Bundesamt
für Strahlenschutz

**Spotlight on EMF Research**

# Spotlight on “Effects of 5G-modulated 3.5 GHz radiofrequency field exposures on HSF1, RAS, ERK, and PML activation in live fibroblasts and keratinocytes cells” by Joushomme et al. in Scientific Reports (2023)

**Category [radiofrequency, in vitro study]**

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Competence Centre Electromagnetic Fields (KEMF)

## 1 Putting the paper into context by the BfS

The new mobile communications technology 5G currently operates at frequencies between 700 MHz and 4 GHz. An important frequency band for 5G use is at 3.5 GHz. To date, there are not as many studies investigating possible health effects at 3.5 GHz as there are for lower frequency ranges. As 5G will also use frequency ranges higher than 3.5 GHz, and the penetration depths of electromagnetic fields (EMF) into the human body further decreases with increasing frequency, skin cells become a primary target when studying possible health effects of 5G exposure. One way to investigate if 5G exposure has negative effects on skin cells, is to check the activation of different cellular stress response pathways. A closer look at irregularities of these pathways will contribute to an improved health risk assessment of 5G.

## 2 Results and conclusions from the authors’ perspective

The authors investigated the hypothesis whether exposure to 5G-modulated 3.5 GHz radiofrequency (RF)-EMF can affect stress response in human skin cells. For that purpose, the activity of four proteins involved in environmental cell-stress response pathways were studied: i) Heat Shock Factor 1 (HSF1), a main regulator of the transcription of heat shock proteins in eukaryotes; ii) Rat Sarcoma virus (RAS) and iii) Extracellular signal-Regulated Kinase (ERK), both are key elements in the RAS/MAPK signaling pathway important for various cellular processes such as gene expression, growth and survival; and iv) Promyelocytic

Leukemia Protein (PML), which forms the basis of PML nuclear bodies that form in response to various stress conditions, including oxidative stress, and are important for apoptosis and DNA repair. Because skin becomes the primary target tissue of 5G exposure, keratinocytes (HaCaT cell line) and human skin fibroblasts (XP6BE cell line) were used as cellular research models. Keratinocytes are the cells of the uppermost, protective and air-exposed layer of the skin, while fibroblasts are part of the connective tissue below this layer. The inter- and intramolecular protein interactions of engineered proteins and protein domains, reflective of stress signal transmission, were assessed by Bioluminescence Resonance Energy Transfer (BRET), a technique based on nonradiative energy transfer between a donor and an acceptor. To measure BRET in live cells, cDNA expression vectors encoding the proteins in question and marked with acceptor and/or donor BRET-probes were designed. The expression vectors were then inserted into the cells by transient transfection.

The transfected fibroblasts were exposed or sham-exposed to a 5G-modulated 3.5 GHz signal for 24 h at specific absorption rate (SAR) levels of 0.25, 1 and 4 W/kg. A continuous wave (CW) mode as well as intermittent exposure (IE) (5 min ON/10 min OFF) were implemented. To overcome temperature increases induced by exposure, the cell incubator was set up to maintain the biological samples at 37°C throughout the experiment.

To assess the impact of 24 h exposure on basal or chemically-induced activation of HSF1, RAS, ERK and PML, transfected skin fibroblasts were incubated with increasing concentrations of different, pathway-specific chemical substances, and the resulting BRET signal was measured. For comparison of 5G to sham-exposed cells, three values out of each resulting sigmoidal dose-response curve were determined: 1) the bottom plateau as a measure for the basal activity, 2) the top minus the bottom plateau as a measure of the maximal efficacy, 3) log EC50 as the chemicals' potency to trigger activation, i.e. the concentration at which the BRET response was halfway between the bottom and top of the curve.

First, the impact of exposure on basal or chemically-induced HSF1 activation was assessed. Transfected cells were challenged for 18 h with MG132, a proteasome inhibitor that triggers proteotoxic stress. RF-EMF exposure led to a statistically significant and consistent decrease of HSF1 basal BRET at 0.25 W/kg CW and at 0.25 and 1 W/kg IE mode. No differences to sham-exposed cells were found for any other parameter or condition.

Second, chemically induced activation of RAS and ERK stress sensors was achieved by challenging the cells for 15 min with phorbol-myristate-13-acetate (PMA). The only statistically significant effect observed was a slight decrease in the PMA potency to activate ERK at a SAR of 0.25 W/kg CW mode.

Third, cells were challenged with arsenic trioxide, an inducer of oxidative stress, triggering SUMOylation of PML. SUMOylation, the addition of the protein SUMO (**S**mall **U**biquitin-like **Mo**difier) to PML, is a key-event leading to PML activation. A statistically significant decrease of basal SUMOylation was seen in cells exposed to 4 W/kg CW mode and a statistically significant decrease in the maximal efficacy of arsenic trioxide was seen in all exposure groups in CW mode.

The same set of experiments, but in CW mode only, was also done in keratinocytes. The only observed difference between sham- and RF-EMF-exposed keratinocytes was a statistically significant increase in the maximal PMA efficacy to activate ERK when cells were exposed at 1 W/kg.

In the discussion the authors point out that the few statistically significant changes observed are inconsistent across cell types, effective SAR, exposure mode and cellular stress response pathway. No dose-response-dependent effect was seen, except for the arsenic trioxide maximal efficacy to trigger PML SUMOylation, but the magnitude of the effect was small. The decrease of HSF1 basal activity at low SAR levels observed in fibroblasts was also seen in a previous study on HEK293T cells [2]. Because there was no effect on HSF1 at high SAR levels, the authors discuss the possibility of a hormetic dose-response effect that should be further investigated. Hormesis stimulates a biological response in cells at low, subtoxic amounts of a stressor, but leads to detrimental effects at high, toxic levels of the same stressor. Overall, the authors conclude that their study shows no conclusive evidence for molecular effects in skin cells when exposed to 5G RF-EMF signals for 24 h.

# 3 Comments by the BfS

Joushomme et al. present a comprehensive and well-conducted study with a clear hypothesis. The authors focus on four proteins involved in important stress response pathways of cells and use an elegant biosensor engineering approach that enables monitoring the activity of these pathways using BRET. The methods are extensively described and quality standards, including a well-conducted dosimetric evaluation, temperature control, sham control and independent experiments for each experimental condition are implemented.

The authors discuss their results in view of a possible hormetic dose-response relationship, but their argumentation is not fully comprehensible. The decrease in basal and chemically induced activation of the stress response proteins HSF1, ERK and PML in fibroblast cells was seen at either high (4 W/kg for PML) or low SAR levels (0.25 or 1 W/kg for HSF1 and ERK). In keratinocytes, the only effect seen was an increase, and not a decrease, in PMA's efficacy to activate ERK at 1 W/kg. These inconsistent results make it difficult to infer a hormetic dose-response relationship. It is also unclear why there should be a decrease in basal levels of stress response proteins at low levels and not an increase, because a hormetic effect is generally characterized by stimulation of biological responses at low levels of a stressor [3]. It cannot be excluded, however, that RF-EMF exposure has some kind of effect only in specific stress response pathways, like the ones including HSF1.

A limitation of the study, as also mentioned by the authors, is the use of multiple testing, which means that false positive results are likely to occur. Along with the absence of a clear dose-response relationship and contradicting results in fibroblasts and keratinocytes, there is no conclusive evidence for molecular effects of 5G-modulated 3.5 GHz signals. The independently observed decrease on basal HSF1 levels in two cell lines need further validation, including additional SAR levels and exposure durations.

The methodologically sound and comprehensive study makes an important contribution to the risk assessment of 5G. In summary, we agree with the authors' conclusion that the study does not provide conclusive evidence that 24-hour exposure of skin cells to 5G signals triggers a stress response. Similarly comprehensive studies on stress reactions of skin cells in the millimetre wave range (>20 GHz) would be a useful addition.

# References

[1] Joushomme A, Orlacchio R, Patrignoni L, et al. Effects of 5G-modulated 3.5 GHz radiofrequency field exposures on HSF1, RAS, ERK, and PML activation in live fibroblasts and keratinocytes cells. *Sci Rep*. 2023;13(1):8305. Published 2023 May 23. doi:10.1038/s41598-023-35397-w

[2] Poque, E. et al. Effects of radiofrequency field exposure on proteotoxic-induced and heat-induced HSF1 response in live cells using the bioluminescence resonance energy transfer technique. Cell Stress Chaperones 26, 241–251 (2021) {13}

[3] Mattson MP. Hormesis defined. *Ageing Res Rev*. 2008;7(1):1-7. doi:10.1016/j.arr.2007.08.007

**Impressum**

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