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# Effect of cell-phone radiofrequency on angiogenesis and cell invasion in human head and neck cancer cells

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**Abstract**

**Background:** Today, the cell phone is the most widespread technology globally. However, the outcome of cell-phone radiofrequency on head and neck cancer progression has not yet been explored.

**Methods:** The chorioallantoic membrane (CAM) and human head and neck cancer cell lines, FaDu and SCC25, were used to explore the outcome of cell-phone radiofrequency on angiogenesis, cell invasion, and colony formation of head and neck cancer cells, respectively. Western blot analysis was used to investigate the impact of the cell phone on the regulation of E-cadherin and *Erk1/Erk2* genes.

**Results:** Our data revealed that cell-phone radiofrequency promotes angiogenesis of the CAM. In addition, the cell phone enhances cell invasion and colony formation of human head and neck cancer cells; this is accompanied by a downregulation of E-cadherin expression. More significantly, we found that the cell phone can activate *Erk1/Erk2* in our experimental models.

**Conclusion:** Our investigation reveals that cell-phone radiofrequency could enhance head and neck cancer by stimulating angiogenesis and cell invasion via *Erk1/Erk2* activation.

**KEYWORDS**
angiogenesis, cancer progression, cell invasion, cell phone, *Erk1/Erk2*, radiofrequency

## 1 | INTRODUCTION

Cell phones are now in common use and it has been proposed that this may be associated with an increased risk of head and brain cancers.[1–3] Developments in wireless communication technology over the last 2 decades resulted in increasingly widespread use of mobile phones and wireless systems for communication purposes. Today, it is known that exposure to radiofrequency fields induce internal body currents and energy absorption in tissues and cells, depending on frequencies and coupling mechanisms. Thus, several studies suggested an association between cell phone use and the development of brain cancer, especially glioma.[1,4,5] On the other hand, it has been reported that cell-phone radiofrequency can damage DNA and deregulate several genes related to this event and epithelial-mesenchymal transition in brain cell models,[6–9] which is a hallmark of cancer progression.[10] All of this implicates cell-phone radiofrequency in human cancer initiation and/or progression especially in specific parts of the human body, such as the head and neck.

Currently, human head and neck cancers are frequent malignancies that have recently increased in large areas of the world (World Health Organization). These cancers are characterized by a marked propensity for invasion and spreading to local lymph nodes. Thus, the overall 5-year survival rate for patients diagnosed with head and neck cancers

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has not significantly improved over the past decade.[11] Important factors in the development and progression of these cancers are gene mutation, lifestyle, and bacterial and viral infections, in addition to environmental pollutants produced by human activities, including new technologies. So far, little is known about the outcome of cell-phone radiofrequency on human head and neck cancer progression. Thus, in this investigation, we examined, for the first time, the outcome of cell-phone radiofrequency on head and neck cancer progression using the chorioallantoic membrane (CAM) model and 2 human head and neck cancer cell lines. Our data show clearly that cell-phone radiofrequency can enhance angiogenesis of the CAM and cell invasion and colony formation of head and neck cancer cells. Moreover, this investigation pointed out that angiogenesis, cell invasion, and colony formation stimulation induced by cell-phone radiofrequency could be attributed to *Erk1/Erk2* activation.

## 2 | MATERIALS AND METHODS

### 2.1 | Chorioallantoic membrane assay

Fertilized white leghorn chicken eggs were obtained from Arab Qatari for Poultry Production, Doha, Qatar. Eggs were incubated in a MultiQuip incubator at 37°C with 60% humidity. A small window was cut out in the shell on day 4 of chicken embryo development under aseptic conditions; the window was resealed with adhesive transparent tape,[12] and then the eggs were exposed to a cell phone, as described in the following; afterward, embryos were returned to the incubator for 9 days. This study was approved by the QU-IBC Committee of the Qatar University number 15/16-17.

### 2.2 | Cell-phone radiofrequency exposure

In this study, two regular iPhone 6 devices were used; the service provider of the two cell phones was one of the major local cell-phone providers, Vodafone Qatar; the frequency used by this company is 1800 MHz. The CAMs of the embryos were exposed to cell-phone radiofrequency for a total of 30 minutes per day, while the phones were on call mode (with an interval of a 5-minute break for every 10-minute exposure) for 5 days. The distance between the cell phone and embryos was approximately 5 cm during exposure; this distance is necessary to avoid the heat produced by the device. The experiment was repeated 10 times, exposing 4 embryos to cell-phone radiofrequency each time.

### 2.3 | Cell lines

Two human head and neck cancer cell lines, FaDu and SCC25, were used in our investigation. Cell lines were obtained from the American Type Culture Collection (Rockville, MD) and maintained in a humidified atmosphere of 5% $CO_2$ in air at 37°C. The cells were routinely cultured in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% fetal bovine serum (Life Technologies, Ontario, Canada), 2 mM L-glutamine, and 100 μg/mL penicillin-streptomycin. Cancer cell exposure to cell-phone radiofrequency was repeated 3 times.

### 2.4 | Invasion assay

Cell invasion was assayed in 24-well Biocoat Matrigel invasion chambers (8 μm; Becton Dickinson, Ontario, Canada) in accord with the manufacturer's protocol. Briefly, cancer cells ($5 \times 10^4$) were plated in the top chamber. The bottom chamber contained RPMI medium. Cells were exposed to cell-phone radiofrequency, as described previously, for a total of 30 minutes per day for 2 days with an interval of 5 minutes for every 10 minutes' of exposure. After 48-hour incubation, noninvasive cells were removed with a cotton swab, whereas cells that migrated through the membrane and stuck to the lower surface of the membrane were fixed with methanol and stained with hematoxylin. For quantification, cells were counted under a microscope, as previously illustrated by our group.[13]

### 2.5 | Soft agar growth assay

Twenty thousand cells of FaDu and SCC25 were placed in 35-mm dishes in their medium containing 0.4% agar, and plated over a layer of RPMI 1640 medium containing 0.7% agar. Cells were exposed to cell-phone radiofrequency for a total of 30 minutes per day for approximately 10 days with an interval of 5 minutes for every 10 minute's of exposure. The cultures were examined every 2 days for up to 2 weeks.

### 2.6 | Western blot analysis

The FaDu and SCC25 cells were exposed to cell-phone radiofrequency for 3 days, as described above. Afterward, Western blot was performed, as previously described by our group.[13] Briefly, 30 μg of protein from each sample was used in this assay. Protein samples were then blotted on a nitrocellulose membrane and detected with anti-E-cadherin monoclonal antibody (mAb; Bio/Can Scientific, Ontario, Canada) and anti-*Erk1/Erk2* phosphotyrosine mAb (Upstate Biotechnology, Lake Placid, NY).

### 2.7 | Statistical analysis

All analyses were performed using SPSS version 15.0 for Windows software (SPSS, Chicago, IL). The *t*-test and 1-way analysis of variance were used to determine statistical significance of head and neck cancer cell invasion *in vitro*.

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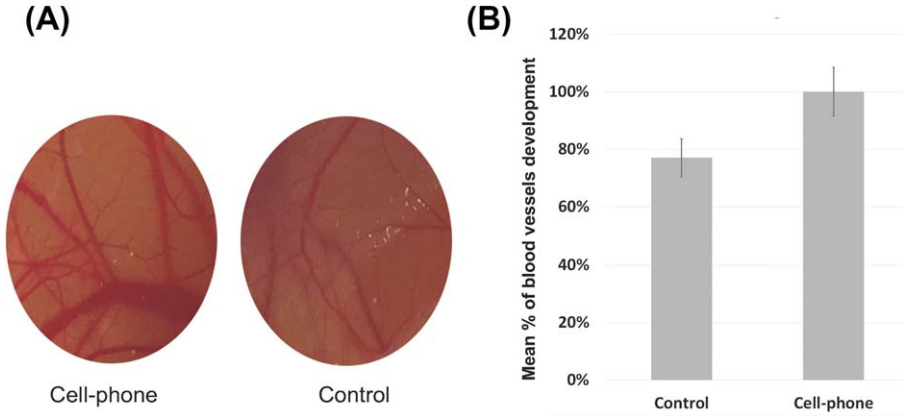


**FIGURE 1** A, Outcome of cell-phone radiofrequency on angiogenesis of the chicken embryo chorioallantoic membrane (CAM) at 9 days of incubation (5 days after cell-phone exposure). We note that cell-phone radiofrequency stimulates angiogenesis of the CAM in comparison with their unexposed controls. For a total of 30 minutes per day for 5 days, 40 embryos were exposed to cell-phone radiofrequency as described in the Materials and Methods section. B, Quantification of CAM angiogenesis of chicken embryos at 9 days of incubation using AngioTool program. Total blood vessels' lengths were compared between cell-phone radiofrequency-exposed CAMs and their unexposed controls ($P \leq .049$). RF, radiofrequency

Statistical significance was accepted at $P < .05$. Angiogenesis was quantified using the AngioTool program. Three main measurements were compared: vessel area, number of junctions, and total vessel length.

## 3 | RESULTS

In order to explore the role of cell-phone radiofrequency in human head and neck cancer progression, we first examined the outcome of cell-phone radiofrequency on angiogenesis, which is well established today as a major event in the progression of human cancer. The CAM of the chicken embryo at 4 days of incubation was used in this study, as illustrated previously by our group.[12] We found that cell-phone radiofrequency increases blood vessel development of the CAM by approximately 25% in comparison with their unexposed control CAMs (Figure 1A,B). Embryos were exposed to cell-phone radiofrequency for a total of 30 minutes per day for 5 days, as described in the Materials and Methods section.

Regarding the outcome of cell-phone radiofrequency on head and neck cancer cells, we exposed 2 human head and neck cancer cell lines, FaDu and SCC25, to cell-phone

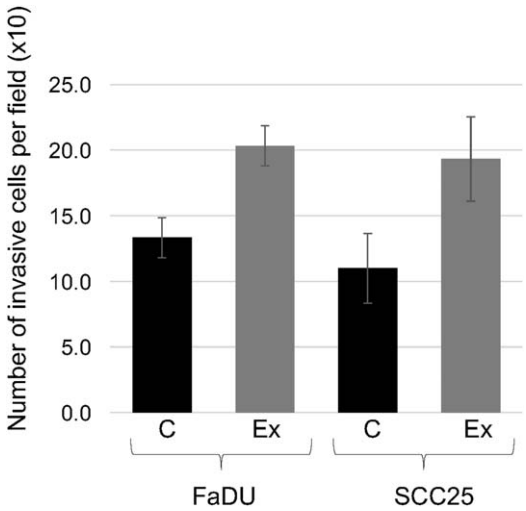


**FIGURE 2** Effect of cell-phone radiofrequency on cell invasion of FaDu and SCC25 cancer cell lines. Cell-phone radiofrequency increases cell invasion ability of both FaDu and SCC25 cells (Ex) by approximately 35% in comparison with their control (C) cells ($P \leq .001$). Boyden chambers were used to assess cell invasion ability of both cell lines. Cancer cells were exposed to cell-phone radiofrequency for a total of 30 minutes per day for 2 days as elucidated in the Materials and Methods section

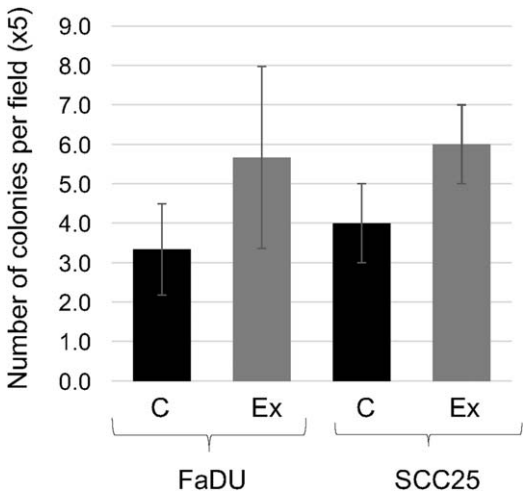


**FIGURE 3** Outcome of cell-phone radiofrequency on colony formation in soft agar of FaDu and SCC25 cell lines. Cell-phone radiofrequency increases the number of colony formations of both cell lines (Ex) by approximately 40% in comparison to their control (C) cells ($P \leq .012$). For this assay cells were also exposed to cell-phone radiofrequency for 30 minutes per day for 10 days, as described in the Materials and Methods section

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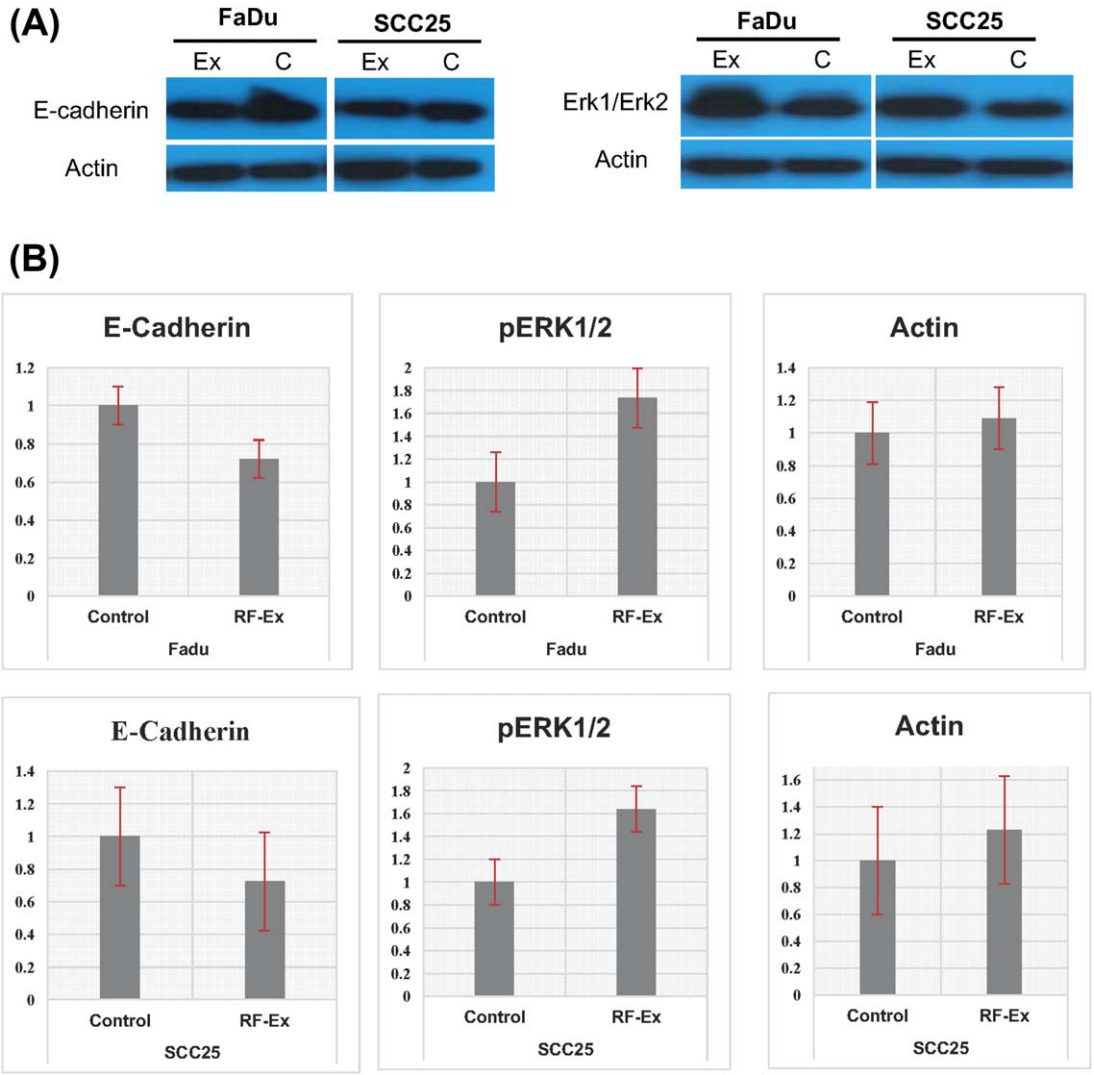


**FIGURE 4** A, B, Western blot analysis of E-cadherin expression and *Erk1/Erk2* phosphorylation in FaDu and SCC25 cells exposed to cell-phone radiofrequency and unexposed cells. We note that cell-phone radiofrequency reduces the expression of E-cadherin in both cell lines (Ex), FaDu and SCC25, in comparison with their control (C) cells. In parallel, cell-phone enhances *Erk1/Erk2* phosphorylation in the 2 cell lines (Ex) in comparison with the control (C) cells. The quantification of both data by the ImageJ program and *t* test confirms the reduction and augmentation of E-cadherin expression and *Erk1/Erk2* phosphorylation, respectively. RF, radiofrequency [Color figure can be viewed at wileyonlinelibrary.com]

radiofrequency for 30 minutes per day for 3 days, as stated in the Materials and Methods section; we noted that cell-phone radiofrequency slightly incites cell proliferation of the 2 head and neck cancer cell lines in comparison to their control (data not shown). Next, we examined the effect of cell-phone radiofrequency on cell invasion and colony formation of FaDu and SCC25 cells. Our data showed that cell-phone radiofrequency enhances cell invasion and stimulates colony formation of both cell lines in comparison with their unexposed control cells (Figures 2 and 3). Boyden chambers and soft agar assays were used for this purpose, as described by our group,[13] to assess cell invasion and colony formation abilities of our 2 cell lines under the effect of cell-phone radiofrequency. Cancer cells were exposed to cell-phone radiofrequency for a total of 30 minutes per day for 2 days for the cell invasion assay, and approximately 10 days for the colony formation in soft agar.

Next, and based on our cell invasion data, E-cadherin expression was explored in FaDu and SCC25 cell lines, because it is a major key regulator of cell invasion and metastasis[10]; we found that cell-phone radiofrequency induces a downregulation of E-cadherin expression in both cell lines (Figure 4A,B). The expression of E-cadherin was assessed by Western blot assay using an mAb, as elucidated in the Materials and Methods section.

Finally, and regarding the molecular pathways of cell-phone radiofrequency on angiogenesis, cell invasion, and colony formation, we assumed that the main pathway behind these events could be *Erk1/Erk2* signaling pathways, because it is well known that *Erk1/Erk2* activation can enhance

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cancer progression via the stimulation of cell proliferation, cell invasion, and angiogenesis.[14–16] Thus, *Erk1/Erk2* activation was evaluated in FaDu and SCC25 cell lines exposed to cell-phone radiofrequency in comparison with their unexposed control cells. We found that *Erk1/Erk2* is activated, under the effect of cell-phone radiofrequency, in both cell lines in comparison to their control (Figure 4A,B).

## 4 | DISCUSSION

In this investigation, we demonstrate, for the first time, that cell-phone radiofrequency can enhance cell invasion and colony formation of 2 human head and neck cancer cell lines, FaDu and SCC25; this is accompanied with a reduction of E-cadherin's expression in both cell lines. Indeed, numerous investigations suggested an involvement of cell-phone radiofrequency in the development of brain cancer, especially glioma.[1,4,5] More specifically, it has been reported that cell phone radiofrequency can damage DNA and deregulate gene expression of apoptotic and antiapoptotic pathways, including signal transducer and activator of transcription 3 (STAT3), tumor necrosis factor α, and interleukin-6 in brain cells.[6–9] On the other hand, it has been shown that these genes can play an important role in the progression of several types of human carcinomas via the initiation of the epithelial-mesenchymal transition event.[10] Nevertheless, there is no study about the outcome of cell-phone radiofrequency on human cancers' progression, including head and neck malignancy. Therefore, we herein investigated, we believe for the first time, the effect of cell-phone radiofrequency on head and neck cancer progression; our study revealed that cell-phone radiofrequency might enhance head and neck cancer progression and metastasis via the stimulation of angiogenesis and cell invasion. More significantly, we report that cell-phone radiofrequency can activate *Erk1/Erk2*, which could be the main pathway behind the reduction of E-cadherin expression and consequent cell invasion stimulation; this is supported by several recent studies that point out that *Erk1/Erk2* activation enhances cell invasion via the down-regulation of E-cadherin and up-regulation of matrix metalloproteinase, which are important controllers of cell invasion and metastatsis.[17–19] Meanwhile, it is important to emphasize that STAT3 could also contribute in angiogenesis, cell invasion, and colony formation stimulation in our experimental models, because cell-phone radiofrequency is found to activate STAT3; and it has been reported that the STAT3 signaling pathway can enhance angiogenesis and downregulate E-cadherin, thereby enhancing cell invasion.[16,20] In addition, tumor necrosis factor-α and interleukin-6 could present other links between cancer progression and cell-phone radiofrequency, as mentioned previously.

## 5 | CONCLUSION

In this investigation we provide evidence, for the first time, that cell-phone radiofrequency can stimulate angiogenesis, which is an important event during cancer progression and metastasis; in addition, our data reveal that cell-phone radiofrequency could enhance cell invasion and colony formation of human head and neck cancer cells. Finally, we are able to show that angiogenesis, cell invasion, and colony formation stimulation induced by cell-phone radiofrequency could occur via the *Erk1/Erk2* signaling pathway. Thus, our data suggest that cell-phone use by patients with head and neck cancer may have an effect on the progression of their disease. However, we believe more studies, including *in vitro*, *in vivo*, as well as epidemiological investigations, are necessary to confirm the outcome of cell-phone use on human health, especially the initiation of brain as well as head tumors, because there is still some controversy regarding this important topic.[21–23] Nevertheless, it is important to highlight that this investigation is the first one regarding the outcome of cell-phone radiofrequency on head and neck cancer "invasion and progression."

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