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Non-contact Electric Field Exposure Provides Potential Cancer Therapy through p53-Independent Proliferation Arrest and Intrinsic Pathway Apoptosis Induction in MG-63 Cell Lines

Omat Rachmat Hasbullah[1*], Ilma Fiddiyanti[2], Dewi Ratih Handayani[2], Endang Sutedja[3], Darmadji Ismono[3], Nucki Nursjamsi Hidajat[3], Wahyu Widowati[4], Ervi Afifah[5], Hanna Sari Widya Kusuma[5], Rizal Rizal[5,6], Firman Alamsyah[7], Warsito P. Taruno[7]

[1]*Faculty of Medicine, University of Sultan Ageng Tirtayasa, Serang, Banten 42124, Indonesia*
[2]*Faculty of Medicine, University of Jenderal Achmad Yani, Cimahi 40531, Indonesia*
[3]*Faculty of Medicine, Universitas Padjadjaran, Jatinangor 45363, Indonesia*
[4]*Faculty of Medicine, Maranatha Christian University, Bandung 40164, Indonesia*
[5]*Biomolecular and Biomedical Research Centre, Aretha Medika Utama, Bandung 40163, Indonesia*
[6]*Biomedical Engineering, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, Indonesia*
[7]*Center for Medical Physics and Cancer Research Ctech Labs Edwar Technology, Tangerang 15143, Indonesia*

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ABSTRACT

Osteosarcoma is a highly malignant primary tumor on bone that mainly attacks children and young adolescents. Until now, osteosarcoma therapy still combines some high costs and invasive therapy modalities that may give side effects, such as p and nausea. Our previous studies suggested t non-contact electric field has anti-proliferative effect on breast cancer cells, *in vitro* and *in vivo*. In this study, we were interested studying alternating current electric field effects on osteosarcoma cells progression as well as its potential cytotoxic effects. MG-63 human osteosarcoma cells were cu ed and treated with 200 kHz for 6 days. Several genes of interest including p53, p21, MDM2, caspase-3, caspase-8, and caspase-9 were analyzed using real-time qPCR method. Apoptotic index was measured using flow-cytometry assay. Apoptosis was observed through p53-independent p21 pathway (p = 0.011). Cells u ergoing apoptosis through internal pathways were shown by the increase of caspase-3 (p = 0.015) and caspase-9 (p = 0.001) levels, but not caspase-8 (p = 0.080). This treatment has successfully reduced the number of living osteosarcoma cells by 14.7% (p = 0.000) and increased cell death up to 4.26% (p = 0.055). Apoptotic index was markedly increased to 16% (p = 0.001). 200 kHz non-contact electric field exposure can disrupt osteosarcoma progression through disruption of normal cell cycle via p53-independent p21 pathway and induction of apoptosis.

1. Introduction

Osteosarcoma is a highly malignant primary tumor on bone that mainly affects children and young adolescents (Solomon *et al.* 2010). In Indonesia, osteosarcoma was recorded to account for 22–25% of all bone and joint cancers (Hutagalung 2005; Ismono 2003) Osteosarcoma cells are the most significant inherent determinant for a bad prognosis in osteosarcoma patients because they are more prone to spreading and developing metastases (Bielack *et al.* 2002). The majority of the time, osteosarcoma spreads to the lungs, while it can also happen to bone and lymph nodes, the progression of osteosarcoma is marked by aggressive growth and local recurrence, resulting in low life expectancy caused by metastasis to lungs in more than 50% cases. So far, osteosarcoma therapy still combines some therapy modalities, including radiation therapy, immunotherapy, chemotherapy which uses chemotherapeutic agent, biological agent or neoadjuvant therapy, and surgical treatment including limb salvage and amputation (Jaffe *et al.* 2013). However, these therapies are

* Corresponding Author
E-mail Address: dr_omatr@yahoo.com

high costs and can cause side effects, such as pain and nausea, and may also affe the healthy cells (Agarwal and Nayak 2012; Chen *et al.* 2013; Dicarpio *et al.* 2003; Hutagalung 2005; Harisson *et al.* 2018; Jaffe *et al.* 2013; Yang 2005).

Several alternative therapies have been proposed to treat osteosarcoma. Numerous cancer medicines that target cancer cells directly and specifically are under clinical development, e.g., multi-target drugs for receptor tyrosine kinase inhibitors, cell cycle and DNA repair proteins, or through indirect targeting of microenvironment modulation, such as macrophage regulator and loaded T lymphocytes (Brown *et al.* 2018). However, not only chemical drugs development, but several physical therapies have also been proposed, such as phototherapy (Yu *et al.* 2017), cold atmospheric plasma (Gumbel *et al.* 2017), ultrasound (Agnese *et al.* 2019), magnetic and water-based hyperthermia (Herea *et al.* 2018), and pulse electromagnetic field (Muramatsu *et al.* 2017). Having ntimitotic effect on cells that ultimately results in cell death (Kirson *et al.* 2004; McCaig *et al.* 2005; Ra mat *et al.* 2017), currently, alternating current, low intensity, intermediate frequency electric field ave been revealed as a novel cancer treatment (Hottinger *et al.* 2016; Kirson *et al.* 200 In our previous study, low intensity (18 Vpp) and intermediate frequency (100 kHz) electric fiel exposure has shown anti-proliferative effect on breast cancer cells, *in vitro* and *in vivo* (Alamsyah *et al.* 2015), and was successfully inhibited in the proliferation of HeLa and oral cancer cells (Mujib *et al.* 2017). This intermediate frequency is used to treat cancer because it specifically targets cancer cells without affecting normal cells. Furthermore, we develop non-contact electric fields to avoid dermatitis or other injuries due to direct contact between the electrodes and the skin of animal model, as reported by Kirson *et al.* (2007).

Apoptosis is the main barrier of oncogenesis and tumor suppressor protein p53 is the main key for the regulation of ap tosis and carcinogenesis which is mediated by two main pathways, extrinsic and intrinsic pathways. In extrinsic pathway, caspase 8 activation occurs to induce apoptosis, whereas in the intrinsic pathway, there is a role of mitochondrial protein in caspase 9 activation to induce apoptosis. Both mechanisms resulted in pro-caspase 3 cleavage to become active caspase 3, the main executioner in the apoptosis. Caspase-3 causes chromosomal DNA degradation and induces cytoskeletal reorganization and cell body disintegration (Fiandalo and Kyprianou 2012; Lahiry *et al.* 2010; Maximov and Maximov 2008; Olsson and Zhivotovsky 2011; Parissh *et al.* 2013; Pirnia *et al.* 2002; Silke and Meier 2013). Protein p53 is an important factor that inhibits tumor progression by activating p21 protein that regulates cell cycle progression (Maximov and Maximov 2008). Mutations of p53 gene is the commonest mutation in more than 50% of cases of malignancy especially in osteosarcoma (Yao *et al.* 2014). The p53 protein in normal cells remains low so that cells keep growing and proliferating (Bai and Zhu 2006). Low p53 protein concentration is caused by the regulation of Murine Double Minute 2 (MDM2) oncoprotein. In most cases of o osarcoma, MDM2 gene is overexpressed and this is related a poor clinical prognosis (Hou *et al.* 2019), MDM2 functions as E3 ubiquitin ligase and is responsible for the continued degradation of p53 in normal cells.

Based on the backgrounds, intermediate frequency and low intensity electric field exposure can be a prospective, less invasive cancer therapy. As far as we know, this kind of novel treatment has not been done on osteosarcoma cancer cells. Based on the backgrounds, intermediate frequency and low intensity electric field exposure can be a prospective, less invasive cancer therapy. Thereby we were interested in studying the effect of this therapy on MG-63 cell line human osteosarcoma in vitro thr h the evaluation of apoptotic markers p53, p21, caspase 3, caspase 8, caspase 9, and MDM2 expression.

2. Materials and Methods

2.1. Cell Culture

MG-63 cell lines (ATCC®CRL-2522), provided by Aretha Medika Utama, Biomolecular and Biomedial Research Center, Bandung, West Java, Indonesia, were cultured in complete dium (Minimum Esential Medium-α (MEM-α) (Biowest L0475) + 10% Fetal Bovine Serum (FBS) (Biowest S181H) + 1% penicillin streptomycin (Biowest L0022) in 37 oC and 5% CO_2 conditions. After reaching 80 to 90% confluence, cells were harvested for treatments (Rachmat *et al.* 2017).

2.2. Treatments

Cells were harvested with 1 mL trypsin EDTA and centrifuged at 2,000 rpm for 5 minutes. Supernatant was discarded an pellet was suspended in 1 ml MEM-a medium. Cells were inserted into a 6-well

plate at 2 × 10^6 cells/well density. Complete medium was added as much as 3 ml and incubated for 24 hours. After 24 hours and the cells were already attached, the medium was replaced. The culture plates were positioned between two capacitive electrodes that were connected to an oscillator (Figure 1) that was provided by Centre for Medical Physics and Cancer Research Ctech Labs Edwar Technology (Alamsyah *et al.* 2015; Mujib *et al.* 2017). Later, the electrodes and plates were placed in incubator at 37°C and 5% CO_2. Cells were treated with 200 kHz for 6 days based on previous research with medium replacement every two days (Rachmat *et al.* 2017).

2.3 RNA Isolation and cDNA Synthesis

Aurum Total RNA Mini kit (Bio-Rad, 1708841) [California, USA] was used to perform RNA isolation. All procedures were referred to Afifah *al.* (2019) and Widowati *et al.* (2020a) protocol. The iScript cDNA synthesis kit (Bio-Rad, 1708890) [California, USA] was used on performing cDNA synthesis according to the manufacturer's protocol. Temperature reaction for cDNA synthesis was 25°C for 5 minutes; reverse transcription at 42°C for 30 minutes; RT Inactivation at 85°C for 5 minutes; and the last hold at 4°C for 5 min (Afifah *et al.* 2019; Widowati *et al.* 2020a).

2.4. Gene Expression Measurement with Real-Time PCR

The qPCR reaction was done using SsoFast Eva Green Supermix kit according to manufacturer's protocols (BioRad, 172-5200) [California, USA] which contained: 5 µl evagreen, 2 µl each reverse and forward primers (See Table 1), 2 µl Nuclease

Figure 1. Capacitive electrodes as electrical field generator. A microplate was flanked between two capacitive electrodes that were connected to low intensity oscillator to generate the alternate electric field

Table 1. Primer sequence and product size of casp9, casp8, casp3, MDM2, p21, and p53 genes

Primer	Forward	Reverse	Size (bp)
casp9	5'-CAT GCT CAG GAT GTA AGC CA-3'	5'-AGG TTC TCA GAC CGG AAA CA-3'	161
casp8	5'-AGG CCA GAT CTT CAC TGT CC-3'	5'-GGT CAC TTG AAC CTT GGG AA-3'	114
casp3	5'-AGA ACT GGA CTG TGG CAT TGA G -3'	5'-GCT TGT CGG CAT ACT GTT TCA G-3'	191
mdm2	5'-CTG ATC CAA CCA ATC ACC TG-3'	5'-AAG CCT GGC TCT GTG TGT AA-3'	130
p21	5'-CGA CCT TCC TCA TCC A-3'	5'-GAC TCC TTG TTC CGC TGC TAA-3'	71
p53	5'- CCA AGG CGC ACT GGC CTC ATC -3'	5'-CAG AGG CTG GGG CAC AGC AGG -3'	205

free water, 1 µl cDNA samples. The master mix was transferred to piko real plate and incubated in the qPCR machine (ThermoScienc [Massachusetts, USA] with the following stages: pre-denaturation at 95°C for 5 minutes, denaturation at 95°C for 1 minute, annealing at 50°C (for all primers) for 40 seconds, elongation at 72°C for 1 minute, post-elongation at 72°C for 5 minutes, melt curve 55–95°C, and hold at 4°C (Afifah *et al.* 2019; Widowati *et al.* 2020a). This stage was done in triplicate. Primer sequences and product sizes are shown in Table 1. Gene expression was measured as relative copy number compared to control using ΔΔCT method (Arya *et al.* 2005).

2.5. Apoptotic Indexs

Approximately 5 × 10 2 × 10^6 cells were taken and centrifuged at 1,600 rpm, at 4°C for 5 minutes. The supernatant was discarded, while the pellet was mixed with 1 mL of PBS (Gibco, 14200075) [Thermo Fisher Scientific, Massachusetts, USA] contain 5% FBS (Biowest, S181H) [Biowest, Nuaile, France]. Samples were then centrifuged at the same condition. Supernatant was removed and 1 ml binding buffer from Annexin V Fluos Kit (Sigma Aldrich, ab14085) [Sigma Aldrich, Missouri, USA] were added to the pellet. Then 5 ml of PI and FITC was added in the sample and it was incubated at 4°C for approximately 30–40 minutes (Gloushankova *et al.* 2017). Samples were later analyzed in MACSquant Flowcytometry (Miltenyi Biotech) [Bergisch Gladbach, Germany] (Lister *et al.* 2020; Widowati *et al.* 2020b).

2.6. Statistical Analysis

Independent t-test was used to compare significance between control and treaments group at confidence interval 95% ($p = 0.05$).

3. Results

3.1. Non-contact Electric Field Alters Cell Morphology

The alternating current electric field alters cells' morphology. The fibroblastic, spindle shaped osteosarcoma had changed into spherical shaped cells. As also can be seen from (Figure 2), the gap between cells was seen at all treated groups.

3.2. Non-contact Electric Field May Causes Cell Cycle Arrest

The 200 kHz frequency of alternate current electric field in our experiment had caused significant changes on p21 gene and MDM2 gene expression (Table 2). The expression of p21 gene increased significantly compared to control which indicated increase inhibitory activity in cell cycle progression Due to the MDM2 gene's role as a p53 negative regulator, its expression was lower than that of the control. Cell cycle arrest and apoptosis result from an increase in p53 levels, which also increases p53 transcriptional activity. Insignificant variations in p53 expression levels imply potential alternate p21 regulatory mechanisms protein activity that implicated increased p21 response and activity. However, insignificant changes in p53 expression level indicated that there might be an alternative pathway in p21 regulation

3.3. Non-contact Electric Field Induces Apoptosis through Intrinsic Pathway

Electric field exposure had caused decrease in MG-63 human osteosarcoma cell survival by 14.7% ($p = 0.000$), referring to the percentage of live cells, The dead of the cells in treatment group was thought mainly due to apoptosis (Figure 3). The 200 kHz frequency of alternate current electric field had caused significant difference increase of apoptotic index from 1.95(1.77–2.07) to 17.53 (17.36–18.91) ($p = 0.001$), which means that the electric field induced cell apoptotic activity (Table 3). Caspase 9 expression difference significantly ($p = 0.001$), while changes in caspase 8 are insignificant ($p = 0.080$), indicates that the cells are mainly induced to undergo apoptotic activity through intrinsic pathway (Table 2). difference in caspase 9 expression implicated in difference of caspase 3 as the apoptotic executioner by four-fold ($p = 0.015$).

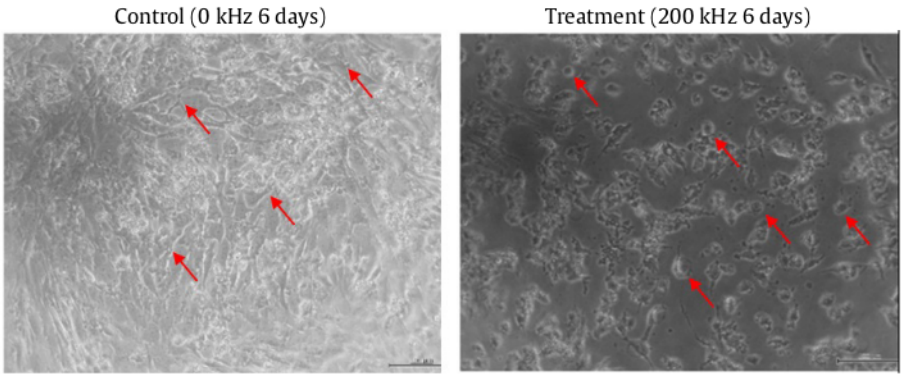

Figure 2. Electrical field reduces MG-63 alters cells' morphology. The figure above shows the MG-63 cell population through an inverted microscope at 6 days incubation at 20 × 20,000 magnification. The non-treated cells show triangular spindle shape morphology. The cells undergone massive proliferation and already reached 100% confluency at 6 days in the control group. Electrical field induction causes alteration on cells' morphology where they seem to shrink and have spherical morphology and show the gap between cells in the treatment group

Table 2. Effect of 200 kHz electrical field induction to p53, p21, MDM2, casp3, casp8, casp9 relative gene expression 24 MG-63 human osteosarcoma cells. Data was presented mean±standard deviation. The single star (*) show statistically significant differences based on the t-test ($p<0.05$)

Variable	Group		Significance (p-value)
	200 kHz treated	Control	
p53 Expression			
Mean (SD)	1.37 (0.06)	1 (0.0)	0.198
Median (range)	1.36 (1.31-1.43)	1 (1-1)	
p21 Expression			
Mean (SD)	9.96 (0,06)	1 (0.0)	0.011*
Median (range)	9.65 (11.71–8.51)	1 (1-1)	
MDM2 Expression			
Mean (SD)	0.67 (0.12)	1 (0.0)	0.065
Median (range)	0.63 (0.57-0.81)	1 (1-1)	

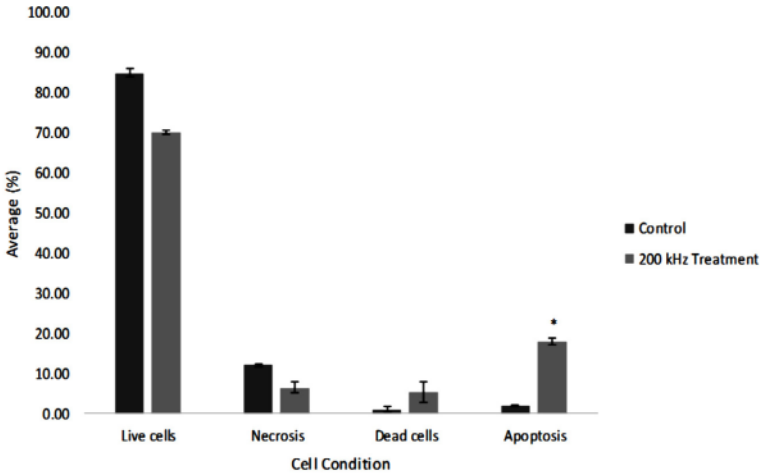

Figure 3. Percentage of MG-63 human osteosarcoma cell conditions with or without 200 kHz electrical induction treatment. The graph shows the proportion of live cells, dead cells, cells that underwent apoptosis, and necrotic cells based on the Flow cytometry result. Electrical induction can reduce MG-63 human osteosarcoma cells survival and induce them to undergo apoptosis resulted in cell death. Data was presented mean ± standard deviation. The single star (*) show statistically significant differences based on the t-test ($p<0.05$)

Table 3. Effect of 200 kHz alternate current in MG-63 human osteosarcoma cells apoptotic activity. Data was presented mean±standard deviation. The single star (*) show statistically significant differences based on the t-test (p<0.05)

Variable	Group		Significance (p-value)
	200 kHz treated	Control	
Caspase 3 Expression			
Mean (SD)	4.05 (0.96)	1 (0.0)	0.015*
Median (range)	4.08 (3.07-4.99)	1 (1-1)	
Caspase 8 Expression			
Mean (SD)	1.55 (0.34)	1 (0.0)	0.080
Median (range)	1.47 (1.25-1.93)	1 (1-1)	
Caspase 9 Expression			
Mean (SD)	8.71 (1.69)	1 (0.0)	0.001*
Median (range)	8.82 (6.96-10.34)	1 (1-1)	
Apoptotic Index			
Mean (SD)	17.93 (0.85)	1.93 (0.15)	0.001*
Median (range)	17.53 (17.36-18.91)	1.95 (1.77-2.07)	

4. Discussion

200 kHz non-contact electric field is the effective frequency to treat osteosarcoma and the therapy should be performed for at least six days to give significant effect (Rachmat *et al.* 2017). As indicated in (Figure 2) the TTFields causes spindle shaped osteosarcoma cells to undergone morphological changes to sphere-like shapes with gap between cells during six days of treatment. The morphological changes indicate cell stress, which is caused by microtubule and actin filament network misalignments. These cytoskeletons also playing roles in maintaining cells adherent junctions and junction associated proteins such as cadherin/cathenin that attach to F-actin which are maintained by microtubules (Gloushankova *et al.* 2017; Kasioulis *et al.* 2017), microtubule and actin filaments give mechanical strength and membrane stiffness to the cells (Kelliher *et al.* 2019).

Mitotic aberrations related to centrosome and mitotic spindle defects causes premature p53–p21 and p21–p16 premature senescence phenotype (Dikovskaya *et al.* 2015; Schmidt *et al.* 2010). Our result shows that p21 is significantly upregulated due to electrical field exposure, however p53 is not. This may indicate that there is a mechanism of p53-independent p21 upregulation pathway, although it has been known that p21 is p53 target. MELK (Maternal Embryonic Leucine Zipper Kinase) inhibition could cause cell cycle arrest at G1 stage through p21 expression in p53-knockdown cancer cells. Further investigation demonstrated two transcriptional regulators of p21, FOXO (Fork head box O) -1 and -3 which were de-activated through phosphorylation by MELK, is upregulated in MELK-knock down cells (Matsuda *et al.* 2017). Another proposed mechanism is upregulation of p21/p27 through autophagy-mediated downregulation of F box protein Skp2 and p62/SQSTM1 expression independent of p53 status due to Quinacrine induction in ovarian cancer (Jung *et al.* 2018). Our data also shows that MDM2 is not significantly downregulated due to electrical field exposure. MDM2 is a protein that is responsible in Akt-MDM2-p53 signaling pathway in p21 regulation (Ignacio *et al.* 2018). The thing that drives apoptosis is from p53 signaling which transcribes and activates a large number of genes so that it undergoes apoptosis which in turn leads to tumorigenesis that causes DNA damage. a study conducted by Mujib *et al.* (2017) regarding the p53 expression of cancer cells under exposure to a low-intensity and medium-frequency non-contact electric field, found that 80-85% of positively stained cancer cells prevented cell division through p53 protein in oral cultured cells and HeLa. From (Figure 3) necrotic index is significantly higher ($p = 0.003$) in non-treated cells compared to treated cells. Induced by oxygen and glucose deprivation (OGD) and beginning with cell swelling, leading to membrane rupture and release of cytoplasmic contents into the extracellular space, necrosis has been considered as an accidental and genetically unprogrammed form of cell death. Cells in the inner regions of solid tumors (including osteosarcoma) display higher OGD because of insufficient blood supply. Necrosis has a promotion potential by releasing Damage-Associated Molecular Pattern molecules including HMGB1 (High Mobility Group Box1) which resulted in enhanced inflammatory responses, reactive oxygen formation, and metastasis

through the release of proinflammatory cytokines by activating NFκB and inflammasomes pathway44. While our result shows that non-contact electric field exposure might potentially inhibit necrosis while promoting apoptosis, potentially, the necrosis inhibition in 200 kHz electric field treated cells is caused by inhibition of miR-23a-3p expression (Dong *et al.* 2017). The mechanism probably lies in miR-23a-3p because there is a sharing pathway between miR-23a-3p and pro-apoptotic protein bax (Grieco *et al.* 2017).

Our result suggests that non-contact electric field exposure causes intrinsic pathway apoptosis, marked by significant higher of casp3 and casp9 expression. Intrinsic pathway is a non-receptor-mediated pathway that cause intracellular cascades within the cell and is initiated within mitochondria. While some studies have suggested that electrical pulse stimulation may cause membrane permeabilization (Nuccitelli *et al.* 2007; Silve *et al.* 2015, 2016), the inner mitochondrial membrane could change because of pore opening of mitochondrial permeability transition, mitochondrial transmembrane potential loss, and pro-apoptotic proteins release from the intermembrane space into the cytosol (Elmore 2007). We hypothesized that the same mechanism might cause mitochondrial membrane permeabilization that leads to initiation of apoptosis intrinsic pathway.

Control and regulation of the mitochondrial-mediated apoptotic event are initiated by members of the Bcl-2 protein family. Bax (Bcl2-associated X protein), one of the family members plays a role in cytochrome c release that leads to activation of pro-caspase-9. Later caspase-9 will cleave caspase-3 as the executioner. This protein will act through activation of CAD (Caspase-Activated DNAse) in chromosomal DNA degradation. In addition to the tumor suppressor protein p53 having a critical role in the Bcl-2 proteins family regulation (Elmore 2007), Caspase 3 induces cytoskeletal reorganization and the cell disintegration into apoptotic bodies. Tumor treating fields (TTFields) prevent DNA damage repair by hindering homologous recombination repair, which may enhance Bax expression and enhance intrinsic pathway apoptosis (Giladi *et al.* 2017). The insignificance between p53 level in electric field treated tumor cells and control may suggest that there might be another mechanism of intrinsic pathway apoptosis which is p53-independent.

In osteosarcoma patients, the microRNA-95-3p is upregulated. When compared to normal controls, miR-95-3p expression was decreased in both high-grade and low-grade osteosarcoma tissues (Novello *et al.* 2013). Reduced expression of miR-95-3p is a statistically significant risk factor that lowers overall survival in patients with osteosarcoma and works as an anti-oncogenic miRNA in osteosarcoma (Niu *et al.* 2016). The miRNA-95-3p expression is probably inhibited during electric field exposure, in osteosarcoma cells, micro-RNA-95-3p inhibition increases caspase-3 and caspase-9 activities and Bax/Bcl-2 protein expression, and eventually suppresses cell growth and induces apoptosis. MiRNA-95-3p suppression also gives anticancer effects on cell growth by supressing TGF-b and p-Smad2 while inducing p21 protein expression and suppressing cyclin D1 protein (Zhao *et al.* 2018).

From our result and literature review, we proposed the possible mechanism of TTField induction in inhibiting osteosarcoma progression as shown in (Figure 4). The alternating current can affect osteosarcoma cells through several mechanisms: 1) It causes mitotic aberration that causes p21 senescence phenotype through p53-independent pathway. This pathway is probably initiated by MELK inhibition that causes FOXO1 and FOXO3 transcription factor activation which upregulates p21. The upregulation of p21 resulted in inhibition of cyclin D1 that causes G1/S phase progression delay (senescence); 2) miR-23a-3p inhibition that resulted in necrosis inhibition and apoptosis progression; 3) inhibition of miR-95-3p that upregulate p21 and Bax, caspase9, and caspase3, marker proteins of apoptosis intrinsic pathway; 4) increased mitochondrial permeability that resulted in intrinsic pathway upregulation. In the intrinsic pathway apoptosis, protein Bax will cause cytochrome c release and together with caspase9 will create apoptosome that will result in caspase3 activation. Caspase3 later will cause cytoskeletal disorganization and DNA degradation through CAD activation; and 5) Inhibition of homologous repair that cause in DNA repair delay.

In conclusion, non-contact electric field exposure can disrupt osteosarcoma progression through disruption of cell morphology and adhesion between cells. Osteosarcoma treated with 200 kHz for 6 days gave the cell senescence through p53-independent p21 pathway. Cells undergone apoptosis through

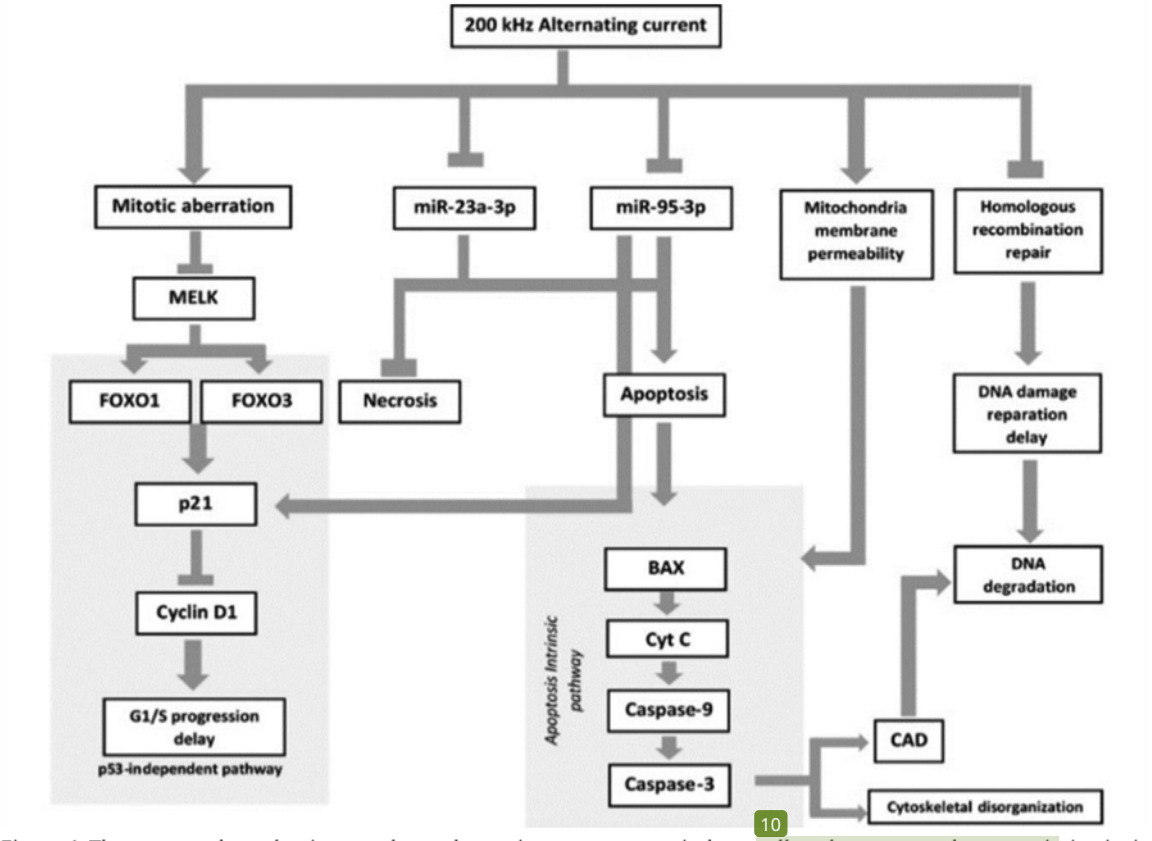

Figure 4. The proposed mechanism on how alternating current can induce cell-cycle arrest and apoptosis intrinsic pathway in osteosarcoma cells

intrinsic pathway by the marked increase of casp-3 and casp-9 levels. This treatment had successfully reduced the number of living osteosarcoma cells by 14.7% and increased cell death by 4.26%. The non-contact electric field is an attractive candidate to serve as a novel, non-invasive treatment for osteosarcoma.

Conflict of Interest

The authors declare that they have no competing interests.

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References

Afifah, E., Mozef, T., Sandra, F., Arumwardana, S., Rihibiha, D.D., Nufus, H., Rizal, R., Amalia, A., Bachtiar, I., Murti, H., Widowati, W., 2019. Induction of matrix metalloproteinases in chondrocytes by interleukin IL-1β as an osteoarthritis model. *J. Math. Fundam Sci.* 5, 1–9. https://doi.org/10.5614/j.math.fund.sci.2019.51.2.1

Agarwal, M.G., Nayak, P., 2012. Limb salvage for osteosarcoma: current status with a review of literature In: Agarwal M (Eds.). *Osteosarcoma*. Shanghai: InTech. pp. 109–136.

Agnese, V., Costa, V., Scoarughi, G.L., Corso, C., Carina, V., De Luca, A., Bellavia, D., Raimondi, L., Pagani, S., Midiri, M., Stassi, G., 2019. Focused ultrasound effects on osteosarcoma cell lines. *BioMed Res. Int.* 2019, 1–14. https://doi.org/10.1155/2019/6082304

Alamsyah, F., Ajrina, I.N., Dewi, F.N.A., Iskandriati, D., Prabandari, S.A., Taruno, W.P., 2015. Antiproliferative effect on electric fields on breast tumor cells *in vitro* and *in vivo*. *Indones. J. Cancer Chemoprevention*. 6, 71–77. https://doi.org/10.14499/indonesianjcanchemoprev6iss3pp71-77

Arya, M., Shergill, I.S., Williamson, M., Gommersall, L., Arya, N., Patel, H.R., 2005. Basic principles of real-time quantitative PCR. *Expert Rev. Mol. Diagn.* 5, 209–219. https://doi.org/10.1586/14737159.5.2.209

Bai, L., Zhu, W.G., 2006. p53: Structure, function and therapeutic applications. *J. Cancer Mol.* 2, 141–153.

Bielack, S.S., Kempf-Bielack, B., Delling, G., Exner, G.U., Flege, S., Helmke, K., Kotz, R., Salzer-Kuntschik, M., Werner, M., Winkelmann, W., Zoubek, A., 2002. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J. Clin. Oncol.* 20, 776–790. https://doi.org/10.1200/JCO.2002.20.3.776

Brown, H.K., Schiavone, K., Gouin, F., Heymann, M.F., Heymann, D., 2018. Biology of bone sarcomas and new therapeutic developments. *Calcif. Tissue Int.* 102, 174–195. https://doi.org/10.1007/s00223-017-0372-2

Chen, Y., Yang, Y., Wang, C., Shi, Y., 2013. Radiographic response to neoadjuvant therapy and its impact on scope of surgery and prognosis in stage IIB/III soft tissue sarcomas. *BMC Cancer.* 13, 1-9. https://doi.org/10.1186/1471-2407-13-591

Dikovskaya, D., Cole, J.J., Mason, S.M., Nixon, C., Karim, S.A., McGarry, L., Clark, W., Hewitt, R.N., Sammons, M.A., Zhu, J., Athineos, D., 2015. Mitotic stress is an integral part of the oncogene-induced senescence program that promotes multinucleation and cell cycle arrest. *Cell Rep.* 12, 1483–1496. https://doi.org/10.1016/j.celrep.2015.07.055

Dong, Y., Li, T., Li, Y., Ren, S., Fan, J., Weng, X., 2017. MicroRNA-23a-3p inhibitor decreases osteonecrosis incidence in a rat model. *Mol. Med. Rep.* 16, 9331–9336. https://doi.org/10.3892/mmr.2017.7808

Elmore, S., 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35, 495–516. https://doi.org/10.1080/01926230701320337

Fiandalo, M.V., Kyprianou, N., 2012. Caspase control: protagonists of cancer cell apoptosis. *Exp. Oncol.* 34, 165–175.

Giladi, M., Munster, M., Schneiderman, R.S., Voloshin, T., Porat, Y., Blat, R., Zielinska-Chomej, K., Hååg, P., Bomzon, Z.E., Kirson, E.D., Weinberg, U., 2017. Tumour treating fields (TTFields) delay DNA damage repair following radiation treatment of glioma cells. *Radiat. Oncol.* 12, 1–13. https://doi.org/10.1186/s13014-017-0941-6

Gloushankova, N.A., Rubtsova, S.N., Zhitnyak, I.Y., 2017. Cadherin-mediated cell-cell interactions in normal and cancer cells. *Tissue Barriers.* 5, 1–15. https://doi.org/10.1080/21688370.2017.1356900

Grieco, F.A., Sebastiani, G., Juan-Mateu, J., Villate, O., Marroqui, L., Ladrière, L., Tugay, K., Regazzi, R., Bugliani, M., Marchetti, P., Dotta, F., 2017.MicroRNAs miR-23a-3p, miR-23b-3p, miR-149-5p regulate the expression of proapoptotic BH3-only proteins DP5 and PUMA in human pancreatic b-cells. *Diabetes.* 66, 100–112. https://doi.org/10.2337/db16-0592

Gumbel, D., Bekeschus, S., Gelbrich, N., Napp, M., Ekkernkamp, A., Kramer, A., Stope, M.B., 2017. Cold atmospheric plasma in the treatment of osteosarcoma. *Int. J. Mol. Sci.* 18, 1–13. https://doi.org/10.3390/ijms18092004

Harrison, D.J., Geller, D.S., Gill, J.D., Lewis, V.O., Gorlick, R., 2018. Current and future therapeutic approaches for osteosarcoma. *Expert Rev. Anticancer Ther.* 18, 39–50. https://doi.org/10.1080/14737140.2018.1413939

Hutagalung, E.U., 2005. Overview of bone tumours in Cipto Mangunkusumo Hospital Jakarta management and problems. *In: Proceedings of 10th National Conference Pathology and Anatomy Professionals Associations in Indonesia*. Surabaya: National Conference Pathology and Anatomy Professionals Associations in Indonesia, pp. 24–51.

Herea, D.D., Danceanu, C., Radu, E., Labusca, L., Lupu, N., Chiriac, H., 2018. Comparative effects of magnetic and water-baed hyperthermia treatments on human osteosarcoma cells. *Int. J. Nanomed.* 13, 5743–5751. https://doi.org/10.2147/IJN.S174853

Hottinger, A.F., Pachero, P., Stupp, R., 2016. Tumour treating fields: a novel treatment modality and its use in brain tumours. *Neuro-Oncol.* 18, 1338–1349. https://doi.org/10.1093/neuonc/now182

Hou, H., Sun, D., Zhang, X., 2019.The role of MDM2 amplification and overexpression in therapeutic resistance of malignant tumours. *Cancer Cell Int.* 19, 216–223. https://doi.org/10.1186/s12935-019-0937-4

Ignacio, R.M., Dong, Y.L., Kabir, S.M., Choi, H., Lee, E.S., Wilson, A.J., Beeghly-Fadiel, A., Whalen, M.M., Son, D.S., 2018. CXCR2 is a negative regulator of p21 in p53-dependent and independent manner via Akt-mediated MDM2 in ovarian cancer. *Oncotarget.* 9, 9751–9765. https://doi.org/10.18632/oncotarget.24231

Ismono, D., 2003. The profile of musculoskeletal tumour in Hasan Sadikin Hospital. *In: Proceedings Scientific Meeting and Workshop of Musculoskeletal Pathology*. Surabaya: Scientific Meeting and Workshop of Musculoskeletal Pathology, pp. 181–192.

Jaffe, N., Puri, A., Gelderblom, H., 2013. Osteosarcoma: evolution of treatment paradigms. *Sarcoma.* 2013, 1-7. https://doi.org/10.1155/2013/203531

Jung, D., Khurana, A., Roy, D., Kalogera, E., Bakkum-Gamez, J., Chien, J., Shridhar, V., 2018. Quinacrine upregulates p21/p27 independent of p53 through autophagy-mediated downregulation of p62-Skp2 axis in ovarian cancer. *Sci. Rep.* 8, 1-12. https://doi.org/10.1038/s41598-018-20531-w

Kasioulis, I., Das, R.M., Storey, K.G., 2017. Inter-dependent apical microtubule and actin dynamics orchestrate centrosome retention and neuronal delamination. *Elife.* 6, 1–31. https://doi.org/10.7554/eLife.26215

Kelliher, M.T., Saunders, H.A., Wildonger, J., 2019. Microtubule control of functional architecture in neurons. *Curr. Opin. Neurobiol.* 57, 39–45. https://doi.org/10.1016/j.conb.2019.01.003

Kirson, E.D., Gurvich, Z., Schneiderman, R., Dekel, E., Itzhaki, A., Wasserman, Y., Schatzberger, R., Palti, Y., 2004. Disruption of cancer cell replication by alternating electric fields. *Cancer Res.* 64, 3288–3295. https://doi.org/10.1158/0008-5472.CAN-04-0083

Kirson, E.D., Dbalý, V., Tovaryš, F., Vymazal, J., Soustiel, J.F., Itzhaki, A., Mordechovich, D., Steinberg-Shapira, S., Gurvich, Z., Schneiderman, R., Wasserman, Y., 2007. Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. *Proc. Natl. Acad. Sci. U. S. A.* 104, 10152–10157. https://doi.org/10.1073/pnas.0702916104

Lahiry, L., Saha, B., Chakraborty, J., Adhikary, A., Mohanty, S., Hossain, D.M.S., Banerjee, S., Das, K., Sa, G., Das, T., 2010. Theaflavins target Fas/caspase-8 and Akt/pBad pathways to induce apoptosis in p53-mutated human breast cancer cells. *Carcinogen.* 31, 259–268. https://doi.org/10.1093/carcin/bgp240

Lister, I.N.E., Ginting, C.N., Girsang, E., Nataya, E.D., Azizah, A.M., Widowati, W., 2020. Hepatoprotective properties of red betel (*Piper crocatum* Ruiz and Pav) leaves extract towards H_2O_2-induced HepG2 cells via anti-inflammatory, antinecrotic, antioxidant potency. *Saudi Pharmaceutical Journal.* 28, 1182-1189. https://doi.org/10.1016/j.jsps.2020.08.007

Matsuda, T., Kato, T., Kiyotani, K., Tarhan, Y.E., Saloura, V., Chung, S., Ueda, K., Nakamura, Y., Park, J.H., 2017. P53 independent p21 induction by MELK inhibition. *Oncotarget.* 8, 57938–57947. https://doi.org/10.18632/oncotarget.18488

Maximov, G.K., Maximov, K.G., 2008. The role of p53 tumour-suppressor protein in apoptosis and cancerogenesis. *Biotechnol. Biotechnol. Equip.* 22, 664–668. https://doi.org/10.1080/13102818.2008.10817532

McCaig, C.D., Rajnicek, A.M., Song, B., Zhao, M., 2005. Controlling cell behavior electrically: current views and future potential. *Physiol. Rev.* 85, 943–978. https://doi.org/10.1152/physrev.00020.2004

Mujib, S.A., Alamsyah, F., Taruno, W.P., 2017. Cell death and induced p53 expression in oral cancer, HeLA, and bone marrow mesenchyme cells under the exposure to noncontact electric fields. Integr. *Med. Int.* 4, 161–170. https://doi.org/10.1159/000485186

Muramatsu, Y., Matsui, T., Deie, M., Sato, K., 2017. Pulsed electromagnetic field stimulation promotes anti-cell proliferative activity in doxorubicine-treated mouse osteosarcoma cells. *In vivo.* 31, 61–68. https://doi.org/10.21873/invivo.11026

Niu, J., Sun, Y., Guo, Q., Niu, D., Liu, B., 2016 . Serum miR-95-3p is a diagnostic and prognostic marker for osteosarcoma. *Springerplus.* 5, 1947. https://doi.org/10.1186/s40064-016-3640-0

Novello, C., Pazzaglia, L., Cingolani, C., Conti, A., Quattrini, I., Manara, M.C., Tognon, M., Picci, P., Benassi, M.S., 2013. miRNA expression profile in human osteosarcoma: role of miR-1 and miR-133b in proliferation and cell cycle control. *Int J Oncol.* 42:667–675. https://doi.org/10.3892/ijo.2012.1717

Nuccitelli, R., McDaniel, A., Anand, S., Cha, J., Mallon, Z., Berridge, J.C., Uecker, D., 2017. Nano-pulse stimulation is a physical modality that can trigger immunogenic tumour cell death. *J. Immunother. Cancer*. 5, 1–13. https://doi.org/10.1186/s40425-017-0234-5

Olsson, M., Zhivotovsky, B., 2011. Caspases and cancer. *Cell Death Differ.* 18, 1441–1449. https://doi.org/10.1038/cdd.2011.30

Parrish, A.B., Freel, C.D., Kornbluth, S., 2013. Cellular mechanisms controlling caspase activation and function. *Cold Spring Harb. Perspect. Biol.* 5, 1–24. https://doi.org/10.1101/cshperspect.a008672

Pirnia, F., Schneider, E., Betticher, D.C., Borner, M.M., 2002. Mitomycin C induces apoptosis and caspase-8 and -9 processing through a caspase-3 and Fas-independent pathway. *Cell Death Differ.* 9, 905–914. https://doi.org/10.1038/sj.cdd.4401062

Rachmat, O., Fiddiyanti, I., Sutedja, E., Ismono, D., Hidajat, N.N., 2017. Effect of electric fields on osteosarcoma (MG-63) cells. *Int. J. Sci. Res. Educ.* 5, 6513–6517.

Schmidt, S., Essmann, F., Cirstea, I.C., Kuck, F., Thakur, H.C., Singh, M., Kletke, A., Jänicke, R.U., Wiek, C., Hanenberg, H., Ahmadian, M.R., 2010. The centrosome and mitotic spindle apparatus in cancer and senescence. *Cell Cycle.* 9, 4469–4473. https://doi.org/10.4161/cc.9.22.13684

Silke, J., Meier, P., 2013. Inhibitor of apoptosis (IAP) proteins-modulators of cell death and inflammation. *Cold Spring Harb. Perspect Biol.* 5, 1–19. https://doi.org/10.1101/cshperspect.a008730

Silve, A., Leray, I., Leguebe, M., Poignard, C., Mir, L.M., 2015. Cell membrane permeabilization by 12-ns electric pulses: Not a purely dielectric, but a charge-dependent phenomenon. *Bioelectrochemistry*. 106, 369–378. https://doi.org/10.1016/j.bioelechem.2015.06.002

Silve, A., Leray, I., Poignard, C., Mir, L.M., 2016. Impact of external medium conductivity on cell membrane electropermeabilization by microsecond and nanosecond electric pulses. *Sci Rep.* 6, 1–15. https://doi.org/10.1038/srep19957

Solomon, L., Warwick, D., Nayagam, S., 2010. *Apley's System of Orthopaedics and Fraktures,* ninth ed. Hodder Arnold, London.

Widowati, W., Jasaputra, D.K., Wargasetia, T.L., Azizah, A.M., Subangkit, M., Lister, I.N.E., Ginting, C.N., Girsang, E., Faried, A., 2020a. Apoptotic potential of secretome from interleukin-induced natural killer cells toward breast cancer cell line by transwell assay. *HAYATI J. Biosci.* 27, 186-196. https://doi.org/10.4308/hjb.27.3.186

Widowati, W., Jasaputra, D.K., Sumitro, S.B., Widodo, M.A., Mozef, T., Rizal, R., Kusuma, H.S.W., Laksmitawati, D.R., Murti, H., Bachtiar, I., Faried, A., 2020b. Effect of interleukins (IL-2, IL-15, IL-18) on receptors activation and cytotoxic activity of natural killer cells in breast cancer cell. *African Health Sci*. 20, 822-832. https://doi.org/10.4314/ahs.v20i2.36

Yang, R.S., 2005. Limb salvage operations for patients with malignant bone tumours in the extremities. *Tzu Chi Med. J.* 17, 389–396.

Yao, D., Cai, G.H., Chen, J., Ling, R., Wu, S.X., Li, Y.P., 2014. Prognostic value of p53 alterations in human osteosarcoma: a meta-analysis. *Int. J. Clin. Exp. Pathol.* 7, 6725–6733.

Yu,W., Zhu, J., Wang, Y., Wang, J., Fang, W., Xia, K., Shao, J., Wu, M., Liu, B., Liang, C., Ye, C., 2017. A review and outlook in the treatment of osteosarcoma and other deep tumors with photodynamic therapy: from basic to deep. *Oncotarget.* 8, 39833–39848. https://doi.org/10.18632/oncotarget.16243

Zhao, X., Yang, Y., Xu, J., Luo, Y., Xin, Y., Wang, Y., 2018. Downregulation of microRNA-95-3p suppresses cell growth of osteosarcoma via CDKN1A/p21 expression. *Oncol. Rep.* 39, 289–297. https://doi.org/10.3892/or.2017.6065

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