Original Research

Exposure to 50 Hz electromagnetic fields enhances hair follicle regrowth in C57BL/6 mice

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Impact statement

In this study, our experiments confirmed that 50 Hz EMF affected hair follicle regrowth, and 50 Hz EMF enhanced K15$^+$ stem cells proliferation in the hair bulb and follicular outer root sheath of hair follicles. Those results indicated that 50 Hz EMF may be beneficial for functional healing of hair loss.

Abstract

Many studies have suggested that electromagnetic field activity affects the cellular activity of many types of cells involved in forming hair follicles. However, the bio-effects of electromagnetic fields on hair follicle growth have not been fully elucidated. This present study was designed to determine whether 50 Hz electromagnetic fields increased hair follicle regrowth. In this experiment, C57BL/6 mice were used to present the model of depilation-induced hair follicle cycling, and then those mice were divided at random into the control group and the electromagnetic field group. After electromagnetic field (50 Hz, 5 mT) exposure for 16 days, the skin specimens of the mice were harvested to assess for hair regrowth, and epidermal stem cells proliferation was evaluated by immunofluorescence staining. The expression and location of keratinocyte growth factors were also tested. Our results showed that, compared to the control, the hair club formed faster on the 3rd day, and most of the hair shafts erupted earlier from the pore in the epidermis on the 9th day after depilation, and the hairs length was significantly longer on the 16th day within the electromagnetic field group. After electromagnetic field treatment, there were more Ki67$^+$ cells in the outer root sheath and hair bulb where it co-localized with K15$^+$ cells compared to the control. Keratinocyte growth factors were expressed in the inner root sheath in both groups, and the electromagnetic field group showed more expression of keratinocyte growth factors. Our data suggested that the hair-growth-promoting effect of the 50 Hz electromagnetic field was observed in depilation-induced hair follicles cycling, which was associated with 50 Hz electromagnetic field enhancing K15$^+$ stem cells proliferation and increased keratinocyte growth factor expression.

Keywords: Electromagnetic field, hair growth, epidermal stem cells, keratinocyte growth factor

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Introduction

As an important cutaneous appendage, hair follicles (HFs) produce hairs which have different kinds of functions, being the mechanism of outward transport of environmental signals, decoration, and so on.[1] Mature HFs undergo repeated cyclic regeneration,[2] consisting of phases of growth (anagen), regression (catagen), and quiescence (telogen) in the adult's skin.

The growth of HFs is maintained by a complex controlled balance between cell proliferation, differentiation, and death involving many kinds of cells such as epidermal stem cells (ESCs) and dermal papilla (DP) cells. When DP cells deriving from mesenchyme provide unique and critical signals to ESCs, then ESCs undergo a highly coordinated and stepwise program of differentiation to form *de novo* HFs.[3] As a non-invasive physical therapy, electromagnetic fields (EMFs) exposure is still widely used for the treatment of some pathological conditions to stimulate neural regeneration, tissue, and bone repair.[4–6] Though several studies *in vitro* had suggested that EMF could modify cell

proliferation capabilities of ESCs[7,8] and normal keratinocytes,[9] and interfere with cell differentiation of mesenchymal stem cells,[10] there is little information on the effect of EMF on hair regeneration or regrowth.

Numerous cytokines and growth factors have also been shown to be involved in the process of HF generation.[11] Keratinocyte growth factor (KGF), known as fibroblast growth factor 7, is a paracrine growth factor for keratinocytes, which could inhibit terminal differentiation of cultured keratinocytes,[12] and stimulate ESCs to growth and cell division.[13] Guo *et al.*[14] has shown that KGF was required for hair development.

This present study was designed to determine the effects of 50 Hz EMF on HFs growth. For this purpose, we chose female C57BL/6 mice to present the model of HF regrowth.[10,15] After EMF exposure for 16 days, HF regrowth was evaluated histologically. In this study, we also investigated the effects of EMF on the presence and proliferation of ESCs and the expression and location of KGF in the process of HF regrowth.

Materials and methods

Animals

Animal experiments were approved by the Institutional Animal Care and Use Committee of the Guangdong General Hospital. Eight to nine-weeks-old female C57BL/6 mice (18–20 g) were purchased from the Animal Laboratory of Sun Yat-sen University. The animal room was maintained at approximately 25°C with $50 \pm 10\%$ humidity using a 12-h light/dark cycle. They were fed by the common diet and allowed free access to tap water. A total of 27 mice were used for the experiment.

HF regrowth models

The model of depilation-induced HFs regrowth was made following Paus' classical method.[16] Briefly, the mice were anesthetized by diazepam and ketamine (0.1 mg/g body weight). The mixture of molten wax/rosin (1:1) was applied to the mice's dorsal skin against the direction of the hair grows. After coagulation of the wax/rosin mixture, we depilated the hair shafts, which induced HFs to enter the homogeneous and synchronized anagen. The synchronized mice were judged by the pink color of the mice's dorsal skin.

EMF exposure

For exposure to EMF, we used a Helmholtz coils system with a sinusoidal wave of frequency of 1, 10, 20, 30, 40, and 50 Hz and an intensity of 5 mT.[8] The exposure system was stored in a room at 25°C with $50 \pm 10\%$ humidity. In this study, the mice in the EMF group were exposed to 50 Hz EMF at 5 mT for 30 min, once a day, 16 days in a row. Sham-exposed control samples were kept under similar conditions without exposure to EMF at the same time.

Histological analysis of skin

Dorsal skin was harvested on days 3, 9, and 16 after depilation. Three mice were used at each time point in each group from three independent experiments. Skin specimens were fixed in a 4% paraformaldehyde solution and embedded in Paraffin wax. The blade cut the skin specimens parallel to the vertebral line and the longitudinal axis of HFs. The thickness of paraffin sections was 5 μm. Paraffin sections were tested with hematoxylin and eosin staining or immunofluorescence staining. Hematoxylin and eosin staining was performed on paraffin sections using a standard histological procedure, then the tissue sections were examined by bright-field microscopy (Leica, Germany) at 4× or 10× magnification. In the histology images, the length of HF was described earlier.[16] Briefly, the length of HFs was measured as the distance from the bottom of hair bulbs to the pore in the epidermis from the sections; 20 of HFs on 6 representative sections at days 9 and 16 were measured, and 3 mice were used for each group.

Immunofluorescence staining

The tissue sections from all the groups were processed using a standard histological procedure, then we incubated the sections with the following primary antibody overnight at 4°C anti-Ki67 (1:800; Abcam, San Diego, CA), anti-k15 (1:500; Abcam), or anti-KGF (1:600; Abcam) rabbit monoclonal antibody to mouse. On the following day, sections were washed in PBS and incubated with secondary antibody (Boster, Wuhan, China) at room temperature for 1 h. Nuclei were counterstained with DAPI. Images were captured at 10× magnification using confocal microscopy.

Western blot analysis

Western blot analysis for KGF expression was carried out using the tissue proteins. Briefly, tissue proteins were obtained from the extracts of the epidermis of mice, and Western blot was performed as described in Li *et al.*[13] Antibody reaction was done with antibodies against KGF (Boster, Wuhan, China; dilution 1: 200), GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA; dilution 1: 2,000).

Statistics

Data from three independent experiments were used to calculate the means and standard deviation (SD). Student's t test was used to assess the level of significance for HF length. A value of $P < 0.05$ was considered statistically significant.

Results

Low frequency EMF increased regrowth of HFs

To study the effects of 50 Hz EMFs on the growth of HFs, we chose the female C57BL/6 mice to present the model of depilation-induced HFs regrowth. At nine days after depilation, hair growth was increased in both groups. At 16 days after depilation, most mice in the EMF group were distinctly covered with a significant amount of hair, but not those in the control group (Figure 1(a) to (f)). Subsequently, we found that the hair club formed faster in the 50 Hz EMF group than the control group on the

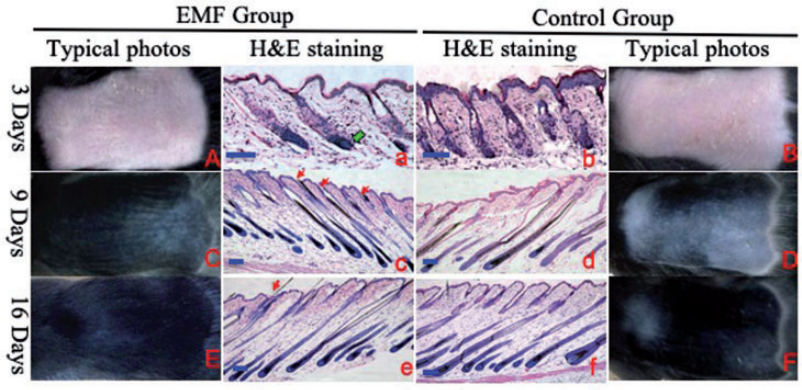

Figure 1. Effects of 50 Hz EMF on hair follicle regrowth. The morphogenesis of the hair follicle was photographed. EMF group (a, c, e) vs. control (b, d, f). H&E staining showed that, compared to the control, the hair club formed earlier in the EMF group than the control on the 3rd day after depilation (green arrow) (a) and most of the hair shafts had erupted from the pore in the epidermis in the EMF group on the 9th day after depilation (red arrow) (c). EMF group (a with magnification 10×, c, e with magnifications 4×) vs. control (b with magnification 10×, d, f with magnifications 4×). Bars 100 μm. (A color version of this figure is available in the online journal.)

3rd day after depilation. Most of the hair shafts erupted from the pore in the epidermis in the EMF group on the 9th day after depilation, but not in the control group (Figure 1(a) to (f)). Next, to quantify the effect of EMF on hair growth, because depilation-induced HFs had the homogeneous and synchronized anagen, so we examined only the hair shaft growth. Selecting 20 hairs at random, and compared to control group, the hairs length was significantly longer at the 16th day in the EMF group (Figure 2).

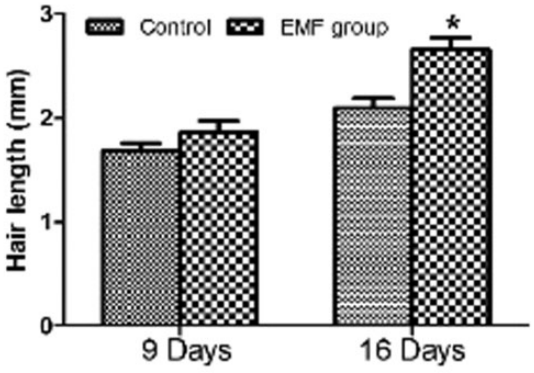

Figure 2. Quantification analysis of the hairs length. Twenty hairs were measured on the 9th, 16th day of the experiment. Data presented as means ± SD. *$P < 0.05$, compared with control (*t*-test).

Low frequency EMF promoted K15$^+$ stem cells proliferation

K15 has become one of the most widely used markers to identify ESCs, and K15 is expressed not only in cells of the bulge but also in the basal layer of the epidermis. In these experiments, immunofluorescence staining showed that more K15$^+$ cells are expressed in cells of the follicular bulge in 50 Hz EMF groups compared with that of the control group at the 9th and 16th day after depilation (Figure 3). Furthermore, we know that Ki67 labeling is used to evaluate cell proliferation. To demonstrate the effects of 50 Hz EMF on the cell proliferation, the authors marked the Ki67$^+$ cells through immunofluorescence staining. Compared to the control group, the results showed a higher numbers of Ki67$^+$ cells standing between DP and the hair bulb in mice on the 3rd day after depilation in the 50 Hz EMF group, and there were more Ki67$^+$ cells in the outer root sheath (ORS) and hair bulb compared to the control group at the 9th and 16th day (Figure 4). In those experiments, Ki67 immunofluorescence showed proliferating cells localizing to the regions where it co-localized with K15$^+$ cells (Figures 3 and 4).

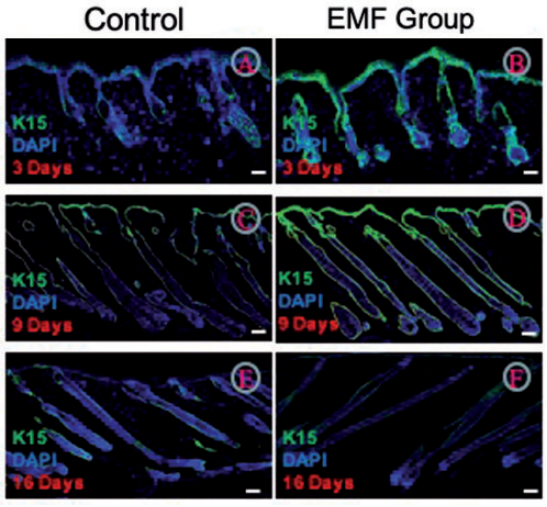

Figure 3. The influence of 50 Hz EMF on the development of anagen hair follicles. K15$^+$ cells were expressed not only in cells of the bulge but also in the basal layer of the epidermis. More K15$^+$ cells are expressed in cells of the follicular bulge in 50 Hz EMF group compared to the control group. Control (a, c, e with magnifications 4×) vs. EMF group (b, d, f with magnifications 4×). Bars 100 μm. (A color version of this figure is available in the online journal.)

Low frequency EMF enhance KGF expression

Increasing evidence suggests that several factors are involved in the hair growth cycle, such as cytokine and growth factors, and that KGF is required for hair development but not for wound healing. Thus, we became interested in the effect of EMF on KGF expression. In this study,

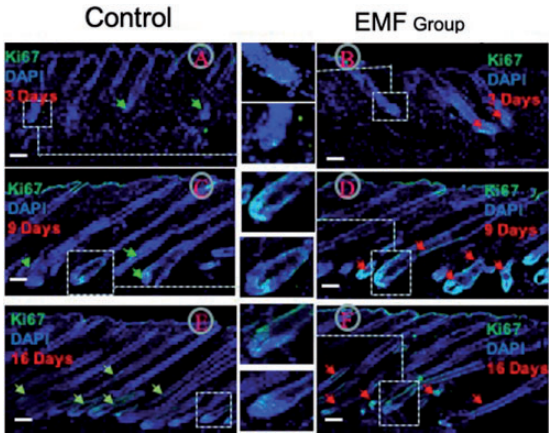

Figure 4. A comparable percentage of Ki67-positive cells were detected in 50 Hz EMF group and control group. The percentage of proliferating cells was higher than controls between dermal papilla and hair bulb in mice on the 3rd day, and in the ORS on the 9th and 16th day (arrow). Control (a, c, e with magnifications 4×) vs. EMF group (b, d, f with magnifications 4×). Bars 100 μm. (A color version of this figure is available in the online journal.)

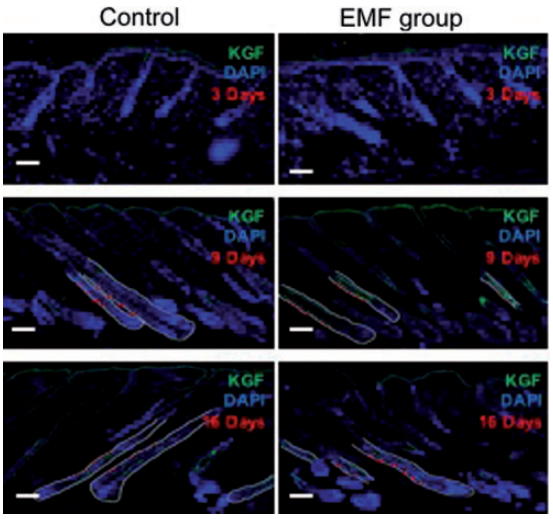

Figure 5. Immunofluorescence staining analysis of KGF protein localization on the skin during hair follicle regrowth. KGF expression was seen in the cells of the inner root sheath in both groups on the 9th and 16th day of the experiment (arrow). Bars 100 μm. All magnifications 4×. (A color version of this figure is available in the online journal.)

KGF was expressed in the inner root sheath (IRS) in both groups at the 9th and 16th day, and the EMF group showed more expression of KGF (Figure 5). Those results were associated with Western blot (Figure 6). Though there was a significant difference in KGF expression between the two groups indicated by Western blot, localization and expression of KGF were not found by immunofluorescence staining on the 3rd day after depilation (Figures 5 and 6).

Discussion

In this study, we found that 50 Hz EMF treatment lead to an increase in hair length in HF cycling models. In line with this, we observed that 50 Hz EMF enhanced $K15^+$ stem cell proliferation in the hair bulb and follicular ORS of HFs and increased KGF expression in IRS.

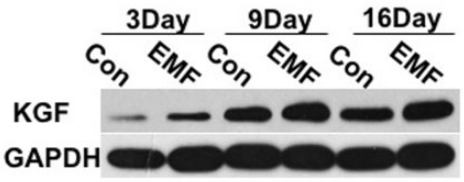

Figure 6. The expression of KGF proteins in the development process of the new hairs. KGF expression increased gradually and then waned at a later anagen phase. There was a higher level of KGF expression after EMF exposure. The difference of KGF expression between the two groups was first identified on the 3rd day of the experiment.

The hair cycle is considered a complex process that involves many factors,[17] and ESCs are considered as the main source of cells for the regrowth of HFs. The deficiency or failure of activation of remaining hair stem cells during the hair cycle is one of the major reasons for hair loss, just like androgenetic alopecia.[18] Thus, methods for influencing proliferation of those stem cells are a great benefit for hair regrowth. In physical medicine, He–Ne laser irradiation of the mice's skin was observed to lead to a significant increase in hair growth.[19] The evidence from some studies *in vitro* showed 50 Hz EMF enhanced ESC proliferation,[7,8] meaning that EMF may be useful for HF growth. Interestingly, low-intensity EMF and essential oils in the treatment of androgen-dependent alopecia exhibited a decrease in hair loss and a hair count increase.[20] We also found in this study that 50 Hz EMF treatment leads to an increase in hair length in depilation-induced hair cycle models. Those findings in the above-mentioned studies indicate the therapeutic effectiveness of 50 Hz EMF on HF regeneration.

HF is a very small but extremely unique mini-organ involved in many types of cell. Signals from DP cells promote hair matrix cells proliferation to regenerate the new lower follicle, and the matrix cells are supplied by slow-cycling and multi-potent stem cells which exist in the bulge region.[21] In general, hair growth depends on the matrix cells proliferation at the bulb which is located in the dermis. In this study, more $K15^+$ cells proliferated at the onset of the anagen phase after EMF exposure, which suggests that EMF exposure could enhance hair club forming through the activity of high proliferation of ESCs from the bulge region. When HFs entered the anagen III phase and later the anagen phase, the stem cells, located in the hair bulge were, also responsible for the regeneration of ORS, IRS, and the hair shaft.[16] In our experiment, we found that $K15^+$ cells increased in ORS, and the cells showed a higher proliferation capacity in the EMF exposure group compared to the control group. Those results showed that EMF could enhance $K15^+$ cells proliferation and play an assistant role during the development process of a new lower follicle.

KGF is a paracrine growth factor for keratinocytes, which is synthesized by mesenchymal cells. Generally, the epithelial-mesenchymal interactions are a crux for normal development of the HF as well as during hair

cycling,[22] and KGF has an important role in those interactions.[23] In the current study, we first found that KGF was present in IRS. It was clear that KGF had a function in the IRS. Because the matrix cells located in the IRS subsequently gave rise to the hair shaft, and KGF receptors are present on matrix cells,[24] our data suggest that KGF could enhance the newly forming hair shaft. Furthermore, because the matrix cells form hair bulbs, the unique functions of KGF were a benefit for the developing hair bulb, though the location of KGF was not clear at the onset of the anagen phase. Meanwhile, it was most interesting that Guo *et al.*[14] also confined the effect of KGF on HFs, and discovered that KGF was required for hair development. Additionally, from our study, we observed a higher level of KGF expression after EMF exposure, which indicated that 50 Hz EMF enhanced regrowth of HFs due to partly increased KGF expression.

Further studies will focus on at least three aspects. First, a possible role for KGF on the hair cycle is not clear, further studies will be necessary to assess that role. Second, how the whole HFs cycling changes after exposure to 50 Hz EMF was not explored in this study. It is important to understand this aspect of the 50 Hz EMF on HFs neogenesis. Third, we will investigate whether 50 Hz EMF is beneficial for functional healing of the wounds and hair loss.

In conclusion, we found that the hair-growth-promoting effect of 50 Hz EMF was observed in depilation-induced HF cycling, which is associated with 50 Hz EMF enhancing $K15^+$ stem cells proliferation, and increased KGF expression. These results strongly support that 50 Hz EMF may be beneficial for functional healing of the wounds and hair loss.

Authors' contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; XP Li, X Wang, LM Bai conducted the experiments, XP Li, P Zhao, and MS Zhang wrote the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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