretion of this cytokine by the neoplastic cells themselves but also from recruitment into the tumor microenvironment of other types of cells that produce TGF-β1 including stromal fibroblasts, tumor-associated macrophages, dendritic cells, and immature myeloid cells [29]. As a result, the immunosuppressive character of ELF-EMF may be reduced *in vivo*. Additionally, TGF-β1 has an effect on endothelial cells and upregulates VEGF, which promotes angiogenesis. Increased VEGF levels also appear to recruit more endothelial cells, leading to prolonged angiogenesis. Therefore, further research is needed to confirm obtained results in mice model. In this study, the cytokine VEGF was secreted in trace amounts or was not secreted at all, making it difficult to determine the correlation between the concentration of TGF-β1 and VEGF [31]. Finally, the results of the other evaluated cytokines suggest that ELF-EMF does not have a significant effect on the secretome, probably due to its low energy level. This is consistent with the findings of other researchers.

The cytokine and chemokine secretion between different cell lines was also investigated. Notably, distinct differences in the secretion of IL-2, IL-6, IL-8, TNF-α, and IFN-γ between 769-P (representing a primary ccRCC cell line) and CAKI-1 (representing a metastatic

ccRCC cell line) were observed. Presented research revealed that IL-2, IL-6, and TNF-α secretion in the CAKI-1 line was significantly lower compared to 769-P.

Interleukin 6 is associated with transcriptional regulation, correlates with the histological stage of the tumor, and is associated with poor prognosis in RCC. The role of IL-6 has been strongly emphasized in early-stage cancers, contributing to the formation of a microenvironment that promotes progression and even initiation of tumor processes [32]. What is more, IL-6 also induces angiogenesis and tumor vascularization mediated by the vascular endothelial growth factor VEGF. In the study, elevated levels of IL-6 in the supernatant of 769-P cells were observed, and interestingly, a significant decrease (–8.015-fold change) in the expression of *VEGFC* following exposure to ELF-EMF was also noted. This indicates a critical link between IL-6 and VEGF in ccRCC cell lines, particularly considering the distinct cytokine secretion profile in cells derived from primary and metastatic tumor lesions [33,34].

Interleukin 2 has been utilized in the treatment of ccRCC, particularly in metastatic cases, since the 1990s [35]. In the study, it was found that the secretion of this cytokine in CAKI-1 cells was undetectable both before and after exposure to ELF-EMF, but the secretion of this cytokine in cells derived from primary tumors was observed. Clinically, 1 significant limitation of using IL-2 as a chemotherapeutic agent is its requirement for high doses to achieve potent anti-cancer effects. Unfortunately, such high doses are associated with toxicities, including vascular leak syndrome, pulmonary edema, hypotension, and cardiac toxicity. Some researchers propose that the induction of IL-6, TNF-α, and IFN-γ may potentially contribute to the development of IL-2 induced vascular leak syndrome [36].

Tumor necrosis factor α has been demonstrated to trigger a network of inflammatory cytokines, chemokines, matrix metalloproteinases, and endothelial adhesion molecules. In the context of RCC, TNF-α functions as an autocrine growth factor, promoting tumor growth and metastasis [37,38]. Elevated serum levels of TNF-α have been associated with larger RCC size. The study conducted by Ho et al. further confirmed that TNF-α not only promotes proliferation and angiogenesis but also facilitates tumor transformation, invasion, and metastasis [38].

In present study, IL-8 and IFN-γ concentrations in cell culture supernatant were statistically significantly higher compared to the metastatic CAKI-1 line. Interleukin 8 is associated with disease progression, metastasis, and poor patient prognosis. In esophageal cancer, IL-8 has been implicated as a factor responsible for metastasis, e.g., to the lymph nodes [39]. Interferon gamma, on the other hand, acts as a tumor suppressor by stimulating antitumor immune responses [39]. As a primary product of the Th1 immune response, IFN-γ activates innate immune responses, including macrophages and natural killer cells, via a positive feedback loop [40]. Perhaps it is due to the above-described functions of the proteins, which are primarily important in the initial stages of tumor development, that they are not secreted in such high concentrations after metastasis, as is the case with the CAKI-1 cell line. Nonetheless, in order to obtain accurate answers defining the changes, roles, and relationships of particular cytokines and chemokines in the TME, also after exposure to ELF-EMF, its direct interrelationships with cells directly involved in the immune response should be thoroughly studied.

CONCLUSIONS

This study examined the effect of ELF-EMF exposure on kidney cell lines, including primary cancer cell lines, metastatic cell lines, and a "healthy" cell line. The results suggest that ELF-EMF may inhibit the progression of ccRCC by inducing changes in the *ADAM28, NCAM1* and *VEGFC* expression. However, the inconsistency in the results from various studies that have investigated the effects of ELF-EMF suggests that there may be an unidentified factor that influences the impact of EMF on cells. Moreover, it is important to note that the investigations detailed in this manuscript were conducted using cell lines possessing specific mutations and gene expression profiles, which might not comprehensively mirror the characteristics of primary cells. It should be acknowledged that 2D cell cultures fail to encompass the intricate interactions occurring within the tumor microenvironment, an aspect of particular significance in the context of RCC. Further research is needed to understand the cellular activity of unitary ELF-EMF in different types of human cells and to standardize the experimental model for analyzing the impact of EMF on various types of cells, not just cancer cells.

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Interpretation of results: Aleksandra Cios, Damian Matak, Sławomir Lewicki, Małgorzata Palusińska, Łukasz Szymański
References: Wanda Stankiewicz, Łukasz Szymański

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