

# Tumor-Treating Fields Inhibit the Metastatic Potential of Osteosarcoma Cells

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Ju Yeon Oh, PhD<sup>1,\*</sup>, Yeon-Joo Lee, PhD<sup>2,\*</sup> , and Eun Ho Kim, PhD<sup>3</sup> 

## Abstract

The prognosis of metastatic osteosarcoma (OS) remains poor with a <20% survival rate, particularly in cases of distant (non-lung) metastases. Tumor-treating field (TTF) therapy is a novel electric field-based treatment that causes metaphase arrest and tumor cell death, with the advantage of reduced side effects compared to radiation and chemotherapy. TTF shows promise in glioblastoma and other solid tumors; however, few studies have examined its potential in the treatment of osteosarcoma. Therefore, we explored the mechanism of TTF-induced metastasis inhibition and cell death using *in vitro* models. TTF (1.5 V/cm, 150 kHz) was applied to U2OS and KHOS/NP OS cell lines. In addition, a 3-dimensional culture system was established using these OS cell lines. Cell migration and invasion (i.e., metastatic potential) were examined using a wound-healing scratch assay and transwell assay, respectively. Western blotting of metastasis- and angiogenesis-related proteins was performed. TTF suppressed the migration of and invasion by OS cells and inhibited the expression of epithelial markers, thereby preventing epithelial-mesenchymal transition (EMT), a hallmark of metastasis. Moreover, TTF prevented angiogenesis in human tumor endothelial cells and downregulated matrix metalloproteinase-2 (MMP2) and vascular endothelial growth factor (VEGF) expression. Therefore, TTF shows potential as an improved treatment for osteosarcoma, warranting further preclinical studies in animal models to support clinical trials.

## Keywords

osteosarcoma, TTF, metastasis, MMP-2, VEGF

## Abbreviations

EMT, epithelial-mesenchymal transition; GBM, glioblastoma; MMP-2, matrix metalloproteinase-2; OS, osteosarcoma; TTF, tumor-treating fields; VEGF, vascular endothelial growth factor.

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## Introduction

The most common primary malignant bone tumor is osteosarcoma (OS).<sup>1,2</sup> Metastatic OS is a bone tumor that spreads to distant areas from the initial site of origin in the affected bone. It is typically challenging to control metastatic OS, although a better prognosis is seen in patients with localized metastases as compared to patients with distant metastases.<sup>3,4</sup> As per historical data, significantly fewer metastatic OS patients (20%) have shown no recurrence of their cancer and survived compared to non-metastatic patients. Currently, treatment may consist of radiation, surgery, biological therapy, or chemotherapy, as either single therapies or in combination. Relapse is seen in nearly one-third of OS patients with localized disease at initial diagnosis, irrespective of combination therapy,<sup>5</sup> with 90% of those recurrent due to lung metastases. Conventional therapy demonstrates a 5-year survival of 60-70% for non-metastatic

OS, with little progress for more than 20 years.<sup>6</sup> However, patients with metastatic or recurrent disease have reportedly show a <20% long-term survival.<sup>7</sup> As such, the evaluation of new and innovative therapies for cancer patients is deemed necessary for developing more effective cancer treatments.

<sup>1</sup> Laboratory of Biochemistry, School of Life Sciences and Biotechnology, Korea University, Seongbuk-gu, Seoul, South Korea

<sup>2</sup> Division of Radiation Biomedical Research, Korea Institute of Radiological and Medical Sciences (KIRAMS), Seoul, South Korea

<sup>3</sup> Department of Biochemistry, School of Medicine, Daegu Catholic University, Duryugongwon-ro, Nam-gu, Daegu, Korea

\*These authors contributed equally to this work.

## Corresponding Author:

Eun Ho Kim, Department of Biochemistry, School of Medicine, Daegu Catholic University, 33, 17-gil, Duryugongwon-ro, Nam-gu, Daegu, Korea.  
Email: eh140149@cu.ac.kr



In the field of cancer treatment, tumor-treating field (TTF) therapy is a novel anti-mitotic electric-field-based treatment. In fact, additional features such as being a non-chemical, non-ablative treatment set this therapy apart from radiotherapy, surgery, chemotherapy, and other established cancer treatment modalities. After 10 years of intensive mechanistic research, TTF has entered clinical use quite recently. A portable, battery-operated medical device is used to deliver TTF to patients. The device uses non-invasive transducer arrays that are placed on the skin surface encircling the tumor under treatment. The U.S. Food and Drug Administration (FDA) has approved the use of this drug for newly diagnosed glioblastoma (GBM) and for treating patients with recurrent GBM with no further scope for surgery or radiotherapy. Moreover, with the features of this novel treatment modality, its high effectiveness, and low toxicity profile, the American Society of Clinical Oncology (ASCO) has recognized TTF as an advancement in cancer treatment.<sup>8</sup> In fact, TTF has been recommended in the most recent National Comprehensive Cancer Network (NCCN) guidelines as a category 2A treatment in newly diagnosed GBM for patients with a good performance status.<sup>9</sup> In particular, NCCN is conducting clinical trials of TTF in other solid tumors such as non-small cell lung cancer (NSCLC), ovarian tumors, pancreatic tumors, and malignant mesothelioma.<sup>10</sup>

TTF is similar to other cancer treatments that demonstrate certain biological effects, which are and continue to be researched in several studies. Here, we discuss the currently available knowledge on this mechanism. Highly polarized tubulin dimer dipoles align in the direction of the applied TTF. During metaphase within the cytoplasm, the normal randomized movement of the tubulin subunits is eliminated by TTF and thus the normal polymerization process of microtubules that form the mitotic spindle is disrupted.<sup>11-13</sup> This leads to metaphase arrest, prolonged mitosis, and ultimately cell death or induced autophagic cell death.<sup>14-16</sup>

Ongoing clinical trials and the continued evaluation of new treatments will result in future progress in the treatment of metastatic OS. Treatment improvement in metastatic OS, specifically in areas of active exploration, will include TTF. This pertains to the combined use of 2 or more techniques, which is emerging as a significant approach to augment a patient's cure or survival chances. The most promising treatment can be expected in OS cases by utilizing new and innovative TTF therapies in clinical trials. Reportedly, cells proliferating via an anti-mitotic mechanism are selectively targeted by TTF.<sup>11</sup> However, the effects of TTF on OS metastasis have been examined by very few studies. TTF has inhibited solid tumor metastasis in the lungs, as reported in 2 animal models by Kirson et al,<sup>13</sup> suggesting that primary cancer metastatic spread may be effectively prevented by TTF. Nevertheless, the effects of TTF on the regulation of the metastatic potential of OS or its underlying mechanisms remain unclear. As such, this study is the first to apply TTF to OS cells to explore the molecular mechanisms of metastasis inhibition by assessing migration and invasion.

## Materials and Methods

### Experimental Setup of the Electric Fields

Wires were attached to the bottom of each well, 1 cm apart. The electric field (EF) intensity in the medium was measured using 2 insulated wires with exposed tips, which were connected to an oscilloscope (GDS-2102A, GW Instek; New Taiwan City, Taiwan) through a differential probe (GDP-100, GW Instek). The noise level in the measurement of field intensity, which was determined by the comparison between the EF intensity at 90° (normal to the EF lines) and at 0° (parallel to the EF lines), was less than 30% of the signal. The applied EF intensity was 1.5 V/cm and the applied frequencies ranged from 50 kHz to 300 kHz. Temperature was measured within culture dishes during treatment because EF application can generate non-negligible heat. The measurement showed that the change in temperature due to the application of TTF was not significant, revealing a maximum change of 0.3°C increase under our experimental conditions. TTFs were generated using a pair of insulated wires connected to a functional generator and a high-voltage amplifier, which generated sine-wave signals ranging from 0 V to 800 V, resulting in an applied EF intensity and frequency of 1.5 V/cm and 150 kHz, respectively.<sup>17</sup> We used 1.5 V/cm as the field intensity because of its use in clinical settings. For TTF treatment, cells were plated in 100-mm dishes and incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> until they reached 70–80% confluency.

### Cell Culture

Two human OS cell lines (U2OS, KHOS/NP) and a murine endothelial cell line (2H11) were selected for this study from the American Type Culture Collection (Rockville, MD). OS cell lines were maintained in  $\alpha$ -minimum essential medium (Gibco<sup>®</sup>, Life Technologies, Carlsbad, CA) containing 10% (v/v) fetal bovine serum (FBS; Gibco<sup>®</sup>, Life Technologies) and 1% (v/v) penicillin-streptomycin (Gibco<sup>®</sup>, Life Technologies). The 2H11 cell line was maintained in DMEM supplemented with 10% fetal bovine serum (FBS) in a humidified 10% CO<sub>2</sub> environment.

### Cell Viability Assay

Cell viability was determined by the trypan blue exclusion assay and the MTT assay. An equal volume of trypan blue was added to the cell suspension, and the percentage of viable cells was evaluated by microscopy. Assays were performed in triplicate. The MTT assay (EZ-CYTOX, Daellab Service; Seoul, Korea) was performed according to the manufacturer's standard protocols.

### Colony Formation Assay

Cells were treated with TTF for 48 h and then incubated for 10 days. The resulting colonies were fixed with 100% methanol for 30 min, stained with 0.4% crystal violet (Sigma-Aldrich; St.

Louis, MO), and then counted. The survival fraction, expressed as a function of irradiation, was calculated as follows: survival fraction = colonies counted/(cells seeded  $\times$  plating efficiency/100).

### Three-Dimensional (3d) Culture System

U2OS and KHOS/NP cells were seeded in 96-well plates at  $1 \times 10^4$  cells/well. In the 3D culture model, wells of a 96-well plates were pre-coated with 40  $\mu$ l Matrigel (undiluted; Corning, Tewksbury, MA) as a basement membrane followed by incubation at 37°C for 30 min. Cells were plated onto the gel in an appropriate medium, and wells were photographed using an Eclipse Ti microscope with a DS-Fi1 camera (Nikon, Tokyo, Japan) after 10 days.

### Wound-Healing Scratch Assay and Invasion Assay

OS cells were seeded onto 6-well plates (Corning) at  $2.5 \times 10^4$  cells/well in 3 mL medium supplemented with 10% FBS. At 2 days, monolayers were mechanically disrupted using a sterile 200- $\mu$ l pipette tip. The assay was performed in duplicate. Wells were photographed after 24 h. Cells were then stained with 0.2% crystal violet. Cell migration was monitored using an Eclipse Ti microscope with a DS-Fi1 camera (Nikon), and cells were counted using ImageJ software (United States National Institutes of Health, Bethesda, MD).

Migration and invasion were measured *in vitro* using transwell chambers (Corning) according to the manufacturer's protocol. Briefly, the upper well chamber was precoated with 40  $\mu$ l of Matrigel (diluted 1:14 with opti-MEM media). The cells were seeded onto transwell upper chamber membranes at  $2 \times 10^4$  cells/mL in 200  $\mu$ L of medium and were either left untreated or treated with TTF for 48 h. The medium in the upper chamber was serum-free, while the medium in the lower chamber contained 10% FBS as a chemoattractant. Cells that passed through the coated membranes were stained with a crystal violet solution supplied in the Transwell invasion assay kit (Chemicon, Millipore; Billerica, MA) and photographed after 24 h of incubation.

### Matrigel-Based In Vitro Endothelial Tube Formation Assay

Endothelial cell tube formation was assessed using Matrigel-coated chamber slides, as described previously.<sup>18</sup> Each Cell lines ( $1 \times 10^4$ ) were resuspended in serum-free DMEM and loaded on top of the Matrigel, respectively. After TTF treatment for 48 h, the results of each assay were photographed with an Eclipse Ti microscope with a DS-Fi1 camera (Nikon) at a magnification of  $\times 10$ .

### Western Blot Analysis

Total proteins from OS cells were extracted in RIPA buffer (50 mM Tris-HCl, pH 7.4; 1% NP-40, 150 mM NaCl, and 1 mM

EDTA) supplemented with protease inhibitors (1 mM PMSF, 1  $\mu$ g/mL aprotinin, 1  $\mu$ g/mL leupeptin, and 1 mM  $\text{Na}_3\text{VO}_4$ ) and quantified using the Bradford method. Protein samples (30  $\mu$ g) were separated by SDS/polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane using wet transfer. After blocking non-specific antibody binding sites, the membrane was incubated overnight at 4°C with mouse monoclonal antibodies against the indicated antibodies. Vimentin (sc-6260), Twist (sc-81417), VEGF (sc-507), and MMP-2 (sc-10736) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). And N-cadherin (4061s), Snail (3895s), and  $\beta$ -actin (3700) were obtained from Cell Signaling Technology (Danvers, MA, USA). The secondary antibody was purchased from Invitrogen (Carlsbad, CA). After incubation with peroxidase-conjugated secondary antibodies at 37°C for 1 h, the protein bands were visualized using enhanced chemiluminescence reagent (GE Healthcare Biosciences, Pittsburgh, PA) and detected using the Amersham Imager 680 (GE Healthcare Biosciences).

### Statistical Analysis

Statistical significance was determined using Student's t-test. Differences were considered significant if the *P* value was less than 0.05 or 0.001 (\**p* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001).

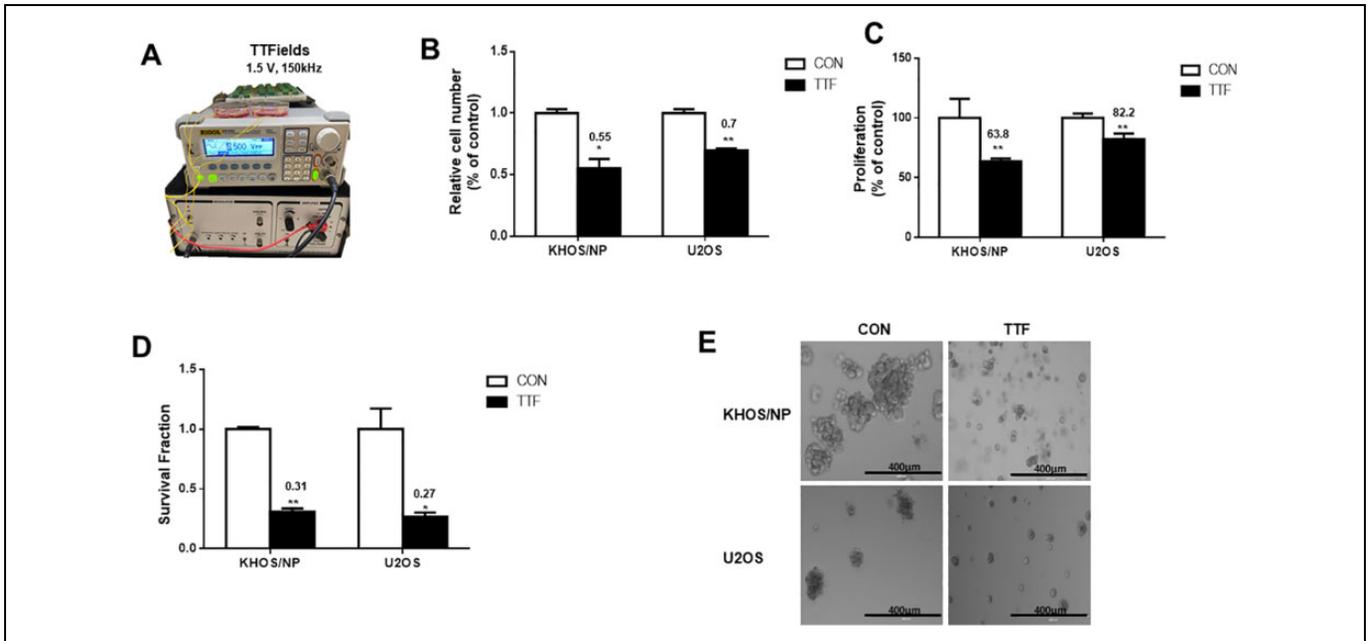
## Results

### TTF Inhibited Cell Proliferation in OS Cell Lines

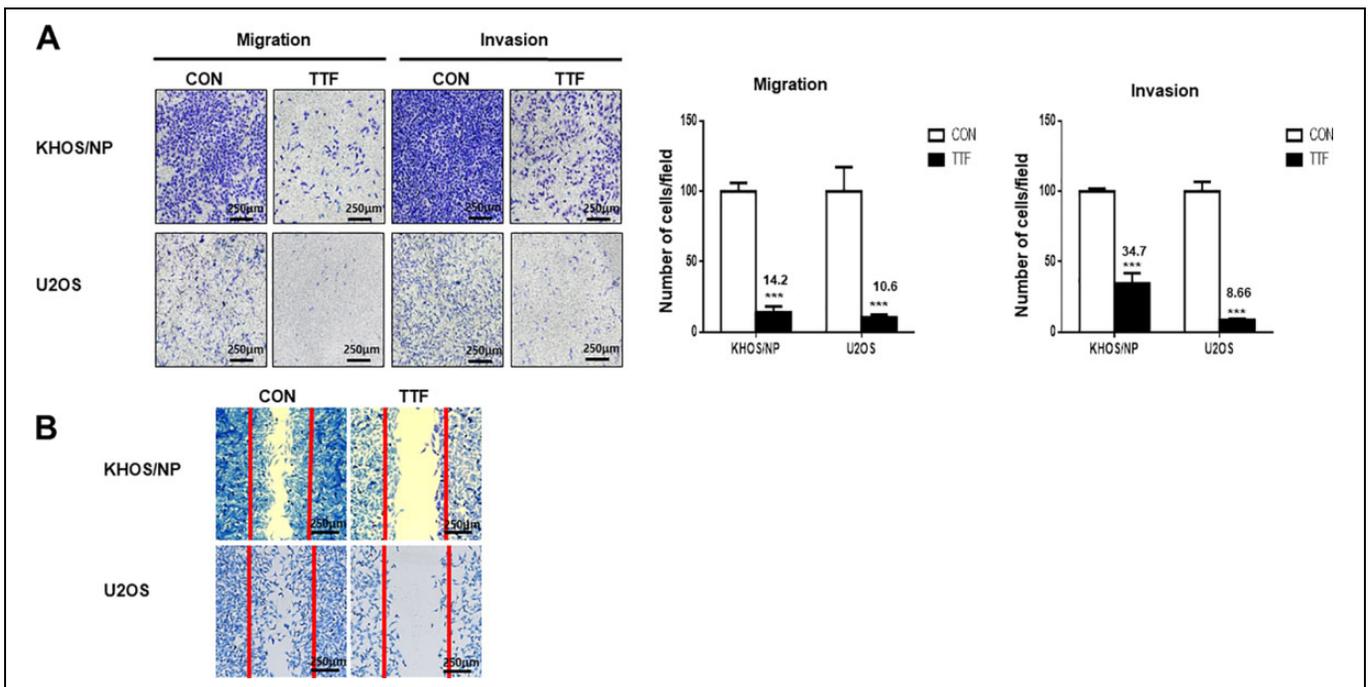
The TTF was aimed using insulated wires connected to a generator and an amplifier to generate a sine-wave signal ranging between 0-800 V (Figure 1A). This section focuses on the detection of TTF effects on KHOS and U2OS cell proliferation, and subsequently sent to the treatment for the counting of cells. From the observations, it was inferred that the application of TTF for 48 h played a significant role in the reduction of proliferation of both KHOS and U2OS cells by the trypan blue assay (Figure 1B) and the MTT assay (Figure 1C). To determine the effects *in vitro*, we applied colony forming measures (Figure 1D). After 10 days, we observed a clonogenic efficiency of 30% in KHOS cells after treatment. Similar to the results observed when applying TTF, we observed a reduction in efficiency by 73% in U2OS cells. The differences between treated and untreated cells were significant (*p* < 0.01). Further, colonies in untreated 3D cultures were larger than those formed by TTF-treated cells (Figure 1E). This suggests that TTF can initiate both anchorage-dependent (cell proliferation) and anchorage-independent (colony formation) growth of OS cells.

### TTF Suppression of Cell Migration and Invasion

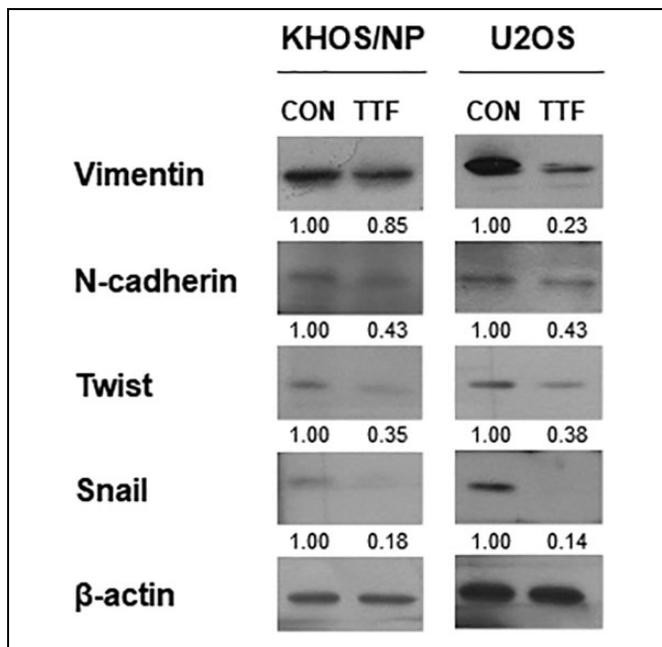
A malignant form of OS was observed in cases of intensified cell growth.<sup>13</sup> Transwell chambers were further employed to detect these effects. TTF-treated cells showed reduced migration and invasion in transwell assays compared with controls, using Matrigel (Figure 2A). Further, the percentage of invading



**Figure 1.** Effect of TTFs on the viability of OS cells (left: KHOS/NP, right: U2OS). (A) Schematic of the experimental setup for TTF treatment. (B) Cell viability was determined by a trypan blue exclusion assay; \* $p < 0.05$ ; \*\* $p < 0.01$ . (C) The viability of cells treated with TTF was significantly lower than that of cells treated with the control. The proliferation rate was detected by the MTT assay; \*\* $p < 0.01$ . (D) The sensitivity of OS cells treated with TTF was measured via a colony formation assay. The survival fraction was calculated as follows: survival fraction = colonies counted/(cells seeded  $\times$  plating efficiency/100); \* $p < 0.05$ ; \*\* $p < 0.01$ . (E) OS cells were treated with TTFs (1.5 V/cm) for 3D colony culture.



**Figure 2.** TTF inhibits the migration and invasion by OS cells. (A) Tumor cell invasion and migration after 48 h of TTF treatment were examined by transwell chamber assays in KHOS/NP and U2OS cells. The number of invading tumor cells that penetrated through the gelatin and Matrigel was counted using 5 high-intensity fields. Values represent the means of 3 experiments  $\pm$  SD; \*\*\* $P < 0.001$ . (B) OS cells were treated with TTF for 48 h and then incubated for 24 h. The cells were then scraped with yellow pipette tips for the scratch assay. Values represent the means of 3 experiments  $\pm$  SD.



**Figure 3.** TTF regulates EMT protein levels in OS cells. (A) OS cells were treated with TTF for 48 h and western blotting was performed using the antibodies indicated for different timepoints.

or migrating cells was significantly reduced by 65.3% and 85.8% for KHOS cells, respectively, and by 91.3% and 89.4% for U2OS cells, respectively. Additionally, a scratch assay was employed to measure the effects of TTF on the invasive and migratory abilities of OS cells. As shown in Figure 2B, invasion and migration were inhibited by TTF in OS cell lines.

### TTF Regulates EMT-Related Protein Levels in OS Cells

In order to reveal the exact mechanism by which TTF modulates the EMT, we performed western blotting (Figure 3). EMT markers, including vimentin, N-cadherin, TWIST, and SNAIL, were significantly affected by TTF in OS cell lines. Our results indicate that TTF has an inhibitory effect on invasive and migratory abilities through EMT-related markers.

### Inhibition of Tumor Endothelial Cell Angiogenesis by TTF

Angiogenesis is an essential factor in cancer metastasis.<sup>19</sup> This was confirmed in this study when 2H11 cells showed suppression of vascular tubule development upon TTF application, which was measured using a Matrigel-based tube formation assay (Figure 4A). Endothelial tube formation was inhibited by TTF in 2H11 cells. We also evaluated the extent to which MMP-2 expression was altered in 2H11 and OS cells treated with TTF. Notably, TTF suppressed the upregulation of MMP-2. This further enhanced and regulated the expression of angiogenesis-related genes, such as VEGF.<sup>20,21</sup> This suggests that TTF has the ability to suppress both VEGF and MMP2 in 2H11 and OS cells via angiogenesis (Figure 4B).

## Discussion

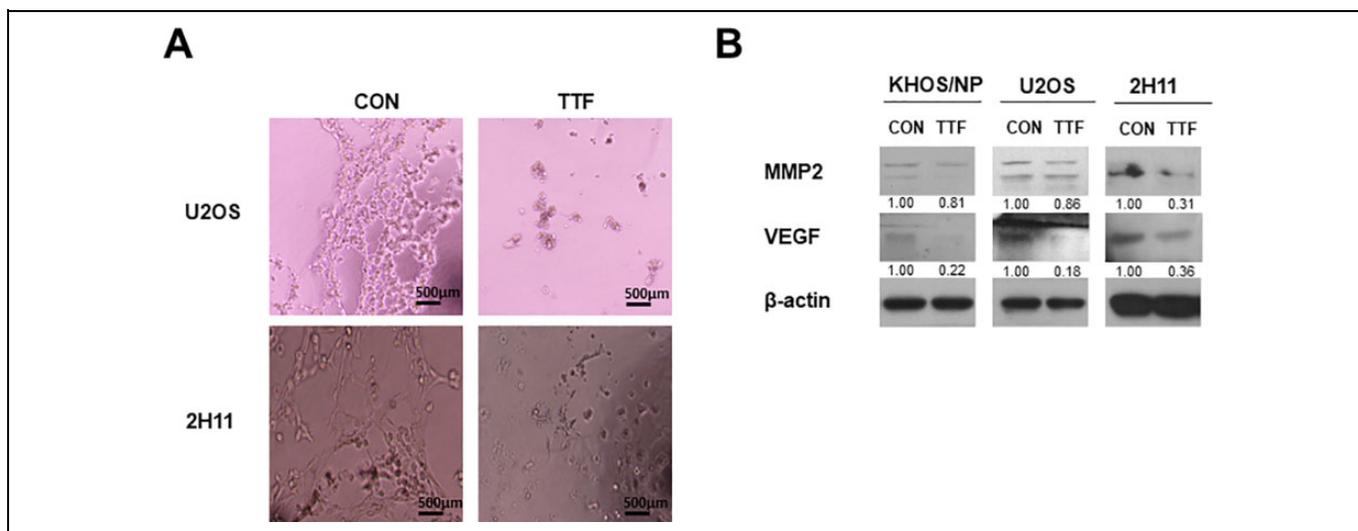
In both adults and children, OS is the most common non-hematologic malignant bone tumor.<sup>22</sup> These malignancies result in a significant decrease in life expectancy, as sarcomas more commonly occur in adolescents and young adults compared to most other forms of cancers.<sup>23</sup> In addition to its poor prognosis, OS is also characterized by a propensity for lung metastasis, resistance to chemotherapy, and a high grade at presentation.<sup>24</sup> Currently, chemotherapy and surgery are both used in OS management. Cure rates showed an improvement from <20% to 65-75% with the incorporation of cytotoxic chemotherapy into therapeutic regimens.<sup>24</sup> Moreover, today, the majority of OS patients are offered limb-salvage surgery.<sup>24</sup> Unfortunately, over the past 3 decades, patient survival has shown no improvement despite advances in chemotherapy and surgical techniques, particularly for metastatic OS patients. This suggests that exploring and identifying directed and novel OS therapies is a critical necessity.

The findings of the present study reveal that, in OS cells, TTF can inhibit cell proliferation, migration, and invasion. Thus, we identified the significance of TTF to demonstrate potential implications regarding the inhibitory effect on not only OS cell proliferation, but also migration and invasion. Herein, MMP-9 was isolated as an invasion-related protein, demonstrated through the digestion of the extracellular matrix surrounding the tumor tissue. Cancer patients show a higher expression of MMP-2 in association with metastasis<sup>25</sup>; similarly, high MMP-2 serum levels are correlated with rapid progression in metastatic cancer patients.<sup>26</sup> As such, it can be safely inferred that by reducing MMP2 expression, TTF suppressed the migration of and invasion by OS cells. Therefore, the suppression of MMP-2 underpins the anti-migration and anti-invasion effects of TTF on OS.

In addition, there is substantial evidence to demonstrate that cancer invasion and metastasis are significantly affected by the EMT. The EMT is marked by 2 primary events: (i) downregulation of vimentin expression and (i) downregulation of N-cadherin expression.<sup>27</sup> TTF treatment resulted in a significant decrease in N-cadherin levels in the current study, indicating a connection between reduced N-cadherin expression and inhibitory effects on OS cell migration and invasion. This inference is in line with a previous report that argued for TTF as a suppressant of GBM cell migration and invasion post-EMT expression inhibition.<sup>28,29</sup>

First, this study identified an inhibitory effect of TTF on OS migration, invasion, and angiogenesis, and propounds the following underpinning mechanisms: (1) reversal of endogenous EMT, (2) suppression of MMP-2 expression, and (3) inhibition of VEGF expression.

As such, this study suggests that TTF is an effective treatment system for metastatic OS. The literature review also shows stagnation in the improvement of patient outcomes despite emerging revolutionary regimens containing conventional cytotoxic chemotherapeutics for OS treatment over the past 3 decades. In addition, the administration of all standard



**Figure 4.** TTF inhibits angiogenesis of OS tumor endothelial cells by inhibiting MMP-2 and VEGF. (A) Representative photomicrographs of *in vitro* tube formation assays for the control and TTF treatment groups after 48 h treatment in 2H11 and U2OS cells. (B) MMP-2 and VEGF expression were analyzed by western blotting of 2H11 and U2OS cells treated with TTF for 48 h.

chemotherapeutic agents in the majority of patients with metastatic and/or recurrent OS during the initial phase of treatment has led to a limitation of secondary therapeutic options for cumulative organ toxicity and/or resistance to previously used agents.<sup>30</sup> This necessitates the need to improve patient survival by focusing on developing novel and effective therapies for OS treatment. More clinical trials are needed to assess the role of TTF in metastatic OS patients. In our opinion, TTF may be considered as a potential modality for OS prevention and therapy.

In conclusion, through the regulation of MMP2, TTF inhibits OS cell proliferation, migration, and invasion. Nevertheless, future studies using animal models are imperative to examine the roles of TTF in OS. Further research will develop an in-depth insight into the underlying mechanisms regulating the anti-tumor effect of TTF, which, in turn, will contribute to the development of improved therapeutic strategies for metastatic OS with improved patient outcomes.

#### 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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#### ORCID iDs

Yeon-Joo Lee  <https://orcid.org/0000-0002-6605-8761>  
Eun Ho Kim  <https://orcid.org/0000-0002-6476-163X>

#### References

- Murphey MD, Robbin MR, McRae GA, Flemming DJ, Temple HT, Kransdorf MJ. The many faces of osteosarcoma. *Radiographics*. 1997;17(5):1205-1231. doi:10.1148/radiographics.17.5.9308111
- Link MP, Goorin AM, Miser AW, et al. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N Engl J Med*. 1986;314(25):1600-1606. doi:10.1056/NEJM198606193142502
- Prater S, McKeon B. *Cancer, Osteosarcoma*. Stat Pearls; 2020.
- Farfalli GL, Albergo JI, Lobos PA, et al. Osteosarcoma lung metastases. Survival after chemotherapy and surgery [in Spanish]. *Medicina (B Aires)*. 2015;75(2):87-90. Metastasis pulmonares en osteosarcoma. Neoadyuvancia, tratamiento quirurgico y supervivencia.
- Longhi A, Errani C, De Paolis M, Mercuri M, Bacci G. Primary bone osteosarcoma in the pediatric age: state of the art. *Cancer Treat Rev*. Oct 2006;32(6):423-436. doi:10.1016/j.ctrv.2006.05.005
- Ferrari S, Palmerini E. Adjuvant and neoadjuvant combination chemotherapy for osteogenic sarcoma. *Curr Opin Oncol*. 2007; 19(4):341-346. doi:10.1097/CCO.0b013e328122d73f
- Ritter J, Bielack SS. Osteosarcoma. *Ann Oncol*. 2010;21(suppl 7):vii320-325. doi:10.1093/annonc/mdq276
- Dizon DS, Krilov L, Cohen E, et al. Clinical cancer advances 2016: annual report on progress against cancer from the American Society of Clinical Oncology. *J Clin Oncol*. 2016;34(9): 987-1011. doi:10.1200/JCO.2015.65.8427
- Gradishar WJ, Anderson BO, Balassanian R, et al. Invasive breast cancer version 1.2016, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2016;14(3):324-354. doi: 10.6004/jnccn.2016.0037
- NOVOCURE®. Updated July 31, 2018. <https://www.novocure.com/our-pipeline/>.

11. Giladi M, Schneiderman RS, Voloshin T, et al. Mitotic spindle disruption by alternating electric fields leads to improper chromosome segregation and mitotic catastrophe in cancer cells. *Sci Rep*. 2015;5:18046. doi:10.1038/srep18046
12. Lee SX, Wong ET, Swanson KD. Mitosis interference of cancer cells by NovoTTF-100A causes decreased cellular viability. *AACR*; 2013;73(8):709-709.
13. Kirson ED, Giladi M, Gurvich Z, et al. Alternating electric fields (TTFs) inhibit metastatic spread of solid tumors to the lungs. *Clin Exp Metastasis*. 2009;26(7):633-640. doi:10.1007/s10585-009-9262-y
14. Jeong H, Sung J, Oh S-I, et al. Inhibition of brain tumor cell proliferation by alternating electric fields. *Applied Physics Letters*. 2014;105(20):203703.
15. Davies AM, Weinberg U, Palti Y. Tumor treating fields: a new frontier in cancer therapy. *Ann N Y Acad Sci*. 2013;1291:86-95. doi:10.1111/nyas.12112
16. Kim EH, Jo Y, Sai S, et al. Tumor-treating fields induce autophagy by blocking the Akt2/miR29b axis in glioblastoma cells. *Oncogene*. 2019;38(39):6630-6646. doi:10.1038/s41388-019-0882-7
17. Jeong H, Sung J, Oh S-i, et al. Inhibition of brain tumor cell proliferation by alternating electric fields. *Appl Phys Lett*. 2014; 105(20):203703.
18. Jo Y, Kim EH, Sai S, et al. Functional biological activity of sorafenib as a tumor-treating field sensitizer for glioblastoma therapy. *Int J Mol Sci*. 2018;19(11). 3684. doi:10.3390/ijms19113684
19. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. *Vasc Health Risk Manag*. 2006;2(3):213-219. doi: 10.2147/vhrm.2006.2.3.213
20. Semenza G. Signal transduction to hypoxia-inducible factor 1. *Biochem Pharmacol*. 2002;64(5-6):993-998. doi:10.1016/s0006-2952(02)01168-1
21. Lee JW, Bae SH, Jeong JW, Kim SH, Kim KW. Hypoxia-inducible factor (HIF-1)alpha: its protein stability and biological functions. *Exp Mol Med*. 2004;36(1):1-12. doi:10.1038/emmm.2004.1
22. Guijarro MV, Ghivizzani SC, Gibbs CP. Animal models in osteosarcoma. *Front Oncol*. 2014;4:189. doi:10.3389/fonc.2014.00189
23. Amankwah EK, Conley AP, Reed DR. Epidemiology and therapies for metastatic sarcoma. *Clin Epidemiol*. 2013;5:147-162. doi: 10.2147/CLEP.S28390
24. Lamplot JD, Denduluri S, Qin J, et al. The current and future therapies for human osteosarcoma. *Curr Cancer Ther Rev*. 2013;9(1):55-77. doi:10.2174/1573394711309010006
25. Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. *Oncol Lett*. 2017;14(5):5865-5870. doi:10.3892/ol.2017.6924
26. Nikkola J, Vihinen P, Vuoristo MS, Kellokumpu-Lehtinen P, Kahari VM, Pyrhonen S. High serum levels of matrix metalloproteinase-9 and matrix metalloproteinase-1 are associated with rapid progression in patients with metastatic melanoma. *Clin Cancer Res*. 2005;11(14):5158-5166. doi:10.1158/1078-0432.CCR-04-2478
27. Loh CY, Chai JY, Tang TF, et al. The E-Cadherin and N-Cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges. *Cells*. 2019;8(10). 1118. doi:10.3390/cells8101118
28. Kim EH, Song HS, Yoo SH, Yoon M. Tumor treating fields inhibit glioblastoma cell migration, invasion and angiogenesis. *Oncotarget*. 2016;7(40):65125-65136. doi:10.18632/oncotarget.11372
29. Kim EH, Kim YH, Song HS, et al. Biological effect of an alternating electric field on cell proliferation and synergistic antimetastatic effect in combination with ionizing radiation. *Oncotarget*. 2016;7(38):62267-62279. doi:10.18632/oncotarget.11407
30. Arndt CA, Koshkina NV, Inwards CY, et al. Inhaled granulocyte-macrophage colony stimulating factor for first pulmonary recurrence of osteosarcoma: effects on disease-free survival and immunomodulation. A report from the Children's Oncology Group. *Clinical cancer research*. 2010;16(15):4024-4030.