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Non-Contact Electro Capacitive Cancer Therapy (ECCT) Modulate the mRNA Expression of PDFG and IL-1 in DMBA-induced Breast Cancer Rat

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ABSTRACT

Background: Breast cancer remains the most prevalent cancer among women globally, with significant mortality rates. Traditional therapies, such as surgery, chemotherapy, and radiotherapy, are associated with severe side effects and resistance, highlighting the need for alternative treatments. Electro-Capacitive Cancer Therapy (ECCT) is a promising non-invasive approach that uses low-intensity electric fields to selectively target cancer cells. **Objective**: This study aims to investigate the molecular mechanisms of ECCT, particularly its effects on key molecules such as PDGF and IL-1 in a DMBA-induced rat breast cancer model. **Materials and Methods**: The study used a post-test-only control group design with four groups: NINT (normal tissue), NIT (untreated tumor tissue), INT (DMBA-induced tumor tissue), and IT (ECCT-treated tumor tissue). ECCT was applied at 150 kHz for 21 days. mRNA expressions of PDGF and IL-1 were quantified using quantitative RT-PCR. **Results**: ECCT significantly reduced the mRNA expression of PDGF and IL-1 in treated tumor tissues (IT) compared to untreated tumor tissues (INT), bringing their levels closer to those observed in normal tissue (NINT). This suggests that ECCT downregulates key pro-angiogenic and pro-inflammatory molecules involved in tumor progression. **Conclusion**: In conclusion, the non-contact ECCT with a frequency of 150 kHz might downregulate PDGF and IL-1 mRNA expression in rat breast tumor tissue.

Keywords : Breast Cancer, ECCT, PDGF, IL-1, Gene Expression.

INTRODUCTION

Breast cancer remains the most prevalent cancer among women globally, with an estimated 2.3 million new cases and 685,000 deaths annually as of 2021(1). It is a leading contributor to cancer-related mortality, particularly in low- and middle-income countries, where limited access to advanced treatment options exacerbates the burden. Current treatments, including surgery, chemotherapy, and radiotherapy, are often associated with significant side effects, therapy resistance, and a failure to address the complexity of advanced-stage malignancies(2,3). These limitations highlight the urgent need for innovative, non-invasive, and effective therapeutic strategies.

Electro-Capacitive Cancer Therapy (ECCT) is emerging as a novel non-invasive therapeutic approach with the potential to revolutionize cancer treatment. ECCT employs low-intensity electric fields to target cancer cells selectively, disrupting their bioelectrical stability, proliferation, and

and survival(4,5). Initial clinical applications of ECCT in breast cancer patients have demonstrated promising outcomes, such as reduced tumor size and improved quality of life, without the systemic side effects associated with conventional therapies. Preclinical studies further support ECCT's potential, showing its ability to inhibit tumor growth and induce apoptosis. However, the molecular mechanisms underlying these effects remain largely unexplored, particularly in the context of breast cancer(6,7).

Previous research has begun to uncover the molecular impact of ECCT on cancer progression. For example, studies have reported that ECCT can modulate the expression of key angiogenic and inflammatory molecules, which are critical in the tumor microenvironment(8). Specifically, it has been suggested that ECCT may downregulate vascular endothelial growth factor (VEGF) and reduce inflammation-associated markers, contributing to its anti-tumor effects(9). However, the impact of ECCT on specific pathways and molecules central to breast cancer pathogenesis, such as platelet-derived growth factor (PDGF) and interleukin-1 (IL-1), has not yet been systematically investigated.

PDGF is a pivotal factor in tumor angiogenesis, stromal proliferation, and metastasis, with elevated expression linked to poor prognosis in breast cancer patients(10). Similarly, IL-1, a pro-inflammatory cytokine, contributes to the creation of a tumor-promoting microenvironment, enhancing cancer cell invasion and immune evasion(11). Targeting these molecules holds promise for disrupting the progression of breast cancer at the molecular level.

Using the 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer rat model, a well-established system that mimics the hormonal and environmental influences of human breast cancer, this study seeks to bridge critical gaps in the understanding of ECCT’s molecular mechanisms(6). Specifically, we investigate how ECCT modulates the mRNA expression of PDGF and IL-1 in this model, providing new insights into its effects on angiogenesis and inflammation pathways.

The urgency of this research lies in its potential to transform the treatment landscape for breast cancer. By elucidating the molecular mechanisms of ECCT, this study not only builds on the growing body of evidence supporting its therapeutic efficacy but also paves the way for its integration into conventional treatment regimens. These findings could position ECCT as a groundbreaking, cost-effective, and accessible alternative for breast cancer patients worldwide, particularly in resource-limited settings.

MATERIAL AND METHODS

Research Design

The design of this study was an experimental-based study with a post-test-only control group design that used four treatment groups, namely NINT: DMBA solvent-induced animal without ECCT group; NIT: DMBA solvent-induced animal group and treated with ECCT; INT: DMBA-induced animal group without ECCT; and IT: DMBA-induced and ECCT-treated animal groups.

Sample and Ethical Clearance

The sample used in this study was nodule breast tumor tissue which was a collection from the Biochemistry Laboratory, Faculty of Biology, Universitas Gadjah Mada. The samples were obtained from mice that had been induced with DMBA at a dose of 20 mg/kgBW, which were administered orally twice a week for 5 weeks. Animal trials in the NIT and IT groups were treated by the ECCT for 2x5 hours per day for 21 days. On the 22nd day, the animals were sacrificed for tissue isolation(4).

This research was carried out at the Genetic Engineering Laboratory, Biotechnology Study Program, Graduate School, Universitas Gadjah Mada. This study used an Ethical Clearance (EC) certificate with

certificate number: 00029/04/LPPT/2018 obtained from the institutional animal care and use committee of the Laboratorium Penelitian dan Pengujian Terpadu (LPPT) Universitas Gadjah Mada.

Quantitative RT-PCR

Total RNA was isolated from tissue samples using Direct-zol RNA Microprep Kits (Cat.No. R2072, Zymo Research, CA, USA) according to the manufacturer's recommendations. Total RNA was quantified by NanoDrop spectrophotometer (Maestrogen, MaestroNano, Hsinchu, Taiwan) respectively. Samples of cDNA were synthesized from total RNA using the cDNA synthesis kit (Cat.No.BIO-65053, SensiFAST, Bioline, London, UK). According to the manufacturer's recommendations, 500 ng/µL of template cDNA was put to the final volume of 20 µL of the reaction mixture. qPCR was performed with the SYBR Green method (Cat. No. BIO-98005, SensiFAST, Bioline, London, UK) by a real-time PCR system (Biorad CFX96, Biorad, California, USA). Quantitative Reverse Transcriptase PCR cycle parameters included 2 minutes at 95°C, then 40 cycles of denaturation (5 seconds at 95°C), annealing (10 seconds at 58-60°C), and elongation process (5-20 seconds at 72°C). GAPDH was used as the reference gene. The sequences of them are in Table 1.

Table 1. The sequences of the primers

Gene	Sequence	Annealing Temperature
PDGF-A	Forward: 5'-CCCCTGCCCATTCGGAGGAAGAG-3' Reverse: 5'- TTGGCCACCTTGACGCTGCGGTG-3'	60 ºC
Apaf-1	Forward: 5'- ATCAGCACCTCACAGCTTCC-3' Reverse: 5'- TCTCCTCCCGATGAGTAGGC-3'	58 ºC
GADPH	Forward: 5'-TGACAACTTTGGCATCGTGG-3' Reverse: 5'-GGGCCATCCACAGTCTTCTG-3'	60 ºC

Statistical Analysis

Data analysis was performed using Biorad CFX managerTM software to obtain quantification cycle (Cq), quantification curve and melting curve values from the qRT-PCR results. Changes in gene expression related to apoptosis and angiogenesis in the therapy and non-therapy groups can be identified by comparing the ΔCq value of the related gene with the ΔCq value of the control or calibrator. The ΔCq value of each therapy and non-therapy group was normalized using the housekeeping gene by the Livak formula(12) and the foldchange were obtained. The foldchange values obtained were processed and analyzed using SPSS 20.0 to test data normality with *Shapiro Wilk* to determine the distribution of data and homogeneity test with the Lavene test. The data was then subjected to a one-way ANOVA parametric different test to compare differences between groups and then continued with the LSD post-hoc test.

RESULTS

ECCT regulate the relative mRNA expression of PDGF and IL-1

Relative mRNA expression of PDGF in untreated breast tumor nodules (INT) showed a significant increase compared to normal breast tissue (NINT), reflecting its role in promoting tumor angiogenesis and stromal proliferation. However, following ECCT therapy at a frequency of 150 kHz, mRNA expression of PDGF in treated tumor nodules (IT) significantly decreased compared to untreated tumor nodules (INT) ($p < 0.05$) (Figure 1). Interestingly, PDGF mRNA expression in treated tumor nodules (IT) did not differ significantly from normal breast tissue (NINT), indicating that ECCT restores PDGF expression to physiological levels without affecting healthy tissue.

As a key pro-inflammatory cytokine, IL-1 exhibited significantly elevated mRNA expression in untreated breast tumor nodules (INT) compared to normal tissue (NINT), consistent with its role in

creating an inflammatory tumor microenvironment that promotes cancer progression. ECCT therapy at a frequency of 150 kHz significantly reduced IL-1 mRNA expression in treated tumor nodules (IT) compared to untreated tumor nodules (INT) ($p < 0.05$) (Figure 1). Furthermore, IL-1 expression in treated tumor nodules (IT) was not significantly different from that in normal breast tissue (NINT), demonstrating ECCT's efficacy in suppressing tumor-associated inflammatory responses without affecting normal tissue.

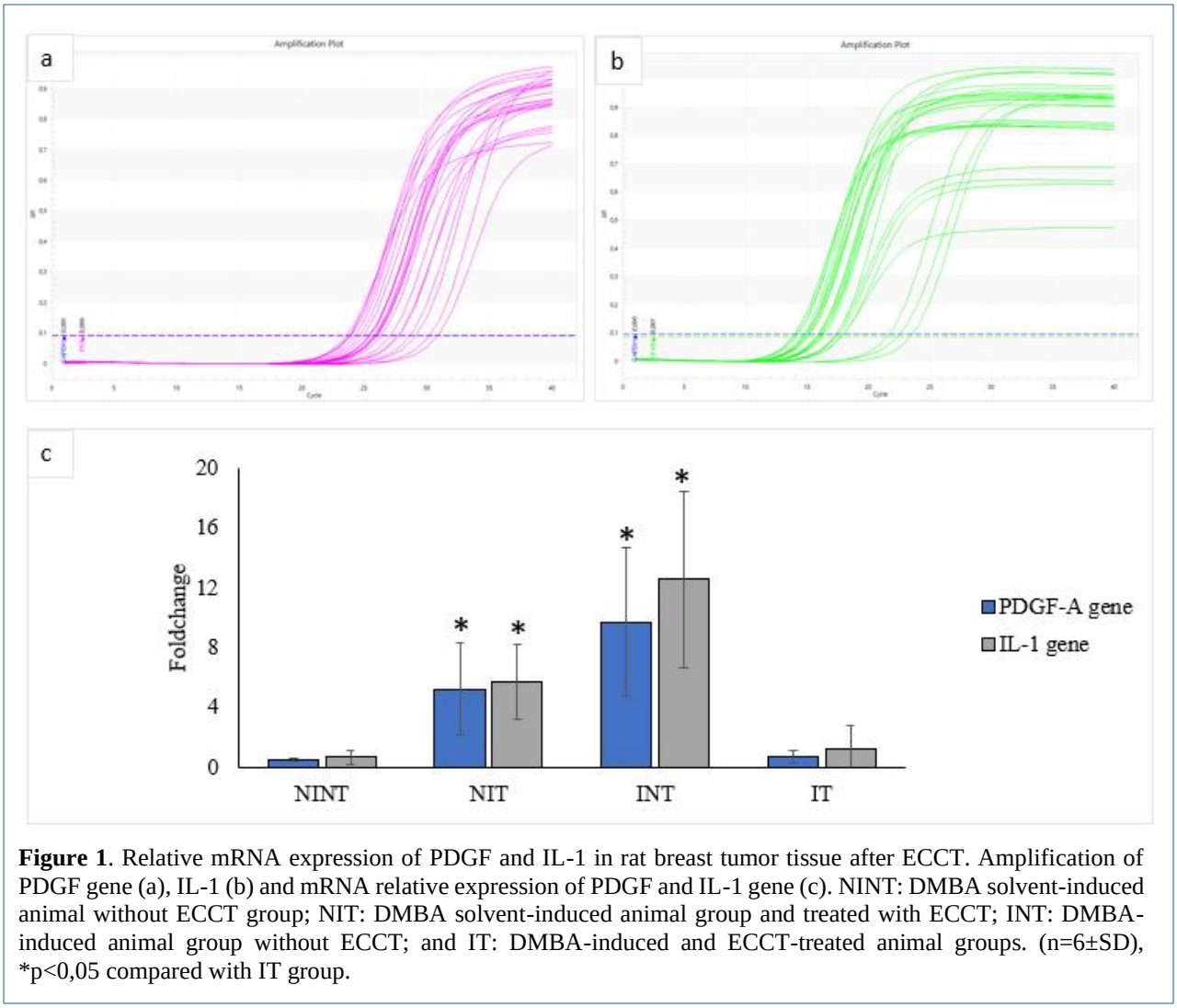

Figure 1. Relative mRNA expression of PDGF and IL-1 in rat breast tumor tissue after ECCT. Amplification of PDGF gene (a), IL-1 (b) and mRNA relative expression of PDGF and IL-1 gene (c). NINT: DMBA solvent-induced animal without ECCT group; NIT: DMBA solvent-induced animal group and treated with ECCT; INT: DMBA-induced animal group without ECCT; and IT: DMBA-induced and ECCT-treated animal groups. (n=6±SD), *p<0,05 compared with IT group.

DISCUSSION

The findings of this study demonstrate that ECCT therapy at a frequency of 150 kHz effectively modulates the expression of PDGF and IL-1, two critical molecules involved in angiogenesis and inflammation, which are central to tumor progression and metastasis.

PDGF is a potent proliferation factor that plays a crucial role in tumor development by stimulating the growth and migration of tumor cells, thereby promoting metastasis(10). In cancer, overexpression of PDGF also facilitates the formation of a robust vascular network, supporting tumor growth and metastasis(13). The significant downregulation of PDGF mRNA expression in treated tumor nodules (IT) compared to untreated tumor nodules (INT) suggests that ECCT disrupts proliferation signaling in the tumor microenvironment. The mechanism underlying this suppression likely involves electric field-induced alterations in cellular signaling pathways. ECCT exposure may influence the

activity of voltage-gated ion channels and membrane potentials, which in turn regulate intracellular calcium levels—a critical mediator of proliferation signaling(14). Calcium-dependent pathways are known to activate transcription factors such as NF-κB and AP-1, which regulate PDGF expression(15). By disrupting these pathways, ECCT may selectively reduce PDGF expression in tumor cells, effectively hindering growth(10). Interestingly, PDGF expression in treated tumor nodules (IT) did not differ significantly from normal breast tissue (NINT), indicating that ECCT restores PDGF levels to physiological norms without disrupting proliferation and angiogenesis homeostasis in healthy tissues. This selective action aligns with previous studies that demonstrate the differential biological responses of cancer cells and normal cells to electric fields, attributable to the distinct electrical properties of their membranes.

IL-1 is a pro-inflammatory cytokine that contributes to the tumor microenvironment by promoting immune evasion, angiogenesis, and the activation of tumor-associated macrophages(5,16). Its overexpression in untreated tumor nodules (INT) compared to normal tissue (NINT) highlights its role in cancer progression. The significant reduction of IL-1 mRNA expression in treated tumor nodules (IT) after ECCT exposure suggests that ECCT disrupts the inflammatory signaling pathways that sustain the tumor microenvironment. One potential mechanism is the effect of ECCT on the NF-κB pathway, a key regulator of IL-1 expression. By modulating membrane potential and calcium signaling, ECCT may inhibit NF-κB activation and its subsequent nuclear translocation, thereby reducing IL-1 transcription(15,17). Furthermore, ECCT may also influence hypoxia-inducible factors (HIF-1α), which are known to regulate IL-1 expression in hypoxic tumor conditions. Similar to PDGF, the lack of significant differences in IL-1 expression between treated tumor nodules (IT) and normal tissue (NINT) suggests that ECCT targets tumor-specific inflammatory responses while sparing normal tissues. This specificity is likely due to the distinct metabolic and electrical properties of cancer cells, such as their reliance on glycolytic metabolism and altered ion channel activity(18).

The interplay between PDGF and IL-1 amplifies their roles in promoting cancer progression. IL-1 can upregulate PDGF expression by activating transcription factors like NF-κB and AP-1, creating a positive feedback loop that enhances angiogenesis and inflammation(19,20). By simultaneously suppressing PDGF and IL-1, ECCT disrupts this synergistic relationship, effectively targeting two key drivers of tumor progression. ECCT's ability to downregulate PDGF and IL-1 may stem from its effects on tumor cell membrane potential and intracellular signal transduction(21). The application of an external electric field alters the plasma membrane's voltage gradient, potentially modulating the activity of ion channels and receptors involved in angiogenic and inflammatory signaling(22). For example, ECCT may reduce calcium influx through voltage-gated calcium channels, disrupting downstream pathways that regulate PDGF and IL-1 expression.

Additionally, the reduction of CCL2 and IL-18 by ECCT in previous study (5) likely contributes to the downregulation of PDGF and IL-1, while TNF-α remains unaffected. CCL2 and IL-18 are key pro-inflammatory cytokines that promote tumor growth by enhancing PDGF and IL-1 expression, which are involved in angiogenesis and immune response. By reducing these cytokines, ECCT may inhibit the activation of PDGF and IL-1, disrupting tumor angiogenesis and inflammation. However, TNF-α may not be as directly influenced by ECCT in this context, possibly due to its involvement in a different inflammatory pathway or its resistance to modulation by the electric field in this experimental setup.

CONCLUSIONS

In conclusion, the non-contact ECCT with a frequency of 150 kHz might downregulate PDGF and IL-1 mRNA expression in rat breast tumor tissue.

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Competing Interests

The authors declare that there is no conflict of interest.

REFERENCES

1. Yu AYL, Thomas SM, DiLalla GD, Greenup RA, Hwang ES, Hyslop T, et al. Disease characteristics and mortality among Asian women with breast cancer. Cancer [Internet]. 2022 Mar 1 [cited 2022 Aug 12];128(5):1024–37. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cncr.34015
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.
3. Weinberg F, Han MKL, Dahmke IN, Campo A Del, de Jonge N. Anti-correlation of HER2 and focal adhesion complexes in the plasma membrane. PLoS One. 2020;15(6):1–15.
4. Pratiwi R, Antara NY, Fadliansyah LG, Ardiansyah SA, Nurhidayat L, Sholikhah EN, et al. CCL2 and IL18 expressions may associate with the anti-proliferative effect of noncontact electro capacitive cancer therapy in vivo. F1000Research [Internet]. 2020 Jul 23 [cited 2021 Mar 25];8:1770. Available from: https://doi.org/10.12688/f1000research.20727.1
5. Pratiwi R, Yudi Antara N, Gunawan Fadliansyah L, Arif Ardiansyah S, Nurhidayat L, Nurwening Sholikhah E, et al. Open Peer Review CCL2 and IL18 expressions may associate with the anti-proliferative effect of noncontact electro capacitive cancer therapy in vivo [version 1; peer review: 1 approved]. 2019 [cited 2022 Jul 15]; Available from: https://doi.org/10.12688/f1000research.20727.1
6. Nurhidayat L, Fajar I, Yati A, Prinanda HH, Irfan M, Afina D, et al. Evaluation of Static Electric Field Exposure on Histopathological Structure and Function of Kidney and Liver in DMBA-Induced RAT (Rattus norvegicus Berkenhout, 1769). Malaysian J Fundam Appl Sci. 2022;18(6):703–13.
7. Fathurrohmah S, Cahyadi GA. YP, Alamsyah F, Pratiwi R. Electric Field-Based Cancer Therapy Induces the Expression of HMGB1 and PD-L1 mRNA Genes on Breast Tumor of Female Rats. Indones J Cancer Chemoprevention. 2022;13(2):128.
8. Alamsyah F, Niswah Ajrina I, Nur F, Dewi A, Iskandriati D, Prabandari SA, et al. Antiproliferative Effect of Electric Fields on Breast Tumor Cells In Vitro and In Vivo. Indones J Cancer Chemoprevention [Internet]. 2015 Oct 31 [cited 2021 Apr 22];6(3):71–7. Available from: https://www.ijcc.chemoprev.org/index.php/ijcc/article/view/88
9. Pratiwi R, Antara NY, Fadliansyah LG, Ardiansyah SA, Nurhidayat L, Sholikhah EN, et al. CCL2 and IL18 expressions may associate with the anti-proliferative effect of noncontact electro capacitive cancer therapy in vivo. F1000Research. 2019;8.
10. Roswall P, Bocci M, Bartoschek M, Li H, Kristiansen G, Jansson S, et al. Microenvironmental control of breast cancer subtype elicited through paracrine platelet-derived growth factor-CC signaling. Nat Med [Internet]. 2018;24(4):463–73. Available from: http://dx.doi.org/10.1038/nm.4494
11. Ferreira-Hermosillo A, Molina-Ayala M, Ramírez-Rentería C, Vargas G, Gonzalez B, Isibasi A, et al. Inflammatory cytokine profile associated with metabolic syndrome in adult patients with type 1 diabetes. J Diabetes Res. 2015;2015.

12. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods. 2001 Dec 1;25(4):402–8.

13. Thies KA, Hammer AM, Hildreth BE, Steck SA, Spehar JM, Kladney RD, et al. Stromal Platelet–Derived Growth Factor Receptor-b Signaling Promotes Breast Cancer Metastasis in the Brain. Cancer Res. 2021;81(3):606–18.

14. Alamsyah F, Fadhlurrahman AG, Pello JI, Firdausi N, Evi S, Karima FN, et al. PO-111 Non-contact electric fields inhibit the growth of breast cancer cells in animal models and induce local immune reaction. ESMO Open. 2018 Jun 30;3:A269.

15. Kim EH, Song HS, Yoo SH, Yoon M. Tumor treating fields inhibit glioblastoma cell migration, invasion and angiogenesis. Oncotarget. 2016;7(40):65125–36.

16. Hillmann A, Paebst F, Brehm W, Piehler D, Schubert S, Tárnok A, et al. A novel direct co-culture assay analyzed by multicolor flow cytometry reveals context- And cell type-specific immunomodulatory effects of equine mesenchymal stromal cells. PLoS One. 2019;14(6):1–21.

17. Baker RG, Hayden MS, Ghosh S. Cell Metabolism Review NF-kB, Inflammation, and Metabolic Disease. Cell Metab. 2011;13:11–22.

18. Moulder DE, Hatoum D, Tay E, Lin Y, McGowan EM. The Roles of p53 in Mitochondrial Dynamics and Cancer Metabolism: The Pendulum between Survival and Death in Breast Cancer? Cancers 2018, Vol 10, Page 189 [Internet]. 2018 Jun 8 [cited 2022 Jul 15];10(6):189. Available from: https://www.mdpi.com/2072-6694/10/6/189/htm

19. Ahmad S, Akhter F, Shahab U, Rafi Z, Khan MS, Nabi R, et al. Do all roads lead to the Rome? The glycation perspective! Semin Cancer Biol. 2018 Apr;49:9–19.

20. Bellei B, Migliano E, Picardo M. A Framework of Major Tumor-Promoting Signal Transduction Pathways Implicated in Melanoma-Fibroblast Dialogue. Cancers 2020, Vol 12, Page 3400 [Internet]. 2020 Nov 17 [cited 2022 Jun 28];12(11):3400. Available from: https://www.mdpi.com/2072-6694/12/11/3400/htm

21. Yang M, Brackenbury WJ. Membrane potential and cancer progression. Front Physiol. 2013;4 JUL(July 2013).

22. Sundelacruz S, Levin M, Kaplan DL. Role of Membrane Potential in the Regulation of Cell Proliferation and Differentiation.