Alexandria University
Medical Research Institute

Medical Research Institute
Department of Applied Medical Chemistry

Effect of Betulinic Acid on Gene Expression of Zinc Finger Protein (Kaiso) in Breast Cancer Cells lines (MCF-7 and MDA-MB-231)

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

In

Applied Medical Chemistry

Submitted by

Esraa Mohamed Abd El Wakeel

Bachelor of Allied Medical Science
Faculty of Allied Medical Science, Pharos University, 2015

2020

VI. SUMMARY, CONCLUSION, AND RECOMMENDATIONS

VI.1 Summary

Cancer is driven by genetic and epigenetic changes which allow cells to escape from the apoptosis mechanisms and continue to proliferate uncontrollably, which leads to increased mass of tumor. Therefore inducing apoptosis in tumer cells without affecting the normal cells is a major process for controlling cancer development and progression.

In this study, The efficacy of BetA in MCF-7 and triple-negative breast carcinoma MDA-MB-231 cell lines was investigated. Bet A has been used to treat human diseases including cancer for thousands of years because of its wide range of biological properties. Which interplay between; the induction of cell cycle arrest, inhibition of angiogenesis, inhibition of topoisomerase I and II function, down-regulate (Sp) transcription factors, Inhibition of NF-κB signaling pathway and overcoming multidrug resistance (MDR). In addition, studies have demonstrated that BA selectively acts on tumor cells, not normal cells.

To achieve our aim, cell viability and cytotoxicity by MTT, cell cycle analysis by flow cytometry as well as apoptotic analysis by annexin were performed in breast cancer cell lines MCF-7 and MDA-MB-231 treated with BA. Also, we tried to explore whether the apoptotic potential of BA is proceeding via modulation of the gene expression of zinc finger protein Kaiso and its targets i.e. as oncogene c-myc as well as the tumor suppressor gene p16 which regulate and subsequently induce cell death in breast cancer cell lines

VI.2 Conclusion

An important finding from our study is that BA has been proposed to be an important factor in breast cell proliferation and differentiation by modulating the methylation state of Kaiso targets; c-myc and p16 in human breast cancer cell lines MCF-7 and MDA-MB-231. In conclusion, our findings are promising since it is well established that BA is one of the most effective anti-cancer therapy in breast cancer cell lines MCF-7, MDA-MB-231 and may have potential as adjuvant therapy or in combination therapy. In particular, a well-tolerated response of MDA-MB-231 upon administration of high dose of BA may add benefit in treatment of TNBC that usually becomes very sensitive during the initial phase of treatment, then successively avoid the cytotoxic effects of the drugs, resulting in a new relapse and metastasis. Thus it may be accepted as a solely or in combination with another therapy to overcome the resistance of breast cancer cells especially TNBC. This finding add benefit in treatment of MDA-MB-231 because its ability to resist the anti-cancer therapy is due to its high ability to avoid apoptosis, a major challenge in breast cancer treatment.

VI.3 Recommendations

We recommend using more treated groups with Betulinic acid for other cancer cell lines, as well as using experimental mice so that we can see the effect of betulinic acid in vivo also.

MCF-7 and MDA-MB-231. The effect of BA on the methylation status of oncogene c-myc and tumor suppressor gene p16 was detected by MSP. Since their epigenetic alterations by methylation affect their regulation by Kaiso, we also studied its gene expression by RT-PCR.

Our results confirmed the cytotoxic activity of betulinic acid on MDA-MB-231 and MCF-7 cell lines, with more sensitivity of MCF-7 to BA than MDA-MB-231.

Our data also revealed that BA promotes the arrest of breast cancer cells at the G0 / G1 phase and forces cells to proceed to irreversible late apoptosis at a lower dose for MCF-7 compared to that needed for the same effect in MDA-MB-231.

By measuring kaiso mRNA expression levels, we observed that IC50 treatment with MCF-7 induces overexpression of Kaiso (14-fold) vs (9-fold) in MDA-MB-231, respectively , indicating that Kaiso acts as a tumor suppressor because its overexpression was parallel to BA cytotoxicity on MCF-7 and MDA-MB-231.

Finally, after analyzing the DNA methylation status within the p16 and c-myc promoter by methylation-specific PCR; we observed the hypermethylation of c-myc and the hypomethylation stronger in MCF-7 upon treatment with 10%IC50 and IC50 compared to these effects in MDA-MB-231. Which indicate that BA may be a crucial modulator of breast cells proliferation and differentiation via modulation of methylation status of the targets of Kaiso; c-myc and p16 in human breast cancer cell lines, MCF-7 and MDA-MB-231 cells.